LITERATURE REVIEW

2.2. LITERATURE REVIEW

2.2.1. Occurrence and Distribution of Acid Phosphatase(s) in Plants :

Among the several phosphomonoesterases, acid phosphatase is cosmopolitan in distribution. The activity of this enzyme has been recorded from the unicellular micro-organisms to higher plants and animals. However, this review will principally be confined to microorganisms and plants only.

Acid Phosphatase of Bacterial Origin :

In bacteria, the activity of acid phosphatase has been documented and studied from a number of sources. Among these the studies on acid phosphatase from *Escherichia coli* (Kozoreva *et al.*, 1984), *Streptomyces hygroscopicus* (Ozegosski and Mueller, 1984), *Rhodotorulla glutinis* (Leelasart and Bonally, 1988), *Pseudomonas aeruginosa* (Garrido *et al.*, 1988), *Zymononas mobilis* (Pond *et al.*, 1984), *Halomonas elongata* (Byhand *et al.*, 1980), *Rhizobium meliloti.*, (Luecheini *et al.*, 1990) are of special importance.

Acid Phosphatase of Algal Origin :

In algae, the activity of acid phosphatase has been detected and biochemically studied from different members like *Nostoc* sp. (Dubois *et al.*, 1984), *Chlorella vulgaris.*, (Gilberto, 1988), *Gigartina teedii.*, and *Chondria lenuissima* (Teskos and Schenpf, 1991) etc.

Acid Phosphatase of Fungal Origin :

Fungal specimens have got immense importance with respect to several biochemical and biotechnological studies centred around acid phosphatase. The activity of this enzyme has been marked from a wide number of fungi having polyphylogenetic diversity. Among these, *Saccharomyces cerevisiae* (Linnenans *et al.*, 1977; Mizunaga, 1980; etc.), *Puccinia graminis tritici* (Bossanyi and Olah, 1974), *Pythium paroccandrum, Botrytis cincrea, Rhizoctonia solani* (Haenssler *et al.*, 1977), *Aspergillus niger* (Komano, 1976;

Samusal and Sheota, 1977, etc), *Ustilago violacea* (Batcho and Cardon, 1980), *Claviceps purpurea* (Vorison, 1977), and in many ectomycorrhizal fungi (Korchler *et al.*, 1988), *Penicillium punicatosum* (Yoshida *et al.*, 1989), *Penicillium brevicompactum* (Krupyanko *et al.*, 1990), *Phytophthora erythro-septica* (Lucas and Pitt., 1975), *Psalliota bispora* (Panzica and Viglietti, 1975) etc.

Acid Phosphatase of Bryophytic Origin :

Studies on acid phosphatase in bryophytes are very inadequate. In this connection, two reports may be pointed out. Haenssler (1977) has shown that SO_2, in higher concentration, inhibits the activity of acid phosphatase in some mosses. And, two monoecious members under the order Marchantiales viz., *Plagiochasma rupestre and Mannia androgyna* possess polymorphic forms of this enzyme (Boisslier and Blischler, 1988). In *Sphagnum pulchrum* isozymes of acid phosphatase have been reported (Daniels, 1982).

Acid Phosphatase from Gymnospermic Origin :

In gymmnosperms, too, the distribution and localization of acid phosphatase have not yet been studied in details. Jonson and Blomquist (1976) reported the presence of this enzyme in two isozymic forms from the needle leaves of *Pinus sylvestris.* In *P. banksiana,* the enzyme has also been detected from the seedlings (Zumzek and Shay, 1988).

Acid Phosphatase from Angiospermic Origin :

The enzymic activity, localization, several physiological role and biochemical regulations of acid phosphatase have well been studied from a large number of angiosperms since the last fifty years. The enzyme is operative in almost all the angiosperms with variable forms in relation with various physiological significance. A brief account of these may be cited here.

Acid phosphatases have been detected from *Zea mays* (Nagahashi and Kumosinski,

1988, etc.), *Nicotiana tabacum* (Kumar and Naherchandani, 1988, etc.), *Oryza sativa* (Ohtsuka and Saka, 1988, etc.), *Cicer arietinum* (Angosto *et al.*, 1988 etc.), *Morus niger* (Lal and Jaiswal, 1988, etc.), *Coccinia grandis* (Gulati, 1989; etc.), *Arachnis* sp. (Hew *et al.*, 1989), *Datura innoxia* (Sangwan *et al.*, 1989), *Phoenix dactylifera* (Chandrasekhar and Demason, 1989), *Musa paradisiaca* (Kanellis *et al.*, 1989), *Vigna radiata* (Kundu and Banerjee, 1990), *Dioscorea alata, D. bulbifera, D. esculanta* (Twyford *et al.*, 1990), *Lycopercicum esculentum* (Tanaka *et al.*, 1990), *Brassica juncea* (Tong and Shiru, 1990), *Solanum tuberosum* (Bingham *et al.*, 1976), *Hordeum vulgare* (Bailey *et al.*, 1976), *Poa pratensis* (Lorence *et al.*, 1975), *Drosera rotundifolia* (Claney and Coffey, 1977), *Pisum sativum* (Weeden and Mark, 1984), *Zamia floridana* (Zavada, 1983), *Acer platanoides* (Szczotka, 1984), *Kalanchoe blossfeldiana* of Crassulaceae (Eckhardt and Engelmann, 1984), *Triticum vulgare* (Mashlowski *et al.*, 1978), *Glycine soja and Glycine max* (Kiang *et al.*, 1985), *Carica papaya* (Carreno and Harvey, 1982), *Annona squamosa* (Vinthanage, 1984), *Rhododendron arboreum, R. barbatum, R. lepidolum, R. anthopogen and R. campanulatum* (Bhadula *et al.*, 1981) etc.

2.2.2. Distribution of Alkaline Phosphatase (s) :

Among the several phosphomonoesterases, alkaline phosphatase is almost cosmopolitan in distribution. The activity of this enzyme has been recorded from the unicellular microorganisms to higher plants and animals.

In **bacteria**, the activity of alkaline phosphatase has been documented and principally studied from *Escherichia coli* (Garen and Levinthal, 1960 and Klibanov *et al.*, 1983). The same enzyme has been detected from *Rhizobium leguminosarum* (WU 235) by Glenn and Dilworth (1980).

In **fungi**, the occurrence of alkaline phosphatase has been reported from yeast (*Saccharomyces cerevisiae*) in different isozymic forms (Mitchell *et al.*, 1981; Kaneko *et al.*, 1987). Adler (1976) detected the enzyme in the halotolerant yeast (*Debasyomyces hansenii*). Multiple forms of alkaline phosphatases have been documented in *Aspergillus oryzae* (Sakurai *et al.*, 1981), and in *Aspergillus niger* (Rokosu *et al.*, 1982). Cultures

of *Aspergillus nidulans* also exhibit alkaline phosphatase activity (Isaas and Gokhale, 1982). In *Neurospora crassa*, under various growth conditions, the activity of alkaline phosphatase has been reported by many workers (Nahas *et al.*, 1982; Auda *et al.*, 1988; Hasunuma and Ishikawa, 1977, etc.).

In **algae**, there are seveal reports of alkaline phosphatase activity covering various divisions of this group. Vincent (1981) demonstrated the enzymic activity in *Scenedesmus quadricauda, Monoraphidium contortum, Anabaena fles-aquae, Melesira quanulata.* Konopka (1982) obtained the enzymatic activity in a Cyanophycean alga, *Oscillatoria rubescens.* enzymic activity has also been documented in an unicellular Chlorophycean alga, *Chlamydomonas reinhardii* (Olsen *et al.*, 1983). In slime mold (*Dictyostelium discoideum*) this enzymic activity has been observed by Atryzek (1976). Alkaline phosphatase activity has also been detected from marine dinoflagellates like *Amphidinium carterae, Ceratium tripus, Prorocentrum minimum and Scrippsiella trochoidea.* Occurrence of the same enzyme in the red-tide dinoflagellate, *Ptychodiscus brevis* has also been confirmed by Vargo and Shannby (1985).

Alkaline phosphatase activity has been detected from a large number of **angiosperms** since the last fifty years. Recently the enzymic activity has been studied from some economically important plants like *Cucumis sativus* (Yamaha and Matsumoto, 1981), *Cucumis melo* (Bansal *et al.*, 1981). There is a significant negative correlation between the alkaline phosphatase activity and extractable total phosphates in leaves of five aquatic plants, namely *Phragmites australis, Typha angustifolia, Stratiotes aloides, Nymphaea alba and Nuphar luteum* (Kufel, 1982). In melon seedlings, alkaline phosphatase activity has been documented by Naguib and Kalil (1989). Activity of the same enzyme has been detected in three different species of *Cuscuta*, a stem parasite (Mattoo and Mattoo, 1974).

In **animals**, activity of alkaline phosphatase has been recorded from a good number of sources. In higher animals, intestine is the principal locale of alkaline phosphatase. Alkaline phosphatase is commercially available in purified form from bovine intestinal mucosa, calf intestinal mucosa and from intestines of chicken, rabbit, dog, horse, sheep, pigion, porcine, eel, trout, guinea-pig, cat etc. (Sigma Catalogue, 1991).

2.2.3. Distribution of L-*myo*-inositol-1-phosphate synthase :

Streptomyces griseus, the microorganism producing the antibiotic streptomycin and mutant strains of this species that cannot synthesize streptomycin, possess L-*myo*-inositol-1-phosphate synthase activity. The enzyme isolated from this organism is extremely unstable *in vitro* and requires a divalent metal ion for its activity and this is the first instance that an enzyme of this specificity is found in a prokaryotic organism (Pittner *et al.*, 1979).

In **algae**, L-*myo*-inositol-1-phosphate has been detected from a number of genera, namely, *Euglena gracilis, Chlorella vulgaris, Spirogyra maxima and Microspora willeana. E. gracilis* exhibits maximum synthase activity from a 7-day old culture (Dasgupta *et al.*, 1984; Dasgupta and Adhikari, 1983).

In **fungi**, appreciable L-*myo*-inositol-1-phosphate synthease activity was recorded in *Saccharomyces cerevisiae* (Donahue and Henry, 1981), *Neurospora crassa* (Pina *et al.*, 1978). *Polyporus anthelminticus, Ganoderma lucidum, Irpex flavus* (Polyporaceae), *Agaricus campestris, Schizophyllum commune, Lentinus subnudus* (Agaricaceae) and *Scleroderma* sp. (Sclerodermataceae) (Dasgupta *et al.*, 1984). Among these members, *Sccharomyces cerevisiae* is considered as a remarkable source of this enzyme from the class of Ascomycetes (Donahue and Henry, 1981) and from the class of Basidiomycetes, *Scleroderma* sp. exhibits highest and *Irpex flavus* bears the lowest specific activity of *myo*-inositol synthase (Dasgupta *et al.*, 1984).

In **bryophytes**, varying degrees of *myo*-inositol synthase activity, usually higher than those found in green algae, were recorded with some of the bryophytes, e.g. *Lunularia* sp., *Targionia* sp. *Marchantia polymorpha, Dumortiera* sp., all of the order Marchantiales, although the enzyme was not detectable in *Plagiochasma* sp. of the same order, or *Pellia epiphylla* of the order Jungermanniales. Interestingly, in *Marchantia polymorpha*, the synthase activity was found to be associated with the development of the reproductive structures. The presence of *myo*-inositol synthesizing activity in algae, fungi and bryophytes, suggests and more ubiquitous occurrence of this cyclitol and a more general physiological significance in the lower plant groups (Dasgupta *et al.*, 1984).

In **Gymnosperms**, the activity of L-*myo*-inositol-1-phosphate synthase was recorded from the pollen grains of *Pinus ponderosa* (Gumber *et al.*, 1984).

In **angiosperms**, L-*myo*-inositol-1-phosphate synthase activity has been detected from a wide number of genera viz., *Vigna radiata* (Majumder and Biswas, 1973, Adhikari and Majumder, 1983), *Acer pseudoplatenus* (Loewus and Loewus, 1971), *Lemma gibba* (Ogunyemi *et al.*, 1978), *Lilium longiflorum* (Schuermann *et al.*, 1981) etc.

The activity of L-*myo*-inositol-1-phosphate synthase of these above plant origin is of soluble or cytosolic in nature. In green plant chloroplast, about 10% of the membrane phospholipids consist of phosphoinositides (Imhoff and Bourdu, 1973) which require an endogenous pool of *myo*-inositol for their synthesis. Impermeability of the chloroplast membrane to the cyclitol (Wang and Nobel, 1971), casts doubt as to the cytoplasmic origin of the chloroplastic inositol and suggests that the plastid is a site for synthesis of *myo*-inositol. Support for the view is found in the report that labelled glucose is enzymatically incorporated into *myo*-inositol by acetone-treated chloroplast preparation from peas (Imhoff and Bourdu, 1973). Loewus *et al.*, (1980) have also found inositol synthase activity present in both soluble and chloroplastic fractions of *Euglena gracilis* Z. Adhikari *et al.*, (1987) have shown that the chloroplasts from 5-to 7-day-old *Vigna radiata* seedling, grown under alternate light/dark conditions or from green *Euglena gracilis* Z. cells harbour L-*myo*-inositol-1-phosphate synthase activity. In contrast, dark grown *Vigna radiata* seedling, or streptomycin-bleached *Euglena* cells exhibit either reduced or no enzyme activity. An apparent enhancement of the choloroplastic inositol synthase by growth in presence of light is observed. According to them, the fundamental difference between the cytosolic and plastidial inositol synthase (s) is associated with this light-dependence. The cytosolic enzyme is not activated or inhibited by light, however, the plastidial one is remarkably light-activated.

Like various microbial and plant systems, the distribution of L-*myo*-inositol-1-phophate in animal systems of a wide phylogenetic diversity has been documented by several workers. L-*myo*-inositol-1-phosphate synthase activity has been monitored in the brain of *Periplaneta americana* (insect), *Channa punctatus* (fish), *Bufo melanostictus* (amphibia), *Calotes versicolor* (reptile), *Columba livia* (bird), *Rattus*

rattus (adult mammal), *Homo sapiens* (fetal mammal) by Biswas *et al.*, (1981). The activity for the same enzyme has also been reported from rat testis (Majumdar and Eisenberg, Jr., 1977), beef testis (Pittner and Hoffmann-Ostenhof, 1979), bovine testis and in rat mammary gland (Naccarato *et al.*, 1975).

2.2.4. Fructose-1, 6-bisphosphatase (s) :

Early in the 20th century a number of workers observed that plant and animal tissues contain enzymes that split phosphoric acid from various phosphate esters (Suzuki *et al.*, 1907; McCollum and Hart, 1908; Grosser and Husler, 1912). These enzymes are, in general, called phosphatases. So, phosphatases are defined as enzymes that catalyze the hydrolysis of phosphoric acid esters. Phosphatases belong to the enzyme class of Hydrolases. Of the different series of phosphoric esters, representatives of only two the phosphomonesters and the phosphodiesters - are so far known as constituents of cells. The formation of orthophosphate from a given phosphodiester involves at least two cleavage steps usually catalyzed by different enzymes : A phosphodiesterase liberates one of the alcohol groups, and a phosphomonoesterase hydrolyzes the phosphomonoester obtained, with formation of orthophosphate. The pronounced specificity of the hydrolases of phosphoryl compounds towards polyphosphates, phosphomonoesters and phosphodiesters contrasts sharply with lack of specificity shown by some phosphomonoesterases towards various phosphomonoesters. Therefore, the numerous individual phosphomonoesterases may be allotted in 2 large groups : nonspecific and specific. The nonspecific phosphomonoesterases are further divided into 2 sub-groups namely alkaline phosphomonoesterases or simply alkaline phosphatases and acid phosphatases.

Fructose-1, 6-bisphosphatase is considered as a specific phosphatase. This enzyme when first described by Gomori (1943) was termed a specific hexose diphosphatase. Later on, for about 30 years, it was known as Fructose-1, 6-diphosphatase. Gomori's preparations from kidney or liver had litle or no activity on glycerophosphate, phenyl phosphate, pyrophosphate etc., whereas hexose diphosphate was rapidly split. Although other sugar phosphates were not tested by Gomori (1943) as possible substrates, later workers showed the enzyme to be, indeed, markedly specific for fructose-1, 6-

diphosphate (McGilvery, 1955; Mokrasch and McGilvery, 1956). Thus glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate, L-sorbose-1-phosphate and phosphoglycerate are not hydrolyzed. Fructose-1-phosphate and L-sorbose-1, 6-diphosphate are hydrolyzed at rates of 0.009 and 0.03, respectively, the rate of fructose diphosphate. As shown by Hers *et al.*, (1953b), the purified liver enzyme decomposes fructose-1, 6-diphosphate as follows :

Fructose-1, 6, diphosphate + H_2O ——> Fructose-6-phosphate + P_i.

Among the general properties that can be mentioned for the catalysis of the above reaction by the specific hydrolase are an optimum pH of about 9.3 to 9.7, activation of the enzyme by Mn^{2+} or Mg^{2+} or both, inhibition by fructose-6-phosphate but not by fructose-1-phosphate, and inhibition by fluoride. The enzyme appears to be a sulfhydryl enzyme as evidenced by the fact that its activity is inhibited by paramercuribenzoate, and purified preparations require mercaptan such as cysteine for activity.

Liver fructose-1, 6-diphosphatase has also been shown to be much more sensitive to quinones than some of the phosphomonoesterases that do not depend on free-SH groups for activity (Walsh and Walsh, 1948). Complete inhibition of the enzyme by 10^{-5}M benzoquinone may be caused by interactioin of an essential-SH group on the enzyme with the quinone to form a thioether addition product (Snell and Weissberger, 1930). Therefore, separation of this substrate-specific alkaline phosphatase also present in kidney is necessary before accurate assessment of fructose-1, 6-diphosphatase activity can be made.

In plants, the enzyme fructose-1, 6-diphosphatase (presently known as fructose-1, 6-bisphosphatase) plays some essential role in the Calvin cycle (and also in Hatch-Slack pathway) of photosynthesis as detected first from the plant tissues by Racker and Schroeder (1958). This enzyme has been purified and extensively characterized from some plant sources (Racker and Schroeder, 1958; Chakrabarty *et al.*, 1959; App and Jagendrof, 1964; Buchanan *et al.*, 1967; Scala *et al.*, 1968; etc.). Scala et al. (1968) have characterized two diferent activities of fructose-1, 6-bisphosphatases from germinated and ungerminated castor beans. The differences are based on the pH optima,

substrate specificity and sensitivity to inhibition by AMP. Among these fructose-1, 6-bisphosphatases, the enzyme isolated from ungerminated castor beans was found to be inhibited by AMP. During germination a second activity appeared which was specific towards both fructose-1, 6-bisphosphate and ribulose-1, 5-bisphosphate, and this enzyme was not inhibited by AMP (Scala *et al.*, 1968). Fructose-1, 6-bisphosphatase may function in non-photosynthetic metabolism in higher plants. In the germinating caster bean acetate is utilized for the synthesis of sucrose (Kornberg and Beevers, 1957; Canvin and Beevers, 1961). The change in patterns of this enzyme in response to changes in physiological conditions suggest a gluconeogenic role for this enzyme (Bianchetti and Satirana, 1967).

Biochemical Reaction Catalyzed by Fructose-1, 6-bisphosphatase :

D-fructose-1, 6-bisphosphate is irrevarisbly hydrolyzed to D-fructose-6-phosphate and inorganic phosphate by a substrate-specific phosphatase, Fructose-1, 6-bisphosphatase (D-fructose-1, 6-bisphosphate 1-phosphohydrolase; $FruP_2$ase; EC 3.1.3.11). The specificity regarding the organic portion of the ester molecule is very high. When H_2O is the only phosphate acceptor, analysis of this phosphatase reaction mixture reveals a stoichiometric release of orthophosphate and the organic sugar moiety containing the single phosphate group at C-6.

There are two principal forms of this enzyme so far detected. Between these two isozymic forms, one is associated with gluconeogenic pathway and localized in cytosol, whereas the second form is confined within chloroplast and has been considered as a major enzyme of Calvin cycle.

The gluconeogenic $FruP_2$ase is an allosteric enzyme; it is strongly inhibited by the negative modulator AMP and stimulated by 3-phosphoglycerate and citrate. The enzyme has at least three binding sites for AMP, which are distinct from the substrate binding site(s). It contains four or more subunits. The enzyme is maximally active and thus favours formation of glucose when the concentration of certain glucose precursors is high and the AMP concentration is low, i.e., when the energy charge is high. $FruP_2$ase of the liver has other properties related to the regulation of gluconeogenesis. It is converted by lysosemal

proteases into a form having a more alkaline optimum pH; this change appears to be the result of endocrine regulation. The unequivocal demonstration of $FruP_2$ase in mammalian brain (Majumder and Eisenberg Jr.; 1977) has been confirmed by others (Hevor and Gayet, 1978). In an attempt to assign a physiological explanation for the existence of this enzyme in brain, a non-gluconeogenic organ, Majumder and Eisenberg, Jr. (1976) speculated that this activity may be vital for regulating the glucose-6-phosphate level in this organ for synthesis of *myo*-inositol by *myo*-inositol-1-phosphate synthase reaction. Free glucose inhibits this gluconeogenic $FruP_2$ase.

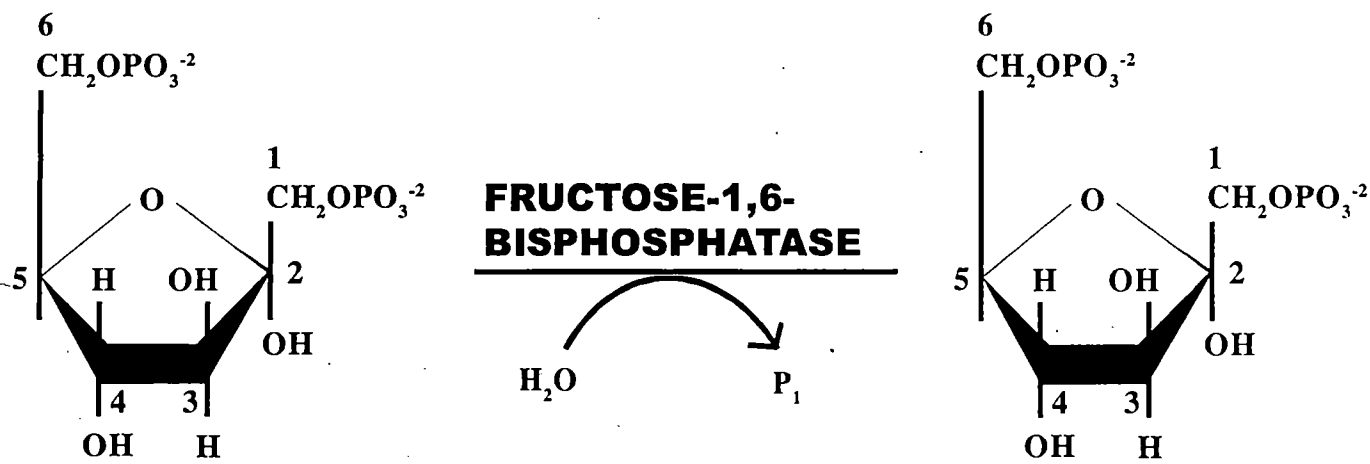
The photosynthetic $FruP_2$ase, on the other hand, is not inhibited by AMP or glucose. However, this plastidial enzyme is remarkably activated by light.

The biochemical reaction which is catalyzed by $FruP_2$ase, either gluconeogenic or photosynthetic, is presented in Figure 2.1. Figure 2.2. and Figure 2.3. depict the specific sequence of operation of gluconeogenic and photosynthetic $FruP_2$ase(s) respectively.

Distribution of Fructose-1, 6-bisphosphatase in Living Organisms:

It has been revealed from a number of studies by several workers that the enzyme, Fructose-1, 6-bisphosphatase, either gluconeogenic or photosynthetic, or both, is distributed almost in all living organisms.

In animal systems, the activity of the cytosolic enzyme has been documented from a number of mammalian and non-mammalian systems. However, most of the experiments were made in the liver, muscle and brain tissues (Pontremoli and Horecker, 1971; Majumder and Eisenberg, Jr. 1976; Biswas *et al.*, 1981, etc). In the present review, $FruP_2$ase of animal origin will not be accounted for practical reasons. Therefore, it will be centered around microbial and plant systems only. Studies on microbial and plant $FruP_2$ase (s) have been started to proliferate since the last twenty five years or so. These may be focused from the present chapter in somewhereelse.



(D-Fructose-1,6-bisphosphate) **(D-Fructose-6-Phosphate)**

Figure : 2.1

Figure 2.1. The biochemical reaction catalyzed by the enzyme Fructose-1, 6-bisphosphatase (D-fructose-1, 6-bisphosphate 1-phosphohydrolase; EC 3.1.3.11).

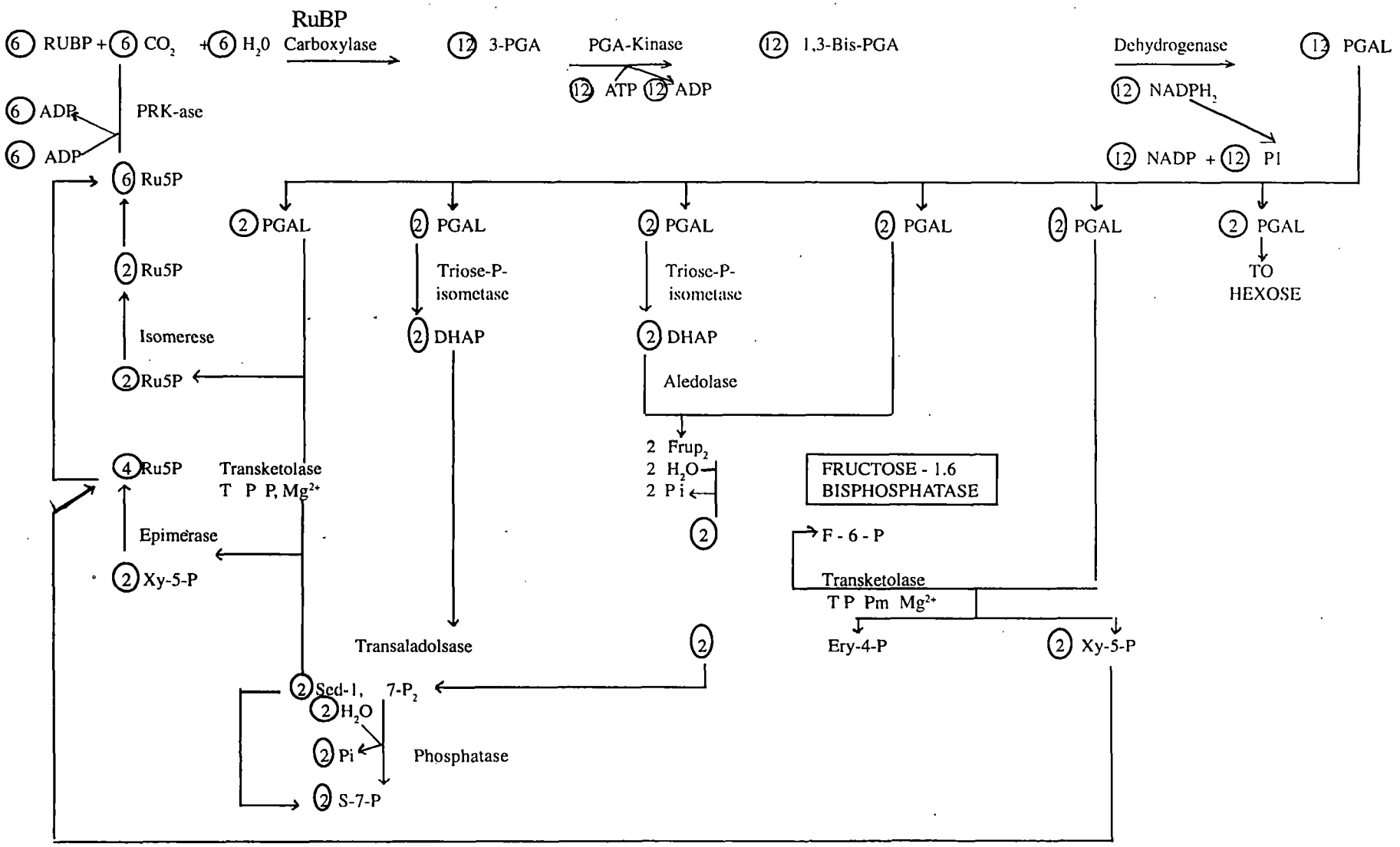


Figure - 2.2

Figure 2.2 Schematic presentation of the Calvin cycle indicating the specific stage of operation of Fructose-1, 6-bisphosphatase (RuBP= Ribulose-1,5 bisphosphate; RuBP-carboxylase = Ribulose-1, 5-bisphosphate carboxylase; 3-PGA = 3-Phosphoglyceric acid; PGA - kinase = Phosphoglycerate kinase; 1.3-Bis- PGA = 1.3-bisphosphoglyceric acid; PGAL - dehydrogenase = 3-Phosphoglyceraldehyde dehydrogenase; PGAL = 3-Phosphoglyceraldehyde; DHAP = Dihydroxyacetone phosphate; $Frup_2$ = Fructose-1, 6-bisphosphatase; F-6-P = Fructose-6-phosphate; Ery-4-P= Erythrose-4-phosphate; Xy-5-P = Xylulose-5-phosphate; Sed-1, 7 P_2 = Sedoheptulose-1, 7-bisphosphate; S-7-P=Se-doheptulose-7-phosphate; R-5-P=Ribose5-phosphate; Ru 5P = Ribulose-5-phosphate; PRK'ase = Phosphoribulokinase).

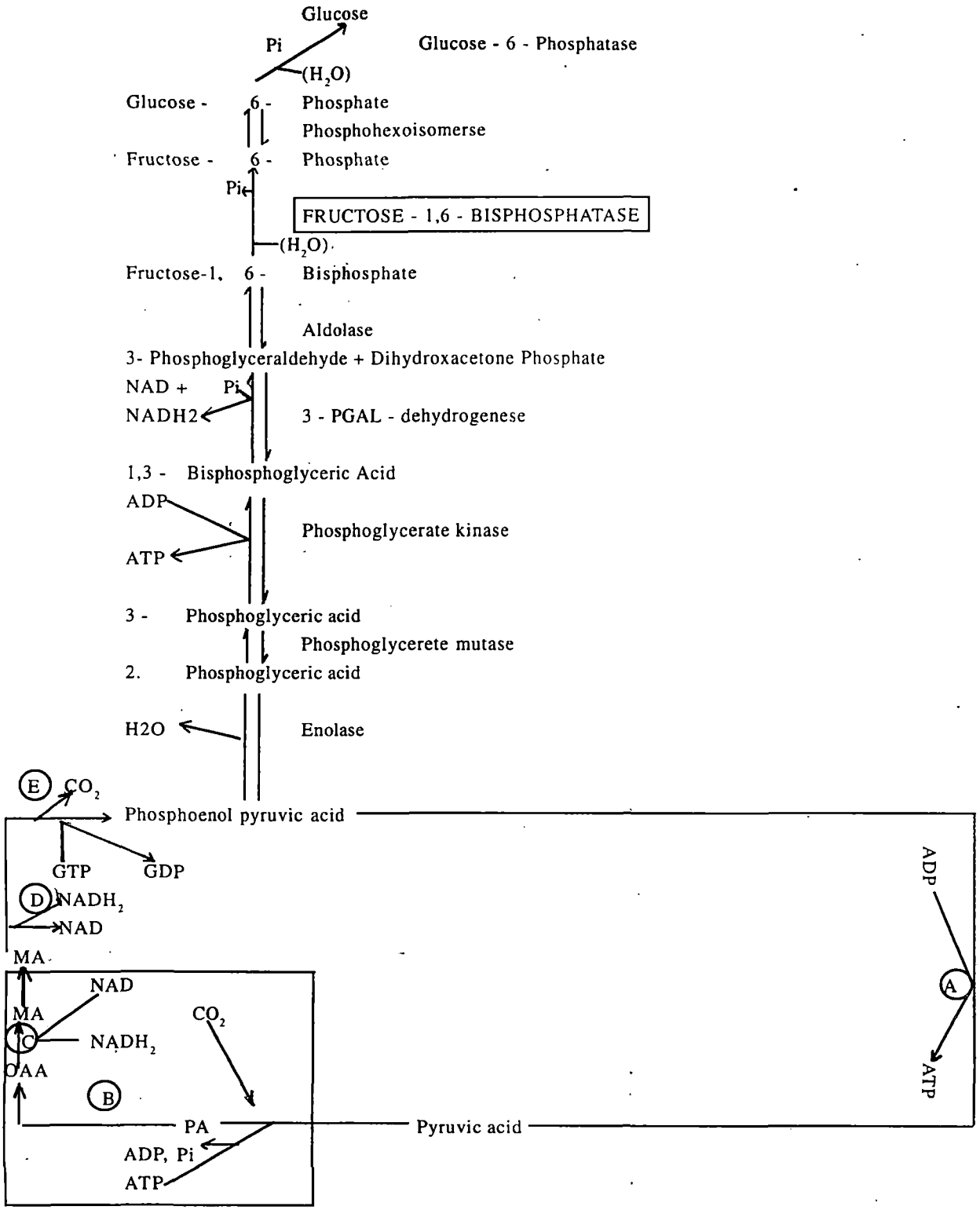


FIGURE - 2.3

Figure 2.3 - Schematic presentation of the Gluconeogenic Pathway during conversion of pyruvate to glucose indicating the specific stage of operation of Fructose-1, 6-bisphosphatase (PA = Pyruvic acid; OAA=Oxaloacetic acid; MA=Malic acid; A=Pyruvate kinase; B=Pyruvate carboxylase; C=Malate dehydrogenase, D=Malate dehydrogenase; E=Phosphoenolpyruvate carboxykinase).

Scala and Semersky (1971) have found an induced fructose-1, 6-bisphosphatase activity from *Acer pseudoplatanus* in suspension culture cells on a medium with glycerol as the carbon source. The enzyme exhibits a neutral pH optimum, insensitive towards AMP and inhibition by EDTA.

Gancedo and Gancedo (1971) have observed that the activity of $FruP_2$ase in yeast can be repressed while the microorganism is grown in sugar medium.

Smith *et al.*, (1978) detected appreciable activity of $FruP_2$ase in germinating seeds of *Sida spinosa*.

Li and Ross (1988) have shown that $FruP_2$ase activity can be detected from the cotyledons of *Corylus avellana*. They have also found that the change in the total enzyme activity is monitored at low temperature and moisture.

Kumar and Selvaraj (1990) have stated that maximum activity of $FruP_2$ase is associated with the ripening of mango (*Mangifera indica*) fruit. This is activated by citrate and inhibited by AMP and Zn^{2+}. They have also suggested that this $FruP_2$ase is a regulatory enzyme in mango.

Recently Botha and Turpin (1990) detected $FruP_2$ase in the green alga, *Selenastrum minutum*. Two iso-forms of this enzyme can be separated by ion-exchange chromatography or acid precipitation. The stability of the two isozymes differ largely. The acid insoluble enzyme exhibits alkaline pH optima in the absence of reductant, a lower affinity for substrate, strong inhibition by phosphate and a low sensitivity to fructose-2, 6-bisphosphate and AMP like the higher plant plastidial enzyme. The second form exhibits high affinity for substrate, sensitivity towards fructose-2, 6-bisphosphate and AMP, but is absolutely dependent on a reductant for stability and activity.

Fructose-1, 6-bisphosphatase in Connection with Gluconeogenesis :

Kobr and Beevers (1971) have shown that the control points of the EMP-pathway in germinating *Ricinus communis* endosperms are sought in two ways : by measuring the

amounts of various glycolytic intermediates at intervals during the germination, by determining the cross-over points appearing during anoxia. A significant departure from thermodynamic equilibrium between substrates and products is found at the level of Fructose-1, 6-bisphosphatase and phosphofructokinase. A definite shift of this ratio is observed at the onset of active gluconeogenesis. The concentrations of phosphoenol-pyruvate and 3-phosphoglyceric acid increase at the same time. Another departure from the expected equilibrium is also observed at the level of the pyruvate kinase. The imposition of anoxia on 5-day-old endosperms reveals two crossover points, at the level of the same enzymes. It is concluded, therefore, that they regulate the glycolytic flow. The maximal glycolytic flow, is however only 1/10 of the gluconeogenic one. The account for this high gluconeogenic efficiency, it is postulated that gluconeogenesis and glycolysis occur in separate intracellular regions. The consistent departure from equilibrium between adenylates observed during the early stages of anoxia supports the concepts that the pools of glycolytic and gluconeogenic intermediates are indeed compartmented and that the two processes are independently regulated.

The locations of the enzymes that convert phosphoenol-pyruvate to fructose-6-phosphate during gluconeogenesis in fatty seeds was determined by Aprees *et al.* (1975). Cotyledons of 5-day-old dark-grown seedlings of *Cucurbita pepo* were used as experimental material. Cytoledons were separated into palisade and mesophyll tissue. Extracts of the two tissues had comparable activities of gluconeogenic enzymes. Extracts were fractionated by density gradient centrifugation to yield mitochondria and glyoxysomes, and by gel filtration to yield proplastides. The isolated organelles retained characteristic ultrastructure and appreciable amounts of marker enzymes. The proportions of the total activities of phosphoglyceromutase and fructose-1, 6-bisphosphatase recovered in the mitochondrial and glyoxysomal preparations were insignificant. The same was true for the activities of phosphoglyceromutase and phosphopyruvate hydratase found in the proplastid preparations. Extracts of a number of other gluconeogenic plant tissues were centrifuged at 2500g to yield particulate preparations. None of the preparations contained a significant proportions of the total activity of phosphoglyceromutase. Therefore, gluconeogenesis from phosphoenolpyruvate in plants probably occurs in the cytoplasm.

The activity of $FruP_2$ase was determined in wild type *Saccharomyces cerevisiae* x 2180 grown in the presence of the glycolytic carbon sources, glucose, fructose and galactose by Roy and Bhattacharyya (1977). The activities of phosphofructokinase, a glycolytic enzyme and phosphoglucoisomerase, an enzyme functioning in glycolysis and gluconeogenesis, were determined for purposes of comparison. A measurable activity of $FruP_2$ase was present in 20-h-old cells grown with moderate shaking in glucose nutrient or minimal medium. This activity increased significantly in 40-h and 60-h-old cells. Similar level of $FruP_2$ase activity was present in 20-h, 40-h, 60-h-old cells grown in 1% fructose nutrient medium. A higher level of $FruP_2$ase activity was present in 20-h-old cells grown in 1% galactose nutrient medium than in 20-h-old cells grown in 1% glucose or 1% fructose nutrient medium. The $FruP_2$ase activity in glucose or fructose grown cells was higher than the corresponding activity in cells grown under similar conditions for 40-h and 60-h in the presence of ethanol, a gluconeogenic carbon source. The phosphofructokinase activity was significantly less in galactose and ethanol grown cells. The phosphoglucoisomerase activity was relatively constant in 20-h, 40-h and 60-h-old cells grown in the presence of glucose, fructose and glactose, but this activity was reduced approximately 50% in ethanol-grown cells. Depending upon the concentration of carbon source and the time of incubation, $FruP_2$ase, a strictly gluconeogenic enzyme, is apparently synthesized by *S. cerevisiae* grown in the presence of glycolytic carbon sources.

The regulatory steps of which the conversion of oxaloacetate to G-6-P during gluconeogenesis in the cotyledons of 5-day-old seedlings of *Cucurbita pepo* was investigated by Leegood and Aprees (1975). The maximum catalytic activities of all the enzymes in this sequence and the amounts of their substrates present *in vivo* were estimated. The results show that the reactions catalyzed by $FruP_2$ase and PEP-carboxykinase are the only ones in the sequence that are substantially displaced from equilibrium *in vivo*. The effect of 3-mercapto-picolinic acid, an inhibitor of gluconeogenesis, on the amounts of the gluconeogenic intermediates present *in vivo* were determined, It has been shown that the enzyme systems, $FruP_2$ase : 2PKase, and the system PEP-carboxykinase : PEP-carboxylase make major contributions to the regulation of gluconeogenesis in the cotyledons.

Hofmann and Polnisch (1990) have found that the activities of $FruP_2$ase, MDH and PEP-carboxykinase from *Candida matlosa* were different on the growth medium between ethanol and glucose. The highest activities of the enzymes were measured in the early log phase of growth on ethanol and the lowest in the early log phase of growth on glucose. They suggested that catabolic repression did not play an essential role in the control of *gluconeogenesis in Candida maltosa.*

Fructose-1, 6-bisphosphatase in Connection with Photosynthesis :

In the so-called "dark phase" or "light-independent" phase of photosynthesis, the enzyme, $FruP_2$ase, plays a pivotal role in C_3 and C_4 plants. Bassham *et al.*, (1970) have found that addition of $FruP_2$ase plus Mg^{2+} in increasing concentrations causes stimulation and then inhibition of photosynthesis in isolated spinach chloroplasts. Thus, the enzyme appears to be exerting its effect through some function other than as an $FruP_2$ase. It is suggested that this other function is in the selective diffusion of metabolites out through the chloroplast outer membrane. Lilley *et al.* (1974) have proposed that Mg^{2+}, PP_i and a protein fraction which exhibits $FruP_2$ase activity may interact to regulate photosynthesis by isolated spinach chloroplasts.

Schuermann and Buchana (1975) have stated that the reduced ferredoxin and a protein factor are required for activation of plastidial $FruP_2$ase in spinach. The $FruP_2$ase is considered as a regulatory enzyme whose activity in chloroplasts is controlled via ferredoxin by light. It has also been observed that the chloroplasts isolated from spinach leaves previously held in darkness contained no fructose-1, 6-bisphosphatase activity measured at pH 7.5 or 7.9, although high activity at pH 8.8 can be detected. Following illumination of these chloroplasts for 7 min, enzyme activity at pH 7.9 was detected, and after 24 min illumination was equal to 36% of the pH 8.8 activity, at this time activity at pH 7.5 also became apparent. The activity at pH 8.8 was not affected by illumination (Kelly *et al.* 1976). Wanger *et al.* (1978) are of opinion that the reduced thioredoxins from microbial and plant cells, both of cytoplasmic or chloroplastic origin, are interchangeable in stimulating diverse enzyme activity of $FruP_2$ase isolated from spinach. Reduced thioredoxins may be nonspecific, multifunctional cellular proteins while in contrast the oxidized forms require specific enzymes for their reduction. Inhibitor experiments

(Anderson *et al.* 1979) indicate that light effect mediator which is reductively activated by transfer of electrons from the photosynthetic electron transport system at or beyond ferredoxin is involved in activation by light of $FruP_2$ase in *Pisum sativum*. Activation proceed optimally when the pH is low and Mg^{2+} is 10 mM. Huber (1979) has observed that millimolar concentrations of Mg^{2+} inhibited CO_2-dependent O_2 evolution by barly (*Hordeum vulgare*) choroplasts and also prevented the activation of $FruP_2$ase by light in intact chloroplasts. Strong reducing agents such as dithiothreitol (DDT), NADH or $Na_2S_2O_4$ could all activate chloroplast $FruP_2$ase, oxidizing agents such as cystine, NAD, FMN and Vitamin K_3 could all inhibit the activity of the activated $FruP_2$ase almost completely. The degree of activation was strictly dependent on pH (Jing *et al.* 1980). Leegood and Walker (1980) have described the mechanism of dark inactivation of $FruP_2$ase in isolated intact chloroplasts of *Triticum aestivum*. Dark inactivation of the enzyme, which is rapid under aerobic conditions, was prevented under anaerobic conditions when chloroplasts were incubated in the absence of an electron acceptor. Electron acceptor like oxaloacetate readily brought about inactivation under anaerobic conditions where chloroplasts were illuminated or in the dark. Inactivation of the enzyme occurred if illuminated on darkened anaerobic chloroplasts were exposed to O_2. Pyocyanine, which catalyzed a cyclic electron flow around Photosystem I, caused inactivation of the enzyme in illuminated, anaerobic chloroplasts. The activity of $FruP_2$ase may be regulated by the availability of electrons and by electron acceptors; dark inactivation may occur by a direct reversal of the activation process. Treatment of spinach chloroplast $FruP_2$ase with subtilisin resulted in a partial loss of enzyme activity measured at pH 8.3 due to a shift of the pH optimum of the enzyme to a more alkaline pH. Although plastidial $FruP_2$ase was considered an enzyme very distinct from gluconeogenic $FruP_2$ase, a resemblance was seen in their behaviour towards subtilisin (Marcus *et al.* 1980). Herting and Wolosiuk (1980) have stated that calcium plays a dual effect on plastidial $FruP_2$ase activation, while the light activatiion of photosynthesis was investigated in spinach by Still *et al.* (1980), it was suggested that $FruP_2$ase might limit flux in the Calvin cycle during induction. Charles and Halliwell (1980) were of opinion that the thiol-treated spinach chloroplast $FruP_2$ase was severely inhibited by H_2O_2 which could be reversed by dithiothreitol, indicating essential thiol groups were oxidized during H_2O_2 inactivation.

The molecular properties of chloroplastic thioredoxin and the photoregulation of the activity of $FruP_2$ase have been elaborately studied by Soulie *et al.* (1981). They isolated and purified thioredoxin to homogeneity from spinach chloroplasts. The protein is a dimer which dissociates in two apparently identical halves when ionic strength is raised. The dimer has a MW of 16,000. In its oxidized state, chloroplastic thioredoxin has no sulfhydril group directly available, even after denaturation of the protein. When reduced by dithiothreitol, 4-SH groups become available on the dimer. Therefore, it can be anticipated there exists one disulfide bridge per monomer. Reduction by dithiothreitol results in the breaking of 2 disulfide bridges and the appearence of 4 sulfhydril groups. Monomeric thioredoxin does not result in a significant activation of $FruP_2$ase. Full activation is obtained in the presence of the dimeric protein. This is, thus, understandable since activation of $FruP_2$ase necessitates reduction of 2 disulphide bridges of the enzyme. Full activation of $FruP_2$ase is obtained in the presence of either an excess of dimeric thioredoxin or in the presence of dithiothreitol. A large excess of oxidised thioredoxin desctivates $FruP_2$ase. These results suggest that there exists an equilibrium.

$FruP_2$ase ——> TH + $_rFruP_2$ase, where $_rTH$ and $_oTH$ reduced thioredoxin and Ca^{2+}, especially in the pH range 7.8 - 8.3.

The light activation of $FruP_2$ase was inhibited in isolated intact chloroplasts of spinach (*Spinacia oleracea*) while exposed to reduced osmotic potentials. Decreases in the velocity and magnitude of light activation correlated with the overall reduction in CO_2-fixation rates. Responses of osmotically stressed chloroplast's enzyme was variable under pH and concentrations of dihydroxyacetone phosphate or 3-phosphoglycerate (Boag and Portis, Jr., 1984).

Etiolated spinach seedlings show a residual photosynthetic $FruP_2$ase activity, which sharply rises under illumination. This increase in activity is due to a light induced *de novo* synthesis; it has been demonstrated by enzyme labelling experiments with ^2H_2O and ^{35}S methionine. The rise of bisphosphatase activity under illumination is strongly inhibited by cyclohexamide, but not by the 70s ribosome inhibitor, lincocin, which shows the nuclear origin of this plastidial enzyme (Chueca *et al.* 1984).

Gontero *et al.* (1984) studied the slow pH-induced conformational transition of chloroplast $FruP_2ase$ and the control of the Calvin cycle. A slow conformation change of chloroplastic reduced $FruP_2ase$ was detected on raising the pH 7.0 to 8.0 or on lowering the pH from 8.0 to 7.0. Stand for reduced and oxidized thioredoxin $_{o}FruP_2ase$ and $_{r}FruP_2ase$ for oxidized and reduced $FruP_2ase$. This equilibrium must be stroungly shifted towards the left. NaDPH in the presence of $NADP^+$-reductase may reduce thioredoxin, and conversely reduced thioredoxin may reduce $NADP^+$ if the reductase is present. These results together with the existence of the above equilibrium provides a tentative scheme of $FruP_2ase$ inactivation *in vivo* in dark when the light has been turned off, electrons are transferred from thioredoxin to $NADP^+$ via $NADP^+$-reductase. Thioredoxin becomes oxidized and then deactivates $FruP_2ase$. Schuermann *et al.* (1981) isolated and purified three isomers of thioredoxin (mb, mc, f). Among these, $FruP_2ase$ is activated by thioredoxin f exclusively.

Cseke *et al.* (1982) have discovered a new compound, fructose-2, 6-bisphosphate, in green leaves. $FruP_2ase$ is completely inhibited by this compound.

Hutcheson and Buchanan (1983) identified $FruP_2ase$ from the crassulacean acid metabolism (CAM) plant. *Kalanchoe daigremontiana*, having a MW of 180,000 and exhibitd immunological cross-reactivity with their counterparts from chloroplasts of C_3 (spinach) and C_4 (corn) plants. *Kalanchoe* $FruP_2ase$ could be activated by thioredoxin f, Mg^{2+} at concentrations greater than 10 mM, however, such activation was considerably less than that observed in the presence of EDTA. These conformation change was fully reversible. Since illumination brought about a pH rise of chloroplastic stroma from 7.0 to 8.0, the results suggested that light activation of $FruP_2ase$ was at least in part due to a slow conformation change of this enzyme.

Marques and Anderson (1985) studied the changing kinetic propertics of $FruP_2ase$ from *Pisum sativum* chloroplasts during photosynthetic induction. After dark-light transitions, there was a delay in photosynthetic CO_2-fixation by isolated pea chloroplasts in the range of some minutes. To assess the physiological significance of light modulation of enzyme activity in the control of induction estimates were made of the kinetic parameters of $FruP_2ase$ immediately upon release from pea chloroplasts in the dark and after

illumination for various time periods. The Michaelis constant for fructose-1, 6-bisphosphate decreased and maximal velocities increased during induction, Light activation of this enzyme might be one of the factors contributing to the overcoming of the lag period in photosynthetic CO_2-fixation.

The activity of $FruP_2$ase was assayed in the etiolated cotyledons of *Brassica* sp. after red light or far red light stimulation. There seemed to be a light sensitivity phase in the course of germination as indicated by the response of leaves to light. During this phase red light stimulated the synthesis of $FruP_2$ase. This effect of red light could be reversed by far red light. Therefore, the initiation of the synthesis of $FruP_2$ase was mediated by phytochrome. The amount of enzyme synthesized was not concerned with the number of light quanta. Phytochrome is only involved in the initiation of the synthesis of $FruP_2$ase, but whether the synthesis will proceed, it depends on many other factors like the availability of substrate, energy etc. (Wu *et al.*, 1987).

Huppa and Buchanan (1989) detected a chloroplast type $FruP_2$ase from *Chlamydomonas reinhardii*. Unlike its counterpart from spinach chloroplasts, the unicellular algal $FruP_2$ase showed a strict requirement for a dithiol reductant irrespective of Mg^{2+} concentration.

Sibley and Anderson (1989) observed that the light modulation of barley chloroplast $FruP_2$ase exhibited a complex response to leaf development status. Light stimulation of $FruP_2$ase activity increased progressively during leaf development at pH 8.0. On the other hand, acid fructose-1, 6-bisphosphatase activity (at pH 6.0) was inhibited by light, and this light inhibition was greater in the base of the leaf than in the tip of the leaf.

Metabolic Regulations of Fructose-1, 6-bisphosphatase in Microorganisms :

The metabolic regulatory aspects of $FruP_2$ase have been studied at length in several microbial, plant and animal systems. However, most of the investigations were made in microbial systems in connection with the gluconeogenic enzyme.

The hydrolysis of glucose-6-phosphate and fructose-1, 6-bisphosphate by their

respective enzymes present in the cell free preparation was significantly inhibited by cycle 3'-5' AMP and 5'-AMP. Cyclic 2'-3'-AMP, 3'-AMP, d5'-AMP, 5'-UMP or 5'GMP could not prevent the hydrolysis of G-6-P and $FruP_2$; but ATP, ADP and PP_i did when used at 10 mM concentration. d AMP, GTP CTP, UTP, orthophosphate and citrate were all inactive. Among all the nucleotides tested, only 5'-AMP was capable of inhibiting the hydrolysis of $FruP_2$ by purified $FruP_2$ase. The hydrolysis of $FruP_2$ was inhibited by ATP, ADP and PP_i when cell-free extract or high-speed supernatant fraction was used as the source of $FruP_2$ase but not when purified $FruP_2$ase was used instead (Sarkar, 1969).

Fructose-1, 6-bisphosphatase was depressed in *Saccharomyces cerevisiae* by incubation in media containing non-sugar carbon sources. Addition of glucose to a derepressed culture led to a rapid loss of the measurable activity of the enzyme. Fructose and mannose also produced inactivation, but 2-deoxyglucose was ineffective. Experiments with cyclohexamide indicated that the inactivation did not require protein synthesis. It was also found that the process was not energy dependent. The reappearence of the enzyme was dependent on an energy source and was prevented by cyclohexamide. These results suggested (Gancedo, 1971) that $FruP_2$ase inactivation was irreversible and that reappearence of enzyme activity replied *de novo* synthesis. Screening of different genera of yeast had shown that the inactivation of $FruP_2$ase was a relatively widespread phenomenon.

Van de Poll *et al.* (1974) isolated a mutant of *Sccharomyces cerevisiae* which did not grow on glucose, fructose, mannose, or sucrose; which had shown long-term adaptation to maltose, but which could grow normally on glactose, ethanol or glycerol. In the mutant, $FruP_2$ase was not inactivated after the addition of glucose, fructose or mannose to the medium, resulting in the simultaneous presence of $FruP_2$ase and phosphofructokinase (PFK) activity. Under these conditions, a cycle is probably catalyzed between $FruP_2$ and F-6-P, resulting in the non-consumption of ATP and on immediate stop of protein synthesis.

Roy and Bhattacharyya (1978) investigated the mode of synthesis and the regulation of $FruP_2$ase and PFK in *Saccharomyces cerevisiae* after growth in presence of different concentrations of glucose of various gluconeogenic carbon sources. The activity of

$FruP_2$ase appeared in the cells after the complete disappearance of glucose from the growth medium with a concomitant increase of the pH and no significant change in the levels of accumulated ethanol. The appearance of $FruP_2$ase activity following glucose depletion was dependent upon the synthesis of protein. The $FruP_2$ase and PFK were present in glucose -, ethanol-, glycerol-, lactate- or pyruvate- grown cells; the time of appearance and the levels of both these enzymes varied. The $FruP_2$ase activity was always higehr in 1% glucose grown cells than in cells grown in the presence of gluconeogenic carbon sources. Addition of glucose to an $FruP_2$ase and PFK synthesizing culture resulted in a complete loss, followed by a reappearance, a $FruP_2$ase activity and an initial increse, followed by a decrease, of PFK activity. In the presence of cyclohexamide the disappearance of glucose and the changes in the levels of $FruP_2$ase and PFK were decreased significantly. Apparently *S. cerevisiae* exhibits a more efficient synthesis of $FruP_2$ase after the exhaustion of glucose compared to the activity present in cells grown in the presence of exogenous gluconeogenic carbon sources. Two metabolically antagonistic enzymes, $FruP_2$ase and PFK, are present during the transition phase, but not during the exponential phase of growth. Decay or inactivation of these enzymes *in vivo* may be dependent upon a glucose-induced protease activity. Roy and Bhattacharyya (1981) also suggested a transcriptional regulation of $FruP_2$ase in *S. cerevisiae* mutant strain A364A blocked in the transport of nuclear RNA at nonpermissive temperature. The level of PFK was reduced more than 25-fold under condition of high citrate accumulation in an aconitase-less, glutamate-requiring mutant strain MO-1-9B. There was a rapid decrease in the levels of ATP and $Frup_2$at the end of log phase of culture growth when both $FruP_2$ase and PFK is present in the cells simultaneously. The changes in the levels of key glycolytic intermediates, but not the changes in ATP, during the simultaneous presence of these two enzymes, can be explained without involving any futile cycling.

Tortora *et al.* (1981) have shown that the addition of glucose to glucose-derepressed yeast (*Saccharomyces cerevisiae*) cells causes disappearance of 60% of the activity of $FruP_2$ase within 3-5 minutes. Reversibility of this catabolic inactivation reaction in a glucose-free medium is independent on *de novo* protein synthesis. The pH -optima of $FruP_2$ase activity in gelfiltrated crude extracts were 8.25 for the enzyme from depressed cells and s.s. for the enzyme from cells treated with glucose for 4 min. In

studies with ^{3}H-leucine labeled glucose-derepressed cells the protein cross-reacting with antibodies against $FruP_2$ase did not disappear within the first 10 min. after addition of glucose. The glucose-induced rapid inactivation of the enzyme is the result of a covalent modification which decreases the $FruP_2$ase activity and changes the pH-activity profile of the enzyme but does not change its immunological reactivity to antibodies. The covalent modification renders the enzyme susceptible to proteinases and initiates its selective proteolysis.

Moson *et al.* (1982) have studied the incorporation of ^{32}P into yeast (*Saccharomyces cerevisiae*) $FruP_2$ase occurred after addition of glucose to a cell suspension incubated with (^{32}P) orthophosphoric acid. The ^{32}P counts were coincident with the enzyme band when immunoprecipitates were subjected to SDS disc gel electrophoresis. The incorporation of phosphate was associated with a decrease in enzyme activity. Approximately 1 mole of phosphate was incorporated per mole of enzyme. The phosphate is bound to the enzyme in a phosphoester linkage with a serine residue. Release of ^{32}P-accompanying enzyme reactivation occurred *in vivo* and in cell free extracts.

Durwin *et al.* (1982) observed in their *in vivo* experiments that concomitant with the glucose-induced inactivation of yeast $FruP_2$ase, a phosphorylation of serine residues of the enzyme occurs. The incorporation of $FruP_2$ase dependent on ATP, Mg^{2+} and cAMP was demonstrated in a cell-free yeast extract suggesting the existence of a cAMP-dependent $FruP_2$ase kinase. When glucose is added to the intact yeast cells within 30 seconds, the cAMP concentration increases from 7 to 3 n mol per gram wet weight. This suggests that upon addition of glucose to yeast cell cAMP functions as mediating signal for the protein kinase catalyzed phosphorylation of $FruP_2$ase.

Yoahino and Murakami (1985) have shown the physiological role of the inhibition of AMP deaminase by Pi in yeast permeabilized cells. $FruP_2$ase was inhibited a little by AMP, which was readily degraded by AMP deaminase under the *in situ* condition. The addition of P_i which showed no direct effect on $FruP_2$ase, effectively enhanced the inhibition on the enzyme by AMP increased through the inhibition of AMP deaminase. P_i activated PFK and inhibited AMP deaminase activity. The AMP deaminase reaction can act as a control system of $FruP_2$ase activity and gluconeogenesis/glycolysis through the change in

the AMP level. P_i may contribute to the stimulation of glycolysis through the inhibition of $FruP_2$ase by the increase in AMP in addition to the direct activation of PFK.

Purified $FruP_2$ase from *Saccharomyces cerevisiae* was phosphorylated *in vitro* by purified yeast cAMP-dependent protein kinase (Pohling and Holzer, 1985). Maximal phosphorylation as accompanied by an inactivation of the enzyme by about 60 *in Vitro* phosphorylation caused changes in the kinetic properties of $FruP_2$ase : (i) the ratio R (Mg^{2+}/ Mn^{2+}) of the enzyme activities measured at 10 mM Mg^{2+} and 2 mM Mn^{2+}, respectively, decreased from 2.6 to 1.2; (ii) the ratio R (pH 7.0/9.0) of the activities measured at pH 7.0 and pH 9.0, respectively, decreased from 0.62 to 0.38, indicating a shift of the pH optimum to the alkaline range. However, the activity of the enzyme for its inhibitors fructose-2, 6-bisphosphate (Fru2, 6-P_2) and AMP, expressed as the concentration (requied for 50% inhibition), was not changed. One maximum amount of phosphate incorporated into $FruP_2$ase was 0.6-0.75 mol/mol of the 40-K Da subunit. Serine was identified as the phosphate-lavelled amino acid. The initial rate of *in vitro* phosphorylation of $FruP_2$ase, obtained with a maximally cAMP-activated protein kinase, increased when Fru-2, 6-P_2 and AMP, both protein inhibitors of the enzyme, were added. As Fru-2, 6-P_2 and AMP did not affect the phosphorylation of histone by cAMP-dependent protein kinase, the inhibitors must bind to $FruP_2$ase in such a way that the enzyme becomes a better substrate for phosphorylation. Nevertheless, Fru-2, 6-P_2 and AMP did not increase the maximum amount of phosphate incorporated into $FruP_2$ase beyond that observed in the presence of cAMP alone.

Toyoda *et al.* (1987) observed that the addition of 6-D-glucose (final conc. 50 mM) to a cell suspension of *Saccharomyces cerevisiae* in stationary phase caused a rapid 3-fold increase in the concentration of cAMP, while a 2-fold increase of cAMP was observed by the addition of alpha-D-glucose. Beta-D-glucose was also more effective than alpha-D-glucose in the inactivation of $FruP_2$ase. It has also been confirmed that cAMP-dependent protein kinase is implicated in the inactivation of $FruP_2$ase.

Doris and Holzer (1987) isolated and purified a protein phosphatase to homogeneity from *Saccharomyces cerevisiae* using reactivation of phosphorylated $FruP_2$ase as assay. Pohling and Holzer (1985) previously observed the phosphorylation of $FruP_2$ase with

cyclic AMP-dependent protein kinase from yeast was accompanied by a 50% decrease in the catalytic activity. Then upon incubation with phosphorylated $FruP_2ase$, the purified protein phosphatase (Doris and Holzer, 1987) not only reverses the 50% inactivation caused by phosphorylation, but also the previously observed change in the pH optimum and in the ratio of activity with Mg^{2+} of Mn^{2+}. The newly identified phosphatase is strongly inhibited by Reparin and fluroide L-carnitine, orthophosphate, pyrophosphate and succinate inhibit to 50% at concentrations from 1 to 10 mM.

Rettenhouse *et al.* (1987) immunopurified labelled $FruP_2ase$ from yeast (*Saccharomyces cerevisiae*) cells that had been incubated in the presence of (^{32}P) orthophosphate. Tryptic peptides from labelled enzyme were mapped by HPLC (high performance liquid chromatography). Most of the radioactivity was found to be associated with the peptide Arg^9 through Arg^{24}, the same peptide which has been previously shown to be phosphorylated *in vitro* by cAMP-dependent protein kinase (Rittenhouse *et al.*, 1984). The amino acid sequence analysis suggests that phosphorylation occurs at the same site, ser^{11}. They have also determined the extent of phosphorylation at ser^{11} of $FruP_2ase$ in yeast cultures grown under various nutritional conditions by measuring the relative amounts of phosphorus. Significant levels of phosphorylation of the enzyme were found in yeast cultures grown under gluconeogenic conditions that varied from 0.15 to 0.50 mol of phosphate per mol of enzyme subunit. However, phosphate incorporation rapidly increased to greater than 0.8 mol after addition of glucose to these cultures.

Schaefer *et al.* (1987) designed a nice set of experiments. Immunoblotting was used to study whether proteolytic degradation of $FruP_2ase$ in yeast cells during catabolic inactivation occur intra-or extravacuolarly. The 40K Da subunits of both the phosphorylated and nonphosphorylated $FruP_2ase$ are rapidly degraded by an extract from isolated vacuoles to a 32K Da intermediate which accumulates and is then slowly further degraded. However, in the intact cells, neither the 32K Da nor any other intermediate reacting with the $FruP_2ase$ antibodies is observed following glucose-induced degradation of the enzyme. These observations are explained as evidence against intravascular degradation of $FruP_2ase$ during proteolytic catabolic inactivation.

Hofmann and Polnisch (1990) have observed that there are about 12 proteins phosphorylated in the presence of cAMP or of a catalytic subunit of cAMP-dependent protein kinase in crude extracts of *Candida maltosa*. A strongly labelled protein spot occured in the position of $FruP_2$ase both after electrophoresis of crude extracts incubated with cAMP and of a partially purified $FruP_2$ase incubated with a catalytic subunit of cAMP-dependent protein kinase. No phosphorylation of the cytoplasmic malate dehydrogenase could be detected by them. From these results it concluded that cAMP-dependent phosphorylation plays an important role in the catabolic inactivation of $FruP_2$ase in *Candida maltosa* as has been reported from other popular species of yeast, i.e., *Saccharomyces cerevisiae*. The inactivation of cytoplasmic $FruP_2$ase was also found to occur after addition of glucose to methanol-grown cells of yeast (Hofmann and Polnisch, 1991).

Purification and Characteristics of the Fructose-1, 6-bisphosphatase (s) :

Fructose-1, 6-bisphosphatase (s) has been purified and extensively characterized from a considerable number of animal, plant and microbial systems which includes both iso-forms, either gluconeogenic or photosynthetic in relevant cases. However, the present review will be centred around the microbial and plant systems excluding the reports from various mammalian and non-mammalian animal systems.

Fructose-1, 6-bisphosphatase was partially purified and some of its properties investigated from a bacterium, *Acinetobacter iwoffi* by Mukkada and Bell (1971). They have found that the enzyme is constitutive for the organism and its level are not affected by the different growth substrates used. Time study experiments showed no appreciable fluctuations during the different growth phases in any medium. The enzyme requires a divalent cation like Mg^{2+} or Mn^{2+}, has a high affinity for the substrate, is stimulated by low concentrations of EDTA, has a pH optimum in the alkaline range and is inhibited by high substrate concentrations. The presence of EDTA, has a pH optimum in the alkaline range and is inhibited by high substrate concentration. The presence of EDTA in the reaction mixture causes a small shift in the pH optimum in different buffers. The enzyme is strongly inhibited by ATP, ITP and UTP and to lesser extents by ADP, IDP and citrate but is relatively insensitive to AMP. A lowering of pH results in some enhancement of

AMP inhibition. The treatment of the enzyme with ATP and citrate probably results in an alteration of the protein leading to reduced affinity for the substrate and consequently reduced activity. At moderate concentrations, the inhibition caused by ATP and citrate together is additive.

Opheim and Bernlohr (1975) purified $FruP_2$ase from the spore forming bacterium *Bacillus licheniformis* to about 800-fold (with a 20% yield of activity) by a procedure that included $(NH_4)_2SO_4$ precipitation, precipitation by $MnCl_2$ and alumina gel absorption. Catalysis of the enzyme *in vitro* was sepcific for fructose-1, 6-bisphosphate (K_m of approximately 20uH) and proceeded optimally at pH 8.0-8.5. This enzyme was rapidly inactivated by incubation in the presence of AMP or in the absence of Mn^{2+}. The AMP-inactivation was prevented by adding phosphoenolpyruvate to the incubation mixture. The enzyme was slowly inactivated when incubated in the presence of stabilizing concentrations of Mn^{2+} (5 nm) at protein concentrations of less than 8 mg of protein/ml. An additional system is produced during sprulation which specifically inactivates $FruP_2$ase *in vitro*. This system, which in ultimately different from the AMP inactivating system, can be blocked by phosphoenolpyruvate. This $FruP_2$ase is strongly inhibited by AMP, exhibiting a K_i of approximately 5' μM. This inhibition could be completely overcome by phosphoenolypyruvate. Phosphoenolpyruvate was also an activator of the enzyme and exhibited a K_m of approximately 2' μM. This activation was prevented in a competitive manner by AMP, exhibiting a K_i of approximately 5 μM. No other effector of $FruP_2$ase was identified in a extensive search. Immunoprecipitation studies indicate that no new species of $FruP_2$ase is produced during gluconeogenic growth or sporulation. The enzyme extracted from cells under a variety of physiological conditions exhibited a molecular weight of about 5×10^5 as determined by sucrose density centrifugation.

Fujita end Freese (1979) have purified and characterized the same enzyme, Fructose-1, 6-bisphosphatase, from another species of *Bacillus* i.e., *B. subtilis*. This enzyme is a constitutive and was purified 1000-fold (30% yield) to 80% purity as judged by SDS-PAGE, where it exhibits a band corresponding to 72,000 daltons. It sediments at 155 in sucrose density gradients, indicating a MW of 380,000, but apparently very asymetric. Its activity is irreversibly inactivated in the absence of Mn^{2+}. The enzyme specifically catalyzes dephosphorylation of $FruP_2$ with a pH optimum of 8.0. It has 40-

60% of full activity in the absence of phosphoenolpyruvate, 60 mM phosphoenol pyravate activates it maximally. High concentrations of monovalent cations also activate, NH_4 being most effective. Inhibitors fall into two groups. Nucleoside monophosphates, phosphorylated co-enzymes and polynucleotides inhibit competitively with phosphoenolpyruvate. The inhibition by nucleoside di - and triphosphates, PP_i and highly phosphorylated nucleotides is not competed by phosphoenolpyruvate but is partially overcome by $FruP_2$.

Shukun (1989) has partially purified chloroplastic $FruP_2$ase from a marine green macro alga *Bryosis corticulans* by employing DEAE-cellulose and Sephadex G-200 steps. This enzyme exhibits a MW of 162,000 and it is highly unstable. Stability cannot be maintained by adding 2-mercaptoethanol only, but the same can be retained by adding Mn^{2+} and glycerol in addition to 2-mercaptoethanol.

Purified $FruP_2$ase from the cyanobacterium, *Syncchococcus leopoliensis*, was S-carboxymethylated and cleaved with trypsin. The resulting peptides were purified by reversed phase high performance liquid chromatography and amino acid sequence of six of the purified peptide was determined by gas phase microsequencing. The results revealed sequence homology with other $Frup_2$ases. The obtained sequence data provide information required for the design of oligonucleotide hybridization probes to screen existing libraries of cyanobacterial proteins may yield important information with the respect of the endosymbiotic theory of mutation (Marcus *et al.*, 1989).

Fructose-1, 6-bisphosphatase was purified from *Candida utilis* by Traniollo *et al.* (1971). The enzyme is a tetramer of MW 130,000. The enzyme is dependent upon the presence of divalent cation for its appreciable activity. The same enzyme as purified from *Saccharomyces cerevisiae* (Funayama *et al.*, 1979) to homogeneity. A MW of 115,000 was obtained by gel filtration. The enzyme appears to be a dimer with identical subunits. The apparent K_m for $FruP_2$ of $FruP_2$ase varies with the Mg^{2+} concentration of the enzyme being 1×10^{-6} M at 10 mM Mg^{2+} and 1×10^{-5} M at 2 mM Mg^{2+}. Other phosphorylated compounds cannot significantly hydrolyzed by the enzyme. An optimum pH of 8.0 is exhibited by the enzyme. This optimum is not changed by addition of EDTA. AMP inhibits the enzyme with a K_i of 8.0×10^{-5} M at 25°C. The inhibition is temperature dependent,

the value of K_i increasing with raising temperature. 2 Deoxy AMP is also inhibitory with a K_i value at 25°C of 1.6 x 10^{-4}M.

Rittenhouse *et al.* (1984) have purified $FruP_2$ase from *Saccharomyces cerevisiae* and the subunit MW of the enzyme has come to about 40,000. Complete immunoprecipitation of specific antibody suggests that there is only one $FruP_2$ase isozyme in *S. cerevisiae*. The MW of the purified enzyme determined by size exclusion HPLC suggests that it has a tetrameric structure of $Frup_2$ases from a broad phylogenetic groups.

Fructose-1, 6-bisphosphatase from the yeast *Kluyveromyces fragilis* was found to have an apparent Mw of 155,000 and to be composed of 4 MW = 35,000 subunits. The extent and rate of phosphorylation of $FruP_2$ by yeast cAMP-dependent protein kinase were dependent on $FruP_2$ inhibitors, 5'-AMP and fructose-2, 6-bisphosphate. In the absence of the inhibitor, the enzyme was slowly phosphorylated with a maximum incorporation of 1 mol of phosphate/mol of enzyme. The estimated sedimentation coefficient of $FruP_2$ was lowered by 5'-AMP from 3.2-5.7 while Fru-2, 6-P_2 increased the S value to 3.5. The presence of either $FruP_2$ or Fru-2, 6-P_2 prevented the 5'-AMP lowering of S value (Toyoda and Jose, 1984).

Bystrykh and Trotsenko (1988) isolated, purified and characterized $FruP_2$ase from the methylotrophic yeast, *Candida boidinii*. The enzyme was found to be homogenous according to grdient PAGE data. The enzyme was a tetramer with a molecular mass of 209 KD. The MW of subunits (46 KD) was determined by SDS-PAGE. Upon gel filtration on Sepharose CL-6B, the tetramer dissociated into di-mers with MW of 115 KD. The enzyme was catalytically active only in the presence of Mg^{2+}. K_m is 2.5 a 10^{-4} M at pH 8.5, the pH optimum of activity was at pH 7.5-8.5. The pH stability change was at 5.8 - 6.6.

Two enzymes capable of hydrolyzing $FruP_2$ have been isolated from the foliose lichen *Pletigera refescens*. These enzymes can be separated using Sephadex G-100 and DEAE-Sephacryl chromatography. One enzyme has a pH optimum of 6.5 and a substrate affinity of 228 micromolar $FruP_2$. This enzyme does not require $MgCl_2$ for activity, and is inhibited by AMP. The second enzyme has a pH optimum of 9.0, with no activity below

pH 7.5. This enzyme responds sigmoidally to Mg^{2+}, with half saturation concentration of 2.0 millimolar $MgCl_2$ and demonstrates hyperbolic kinetics for $FruP_2$ (K_m = 39 micromolar). The enzyme is activated by 20 mM dithiothreitol, is inhibited by AMP, but is not affected by Fructose-2, 6-bisphosphate. It is hypothesized that the letter enzyme is involved in the photosynthetic process, while the former enzyme is a nonspecific acid phosphatase (Brown and Kershaw, 1986).

Lopez and Mayor (1974) have purified $FruP_2$ase from spinach leaves by heating (30 min at 60°C), 'salting out' with $(NH_4)_2SO_4$ (between 30-70% of saturation), filtration through Sephadex G-100 and G-200, fractionation on DEAE-52 cellulose and preparative electrophoresis on polyacrylamide gel. Filtration through DEAE-cellulose led to the isolation of two active fractions (Fractions I and II) with very close MWs. By electrophoresis on acrylamide gel, both fractions gave two active fractions (Fractions Ia - Ib and IIa - IIb). The fractions with low electrophoretic migration rate Ib and IIb are stable in acid and neutral pH, have a MW between 90,000 and 110,000 and constitute the native form of the photosynthetic enzyme. The fractions of faster migration rate Ia-IIa originate from the corresponding fractions Ib and IIb under alkaline conditions, show half the MW of the respective fractions, and behave as subunits of the original dimer form.

Lazaro *et al.* (1975) have purified photosynthetic $FruP_2$ase fractions I and II, from spinach leaves, show a similar amino acid composition, with the exception of a higher glutamic acid content in the latter. In both fractions glutamic and aspartic acids are the main amino acids. pH activity profiles of fractions I and II are similar, with optimum at 8.65 - 8.70, both showing a high specificity for $FruP_2$. These two fractions are Mg^{2+} - dependent for activity, with an optimum concentration of 10 mM in standard conditions, which shifts to 5 mM when Mg^{2+}/ EDTA ratio is increased to 10, Mn^{2+} and Co^{2+} are slightly active EDTA enhance $FruP_2$ase activity slightly, with an optimum at 4-8 mM. Cysteine has no activating effect, and acts as an inhibitor above 10 mM. Both I and II have an optimum substrate concentration of 4 mM and the substrate inhibits at concentrations above this value. Kinetic velocity curves are sigmoidal, with the concave mode located in the range of physiological substrate concentrations (Hill co-efficient 1.75 for both). This suggests a strong regulatory role of $FruP_2$. K_m values are 1.4×10^{-3}M

(Fraction II). The highest activity rate occurs at 60°C in accordance with the high thermostability of both factors.

Baier and Latzko (1975) have purified chloroplastic $FruP_2$ase from spinach. There was no cytosolic enzyme activity. The plastidial fraction was confined in stroma and was light activated.

Zimmermann (1976) developed a relatively straight forward procedure for the purification of chloroplast $FruP_2$ase from spinach leaves to apparent homogeneity and with 80% yield. The MW of the enzyme was about 160,000. Chloroplast $FruP_2$ase consists of four possibly identical subunits and at pH 8.0 easily dissociates into equal halves with lowered activity. Sigmoid saturation curves with Hill co-efficients 3.0 - 3.7 were obtained for $FruP_2$ and Mg^{2+}. Incubation of the enzyme with 20 mM dithiothreitol slowly altered the response to pH from no activity at pH 7.5 and full activity at pH 3.8 to equal activity at each of these pH values; at the same time the number of freely available sulfhydryl groups increased from 4 to 12 per molecule. These properties are considered in the context of the observed activation of this enzyme following illumination of chloroplasts.

Rao and Modi (1976) detected $FruP_2$ase from unripe *Mangifera indica* fruit and they separated two components of the proteins by $(NH_4)_2SO_4$ fractionation, one active at pH 6.0 (acidic $FruP_2$ase) and other at pH 8.5 (alkaline $FruP_2$ase). The alkaline enzyme had a lower K_m (0.15×10^{-3}M) than the acidic components (1.7×10^{-3} M) towards $FruP_2$ and the allosteric inhibitor AMP. It also showed greater heat stability and higher activation in the presence of EDTA as compared to acidic $FruP_2$ase. Both components showed a higher activation with Mn^{2+} ions than with Mg^{2+} ions.

Yovle and Huang (1976) observed the activity of $FruP_2$ase in the fatty endosperm of *Ricinus communis* increased 25-fold during germination and then declined. The enzyme at its peak of development was partially purified. It had an optimal activity at neutral pH (7.8 to 8.0). The apparent K_m value for $FruP_2$ was 8.8×10^{-5}M. The activity as inhibited by AMP allosterically with an apparent K_i value of 2.2×10^{-4}M. The enzyme hydrolyzed $FruP_2$ and not ribulose-1, 5-bisphos-phate or sedoheptulose-1, 7-bisphosphate. Treatment of the partially purified enzyme with acid caused to an 80% decrease in activity. The

remaining activity was insensitive to AMP and had optimal activity at pH 6.7 and a high apparent K_m value (2.5×10^{-4}M) for $FruP_2$.

Zimmermann *et al.* (1978) have purified a cytoplasmic $FruP_2$ase from spinach leaves to apparent homogeneity. The enzyme is a tetramer of MW about 130,000. At pH 7.5, the K_m for the substrate, $Frup_2$, of the enzyme is specific.

Saturation is achieved with lower concentrations at pH 8.0 than at pH 7.0. AMP and high concentration of $FruP_2$ inhibit enzyme activity. $(NH_4)_2SO_4$ relieve the latter inhibition but is itself inhibitory when substrate concentrations are low. Acetylation studies have demonstrated that the AMP regulatory site is distinct from the catalytic site. They suggested that this cytoplasmic $FruP_2$ase may contribute to the regulation of sucrose biosynthesis in plant leaves.

Wu *et al.* (1979) purified chloroplast $FruP_2$ase from spinach leaves by $(NH_4)_2SO_4$ fractionation, Sephadex G-100 chromatography and DEAE-cellulose chromatography. Treatment of the spinach leaves with liquid nitrogen prior to homogenization facilitated the subsequent process. The optimal pH for $FruP_2$ase activity was 8-9 and the enzyme was most stable at pH 6.0, under which it could be stored over several months without appreciable loss of activity. Acrylamide disc electrophoresis of the final enzyme fraction showed only one essential band. The two forms of $FruP_2$ase, purified spinach chloroplast $FruP_2$ase and that in freshly ruptured spinach chloroplast, behaved differently in some of their kinetic properties. Their activities depend throughout on the concentrations of Mg^{2+}, but the K_m (Mg^{2+}) were quite different. The K_m (Mg^{2+}) of the purified enzyme was about 6.0 mM that of $FruP_2$ase in freshly ruptured chloroplasts was about 1.0 mM, which corresponded to the concentration of Mg^{2+} in the stroma of illuminated chloroplasts. Mg^{2+} concentration was a limiting factor for the activity of purified $FruP_2$ase. And the amount of Mg^{2+} in the reaction mixture was lowered, the K_m and V_{max} were both greatly changed. The shortage of Mg^{2+} could not be compensated by increasing the substrate concentration. The purified $FruP_2$ase was completely inhibited by 15 u mol EDTA in the reaction mixture whereas the $FruP_2$ase in freshly ruptured chloroplasts was inhibited only 70% by 30-45 u mol EDTA, which was 2 - to 3- fold of the concentration sufficient to inhibit completely the activity of the purified enzyme. The former was more stable. Its

activity did not decline even after incubation for over 2 hours. The $FruP_2$ase activity was higher in chloroplasts ruptured in 0.2% triton X-100 then that ruptured in water. This phenomenon suggests that this enzyme *in vivo* might be some way associated, at least partly, with chloroplast lamellae.

Spinach photosynthetic $FruP_2$ase was purified to homogeneity by employing affinity chromatography technique also (Pla and Lopez, 1981). Here affinity chromatography was designed on mercaptoethylamino-sepharose.

Bhoo and Hahm (1989) purified and characterized chloroplast $FruP_2$ase from *Pisum sativum* leaves. The approximate MW of the monomer was 40,000 as determined by SDS-PAGE the enzyme was inactive at pH 7.9 while it was activated by dithiothreitol or more alkaline pH, indicating that the purified $FruP_2$ase was originated from chloroplast. The enzyme saturation curves with $FruP_2$ and Mg^{2+} showed sigmoidal shapes with almost same Hill co-efficients suggesting that the enzyme is composed of multimers. The substrate concentration required for half maximum activity was 40 um which was a comparable value (80 μM) for spinach chloroplast enzyme.

Marcus and Harrsch (1990) have determined the amino acid sequence of spinach chloroplast $FruP_2$ase subunit. Placement of the 358 residues in the polypeptide chain was based on automated Edman degradation of the intact protein and of peptides obtained by enzymatic or chemical cleavage. The sequence of spinach chloroplast $FruP_2$ase shows clear homology to gluconeogenic (mammalian, yeast, and *Escherichia coli*) $Frup_2$ases and 80% homology with the wheat chloroplast enzyme. The two chloroplast enzymes show near the middle of the stracture an unique sequence insert probably involved in light-dependent regulation of the chloroplast $FruP_2$ase enzyme activity. The sequence insert contains two cysteins separated by only 4 amino acid residues, a characteristic feature of some enzymes containing redox-active cystoines. The recent X-ray crystallographic resolution has allowed the endorcement of the sequence.

Recently (*Khayat et al.*, 1993) Cytosolic fructose-1, 6-bisphosphatase (FBPase) was purified 472-fold from sugarbeet (*Beta vulgaris* L.) leaves by ammonium sulfate fractionation anion-exchange chromatography (DEAE-Sepharose), cation-exchange

chromatography (S-Sepharose), gel fitration (Sephachyl S-300), and hydrophobic interaction chromatography (Pheoyl Sepharose). The dissociated polypeptide (molecular mass of 37 KD) was used to generate polyclonal antibodies. Western blot analysis revealed a single band that was identified as the cytosolic FBPase. Enzyme activity and protein and transcript levels were measured under various light and dark conditions in growth chamber-grown plants. FBPase protein level remained unchanged during a diurnal cycle, but enzyme activity and transcript levels were highest and lowest at the end of the light and dark periods, respectively. Light-dependent increase in the enzyme activity and transcript level was gradual, occurring sevral hours after the onset of light. At the end of an extended dark period (48 h), FBPase activity was negligible, protein level was unchanged, and transcript level and declined (but considerable amounts of transcript reasin). Neither activity nor protein and transcript were detected in etiolated leaves. Nearly 24 h of continuous exposed to light was required before the FBPase protein and activity reached maximal levels. Unlike the chloroplastic FBPase, which is light activated (direct regulation) changes in the cytosolic FBPase activity and transcription appear to be light dependent in an indirect manner. The data provide first evidence on the course control of this enzyme via a light-dependent modulation of transcription and post-translational modification.