CHAPTER - I

Introduction:

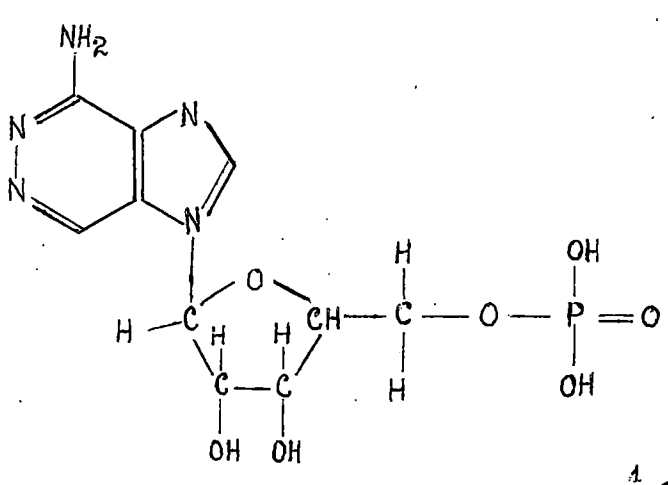
A. General Survey on Nucleotides and Polynucleotides:

Each mononucleotide is composed of a purine or pyrimidine base, a pentose sugar and phosphoric acid. A glycosidic bond unites the pentose and the base at C_1' position of the former. The pentose carbons are conveniently designed as C_1' through C_5' . The term polynucleotide is used in a generic sense for all polymers of nucleotides.

In the case of ribonucleotides, which are produced by alkaline or enzymatic hydrolysis of ribonucleic acid, the pentose is D-ribose. In the case of deoxyribose, which is obtained from deoxyribonucleic acid, the pentose is 2-deoxyribose.

The phosphate may be attached by means of an ester linkage to the 2', 3' or 5' position of ribonucleotides and 3' or 5' positions of deoxyribonucleotides. The trifunctional character of the phosphate makes both linear and branched polynucleotides potentially possible. However, all the natural and biosynthetic polynucleotides so far examined appear to consist of linear chains united by phosphodiester linkages which are exclusively of the 3' - 5' type. Structure I is that of a ribonucleotide, adenosine 5' monophosphate.

The basic ring structures are denoted by II for pyrimidines and by III for purines. The ring positions will be designated as indicated. The R groups represent the usual positions of possible substituents. The points of attachment



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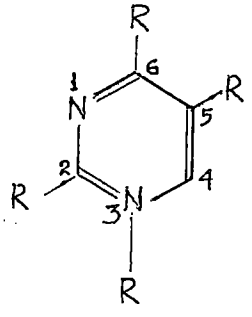

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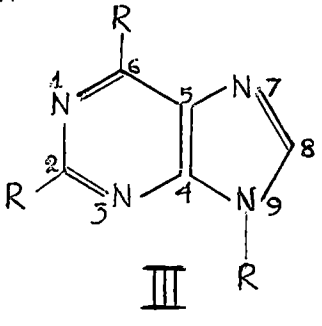


III

of the sugar are at N_3 and N_9 positions for pyrimidine and purine mononucleotides respectively.

The pyrimidines occurring in substantial quantities in deoxyribonucleic acids include thymine and cytosine and considerably less frequently 5-methyl cytosine and 5-hydroxy methyl cytosine. The pyrimidine commonly found in ribonucleic acids are cytosine and uracil. The purines frequently isolated

from nucleic acids are adenine and guanine. In addition to the bases stated above trace quantities of 5-ribosyl uracil (1), 6-methyl amino purine (2-4), 2-methyl amino purine (3), 6,6 dimethyl amino purine (4), 1-methyl guanine and 2-methyl adenine have been reported in nucleic acids.

Properties of the nucleotides:

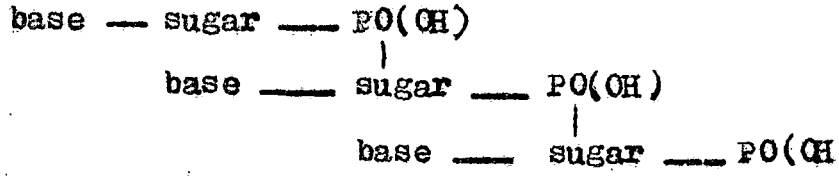
The addition of the phosphate group to the pentose portion of the nucleosides has expected effect upon the electrophoretic and proton-binding properties of these molecules. Thus adenylic, guanylic and cytidylic acids exist largely as zwitter-ion at pH's acid to the pK's of their respective bases.

In addition to the pK arising from the base itself, all of the nucleotides possess a pK close to 1.0 which arises from the primary phosphate ionisation and a second pK near 6.0, which represents the secondary phosphate ionisation. The sugar dissociation at about 12.5, which is observed for nucleosides, is not found for nucleotides.

In general, the state of ionisation of the phosphate group has little influence upon the ultraviolet absorption curve of the base, which is only slightly different for the nucleosides and nucleotides. Minor difference have been reported between 2', 3' and 5' isomers of cytidylic acid (5).

The primary structure of the biosynthetic polynucleotides:

As in the case of protein chemistry, the term primary structure is used to refer to the nucleotide composition, the mode of internucleotide linkage and the sequential arrangement of nucleotides in a macromolecular polynucleotide. When it had become evident that if carefully isolated by mild procedures, both ribonucleic acid and deoxyribonucleic acid could be obtained as polymers of high molecular weight, the question of the manner of linkage of the individual nucleotide units acquired major interest. Once the basic chemistry of the nucleotides themselves was well understood, the number of possibilities became severely restricted. Hydrogen ion titration data upon DNA and RNA clearly indicated that secondary and tertiary phosphate ionisations were present only to a minor extent (6,7). It was thereby apparent that the vast majority of internucleotide linkages in either case must be of the phosphodiester type and that ether or pyrophosphate linkages could be eliminated from consideration. The primary structure of the natural nucleic acids thus emerged from the nucleotides is of the following type:



After it became clearly established that biosynthetic polynucleotides of high degree of polymerization could be produced in vitro, the pressing question come up as to their mode of linkage. In view of the known structure of the nucleotide monomers and the emperical formulae of the polymers any mode of linkage other than the phosphodiester was most unlikely. However, for a direct proof the problem existed to establish whether the phosphodiester linkages were exclusively between the 3' and 5' positions of the ribose ring as is the case with natural DNA and RNA.

In the case of biosynthetic polynucleotides produced by the enzyme from Azobacter Vinelandii, the uncertainties can be said to have been fairly resolved largely through the work of Heppel, Ochoa and co-workers (8-14) involving controlled chemical and enzymatic degradation to identifiable products. In the case of poly A, alkaline hydrolysis under mild condition gave rise to a mixture of 2' and 3' adenosine monophosphate.

Physical properties of polynucleotides in solution:

The dramatic success of the doubly helical model proposed by Watson and Crick (15) and subsequently refined by Wilkins et al (16) in accounting for the observed physical properties of DNA stimulated many investigators to search for similar structures in RNA and in synthetic polynucleotides.

In the following discussion ordered system for the polynucleotides will be reserved for those where a definite periodicity of secondary structure persists over an appreciable fraction of the nucleotide chain while incompletely organised system will be those where definite periodicity of secondary structure can not be found.

The incompletely organised systems:

There was no evidence that poly U exists in any organised fine structure under normal conditions. Very recently, however, Lipsett (17) has obtained evidence for the existence of an ordered state at very low temperature (less than $8^{o}C$). The sharp melting point in the ORD near $10^{o}C$ implies that the structure is probably multistranded (18). But the low stability of this structure has discouraged further study of its geometric nature. The apparent failure of poly U to form any ordered structure is somewhat surprising in view of the fact that Uracil-Uracil hydrogen bonded base pairs are sterically feasible (19). The probable absence of any ordered structure is presumably a consequence of steric difficulties involving other aspects of the molecular organisation.

Polyriboadenylic acid (Poly A) was the first of the biosynthetic polynucleotides to be extensively studied and the first for which X-ray diffraction evidence for the occurrence of an ordered fine structure in the solid state was obtained. A structure involving parallel strands held by salt bridges between the phosphate and the protonated adenine ring has been proposed on the basis of X-ray scattering from fibres (20). Subsequent studies on the ultraviolet absorption and optical activity of acid poly A have revealed at least two distinctly different helix geometries. These forms of acid poly A can be interconverted by changes in salt strength and pH (21,22). The structure of acid poly A determined by X-ray scattering has an appreciable tilt of the base planes from the helix axis (20). It has been suggested that the structures in solution may differ in the amount of base tilting (22).

This high degree of organisation does not appear to persist in solution at alkaline pH. Thus at pH's alkaline to the pK of adenine group (greater than pH 6), poly A has been found to possess many of the characteristics of a relatively unorganised system. The exact pH range corresponding to the limits of stability of alkaline form of poly A is dependent upon ionic strength and temperature (23,24). The hydrodynamic properties of the alkaline form of poly A are

suggestive of a coiled configuration (25,26).

The available data on the macromolecular properties of the (1:1) AU copolymer is indicative of a high degree of coiling at moderate ionic strength, indicating that any helical content it may possess, must be intermittent and can not extend along the entire contour length (27). The observed electrostatically induced expansion of the molecule at low ionic strengths, as reflected by a pronounced increase in intrinsic viscosity also suggests too high a degree of flexibility to be consistent with a rigid, completely helical structure (27).

However, the X-ray diffraction pattern of fibres of the (1:1) AU copolymer is definitely consistent with some degree of ordering and has, in fact, considerable similarity to patterns obtained with natural RNA. There is evidence that some helical content persists in solution as well. Thus there is a high degree of hypochromism (about 40% for the 1:1 copolymer) which is temperature dependent (28).

Guanine residues are particularly prone to aggregation even in short oligo-nucleotides and the mononucleotide (28). Poly G is apparently known only in aggregates in solution. These extremely stable aggregates are not disrupted either by ethylene-glycol or by slightly acidic pH (29,30).

The geometry and the number of strands in this complex are not known clearly. Ionosine often serves as a model for guanosine in polymer systems and poly I.poly C apparently forms a stable double helix similar to poly G. poly C.

Although ionosine does not occur naturally in most RNA's, poly I shows some especially puzzling properties in the optical activity. There is apparently a transition between a multistranded and a single-stranded helix stimulated by changes in salt strength (28). If the ORD curve of poly I in low salt concentration is compared with that in high salt concentration, the melting curve for the low salt form is found to be broad between 25°C and 85°C, while the high-salt form melts sharply at 42°C. An ORD curve at low salt concentration represents a double Cotton effect having negative CD at long wave length. Theoretical considerations imply that for $\pi \longrightarrow \pi^*$ transitions such a pattern should be seen for left-handed helices of single-stranded polymers of identical chromophores. All of the well-known polynucleotide structures are right-handed, but there remains the possibility that some left-handed helices may occur in certain cases.

Poly C may also be protonated to form a double helix. The structure of fibres has been determined by X-ray scattering and the solution structure is assumed to be similar to

it. The CD of the neutral single strand and the acid double strand differ appreciably. It should be noted that the chromophoric properties of a protonated base differ from those of the neutral chromophore. Hence, differences between the CD of the acid double-stranded helix and the neutral single-stranded form arise both from geometric and chromophoric differences.

The Ordered Polynucleotide Systems

(a) Poly A and Poly U system:

It was observed by Warner (31,32) that poly A and poly U could, in presence of sufficient quantity of electrolyte, interact at neutral pH to form molecular complexes. The evidence obtained by Warner was as follows:

It was observed that the absorbance at 259 nm of poly A - poly U mixtures did not correspond to the expected sum for a non-associating mixture. The deviations were in the direction of decreased absorbance, a lowering by 25% being observed for a 1:1 Adenine:Uracil mole ratio. An increase in hypochromism of this magnitude suggested strongly that the interaction was not confined to a few contacts per molecule, but probably involved in an extensive degree of nucleotide pairing.

It was also observed that the equimolar mixture, when examined electrophoretically at a pH 9.6 in the vicinity

of Uracil pK, migrated as a single compound, with a mobility intermediate to that of its constituents. Since the mobilities of poly A and poly U were 10.6 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1} and 13.2 x 10^{-5} cm^2 $volt^{-1}$ sec^{-1} under these conditions (ionic strength 0.1 at $0^{\circ}C$), resolution of the two components would be expected in the absence of any interaction. More direct evidence was obtained by sedimentation. Miles has obtained supplementary evidence for the existence of a poly A -poly U complex by means of infra-red spectroscopy in D_2O solution (33). Using the integrated band intensity method, he was able to show that a mixture of AMP and UMP had an infra-red spectrum which was quantitatively identical within limits of experimental error.

The initial discovery of Warner was soon followed by the still more dramatic observation by Rich and Davies (34) that X-ray diffraction patterns of dried oriented fibres of the equimolar complex were consistent with the existence of a high degree of ordered fine structure and that the pattern was reminiscent of that produced by natural DNA. Subsequent observations have, indeed, provided a consistent confirmation of this view. It was also observed that the equimolar complex displayed the thermal and alkaline denaturation phenomenon characteristic of native DNA (35,36). Furthermore, the observation that formaldehyde pretreatment of the poly A

prevented the interaction is consistent with a direct involvement of the adenine 6-amino group as a hydrogen bond donor (36).

The first detailed ultraviolet spectral mixing curves were obtained by Felsenfeld and Rich (37-39). In the complete absence of added electrolyte no spectral change was observed upon mixing, the mixing curve being simply a straight line corresponding to the expected sum of the optical densities of the two components in the given proportions. At ionic strength of 0.01, a downward bulge in the curve developed slowly with time indicating formation of a complex. However, at an ionic strength of 0.1 in uni-univalent electrolyte at neutral pH, the mixing curve obtained at short intervals after combination was found to be V-shaped, consisting of two straight lines intersecting at a mole-fraction of uridylic residues equal to 0.50.

However, after prolonged standing (48 hours or more) under the above conditions, the slower development of a definite downward bulge at mole fraction of uridylic residues greater than 0.50 was observed. This suggested the formation of a complex of different composition at high U : A mole ratios. The fact that V-shaped mixing curves could be obtained with sharp minima at U : A mole ratios of either 1:1 or 2:1 depending on the conditions was explained on the basis of

stoichiometric formation of two successive complexes corresponding to these U : A ratios. These have been referred to as the (A+U) and the (A+2U) complex species.

Both the kinetics and equilibria of the interaction processes were found to be profoundly dependent upon the nature and concentration of the added electrolyte. Divalent cations, including Mg and Mn, had roughly the same effect as a hundred fold higher concentration of univalent cation. Both the rate and extent of formation of the (A+2U) complex were subsequently found to be very dependent upon the molecular weight of the interacting constituents.

Felsenfeld and Rich were able to follow the stepwise formation of (A+2U) complex in the ultracentrifuge. Working with same poly A and poly U, preparations described earlier, they found that the sedimentation constant for the complex species was 50% greater at a U : A mole ratio of 2:1 than at a ratio of 1:1 under conditions favouring formation of the (A+2U) complex (38).

The X-ray diffraction pattern of fibres of the (A+U) complex is suggestive of a doubly stranded helical structure stabilised by adenine-uracil hydrogen bonding similar to that postulated for adenine and thymine in the Watson and Crick model (38). Corroborative evidence for this mode of

bonding was soon forthcoming from the observation that formaldehyde pretreatment of the poly A, which eliminates the capacity of the C_6 -amino group to serve as a hydrogen bond donor completely blocks the interaction process (35).

Felsenfeld has made an intensive quantitative study of the extent of binding of Mg^{++} or Mn^{++} required to allow formation of the (A+U) complex to proceed to completion in the absence of any other electrolyte (40,41).

In water the binding properties of poly A and poly U were found to be identical. In both the cases the end point occurred sharply when one equivalent of the divalent ion was bound per phosphate group. In view of the similar behavior of poly A and poly U it is quite likely that the site of binding is the phosphate group.

Qualitatively it appears to be clear that the factor dominating the rate of poly A - poly U combination is the degree of masking of the electrostatic repulsion resulting from the similarly charged phosphates. The enhanced effect of the divalent cations are most certainly reflected in the extensive binding of these cations by the phosphates. As a consequence of the charge neutralisation accompanying binding, the electrostatic repulsion arising from the similarly charged phosphates is greatly reduced, permitting the reaction to occur. Binding of the univalent cations is much less

pronounced.

The poly A-poly U interaction has been found to proceed to completion at nucleotide concentration as low as 10^{-5} molar, indicating that the mutual affinity of the two polynucleotides is very high.

Analogous to DNA the absorbancy of the complex is almost independent of temperature at neutral pH until at critical temperature range is attained, at which point the absorbancy increases very sharply to that predicted for the non-associated mixture (42,43). The mid-point of the thermal transition or melting point increases with increasing ionic strength (27).

The (A+U) complex can undergo a denaturation very similar to that of natural DNA. However, unlike the natural DNA case, alkaline denaturation of the (A+U) complex appears to result in actual separation of the strands, manifested by a pronounced drop in molecular weight (35).

It has also been found that copolymers of adenylic and uridylic acid can interact with poly U provided that the fraction of uridylic residues (44) in the poly U is less than about 0.40.

(b) Poly A and Poly I system:

The hypoxanthine base of inosinic acid has the potentiality of hydrogen bonding with adenine (45). It has

been shown by Rich that a complexing reaction between poly A and poly I does occur in solution although it has already been mentioned that poly I has an essentially unorganised structure at pH's alkaline to the zone of titration of its hypoxanthine groups. At more acid pH's there is strong evidence that poly I has some degree of molecular organisation. Poly A and poly I interaction process is reflected by a pronounced alteration in the ultraviolet absorption spectrum like the poly A and poly U interaction process and the drop in absorbancy at 254 nm may be used as a convenient index of the extent of reaction. The rate of interaction of poly A and poly I is strongly dependent upon ionic strength and the rate increases markedly with increasing ionic strength. Divalent cations exerted disproportionate effect in enhancing the rate as in the case of poly A and poly U system.

Formaldehyde pretreatment of poly A results in the abolition of the (A+I) interaction. They strongly suggest that 6-amino group of adenine is essential for the interaction and in all probability as a hydrogen bond donor. The X-ray diffraction pattern of oriented fibres of the A+I equimolar complex has the configuration of a doubly stranded helix stabilised by hydrogen bonding corresponding to pair 7 cited by Donohue (45) with hypoxanthine replacing guanine. The A+I equimolar complex has been found to undergo

denaturation at extremes of pH and at elevated temperature. The addition of Mg^{++} also serves to raise the melting point whereas urea lowers the same. As in the poly A plus poly U case denaturation is accompanied by a pronounced drop in molecular weight suggesting that the process involves an actual separation of the poly A and poly I strands.

(c) Poly I and Poly C system:

Still another example of a highly ordered multi-stranded complex is furnished by the interaction of poly I and poly C (46). This interesting interaction which was discovered by Davies and Rich (46) is, likely process described earlier, reflected by a profound alteration in the ultraviolet absorption spectrum, corresponding at most wave lengths to an increase in hypochromism. The mixing curve at an ionic strength of 0.1 and neutral pH was found to consist of two straight lines intersecting sharply at a 1:1 mole-ratio. No evidence for the formation of (I+2C) species has yet been reported as in the case of other systems both the extent and the rate of reaction are controlled by the concentration of electrolyte present. For the above preparations no reaction at all occurred in 10^{-4} M NaCl. In 0.01 M NaCl the reaction required two hours to reach equilibrium and in 0.10 M NaCl only a few minutes.

The detailed fine structure of the (I+C) complex is still uncertain. The X-ray diffraction pattern of fibres of this material does not seem to resemble that obtained in the (A+U) and (A+I) cases but is quite similar to that of natural RNA (46).

The Secondary Structure of the Ordered Polynucleotides

Poly A plus Poly U:

The X-ray diffraction pattern of fibres of the 1:1 poly A plus poly U complex shows many points of analogy to that of DNA (47-49). Both have the large empty area on the meridian characteristic of helical structures of this type. The poly A plus poly U patterns has a larger spacing of 34 A^{o} and a strong meridional reflexion in the region 3-4 $\mathring{A}$. This indicates that there are ten residues per turn of the helix in both cases. The diameter of the (A+U) complex is slightly larger than that of DNA. This may arise from the presence of an additional hydroxyl group in the ribose moity of the former.

Poly A plus poly U can crystallize in a hexagonal array with a lateral distance between molecules of 28.8 $\mathring{A}$. Both the helical pitch and the separation of the molecules increase with relative humidity. The structure proposed by Rich and Davies (48) is of the doubly stranded helical

type, stabilised by adenine-uracil hydrogen bonding between the C_6 -amino group of adenine and the C_6 -carbonyl of uracil and between the N_1 ring nitrogen of adenine and the N_1 of uracil (48). The bases are in the core of the helix and are roughly perpendicular to the fibre axis.

One difference between the (A+U) and DNA diffraction pattern is the enhanced intensity of the first layer line for the former system (49). This could be accounted for if the chain arrangement were parallel rather than antiparallel or if the diameter were slightly larger than for DNA. The later explanation appears to be more probable.

Poly A plus Poly U in 1:2 ratio:

On stereochemical grounds it has been proposed that the (A+U) structure remains intact and that the second poly U strand lies in the deep helical group of the doubly stranded complex and is stabilised by hydrogen bonds between the C_6 - carbonyl of uracil and the C_6 -amino group of adenine and between the N_1 nitrogen of uracil and the N_7 nitrogen of adenine (50). The available X-ray information is consistent with this model.

Poly A plus Poly I system:

Rich (51) has obtained X-ray diffraction pattern for oriented fibres of the 1:1 complex of poly A plus poly I.

The occurrence of the familiar meridional reflection at 3.4 Å points to a stacking of the planer purine bases at right angles to the fibre axis. This suggests the presence of a doubly stranded helical structure stabilised by inter-base hydrogen bonding. The layer line spacing indicated that such a helix must have a pitch of 38.8 Å. Rich has carried out a very rough Furier analysis by considering the contribution of the phosphate groups alone to the X-ray scattering. Such an analysis has indicated an average diameter of the molecule close to 19 Å. This suggests strongly that the phosphates lie on the periphery of the double helix.

GENERAL SURVEY ON DEOXYRIBONUCLEIC ACID

The nucleic acids provide for some of the most essential functions in the living organism : the storage and transmission of its genetic make-up and the means by which this information is utilised in the synthesis of all cellular proteins, and hence of all cellular constituents. Nucleic acids, like proteins are macromolecules, consisting of a back bone of sugar (ribose in RNA and deoxyribose in DNA) and phosphate to which the various bases are attached. As a matter of fact, among them the former are the largest molecules known. As the name indicates, nucleic acid molecules are acidic and at physiological pH they carry a high

density of negative charge. For this reason, they are found associated in the cell with various species of cations: frequently with basic proteins such as histones and histone-like entities; sometimes with oligoamines, Such as cadaverine or spermine and very commonly with alkaline earth cations.

The bases found in nucleic acids are either pyrimidine or purines-the latter containing a fused pyrimidine-imidazole ring system. In deoxyribonucleic acid, the common bases are the pyrimidines, Thymine (T) and cytosine (C), and the purines, Adenine (A) and Guanine (G). Some 5-methyl cytosine occurs occasionally, especially in the DNA from higher plants and in certain bacteria and bacterial viruses. Uracil and 5-hydoxy methyl uracil have been reported as occurring in certain bacteriophase DNA.

Nucleic acids, being polyanions, must be accompanied by an appropriate number of cations in their vicinity in order to maintain electrical neutrality. The extent of binding of these ions may vary greatly. On the one hand we have inorganic counter cations exemplified by the alkali metals, such as Na^+, Li^+ etc., which serve only to neutralise the negative charges on the phosphate residues electrostatically; this allows the nucleic acid to assume its most compact conformation, as shown by increase in intrinsic viscosity, sedimentation co-efficients, hyperchromicity and specific

rotation and a decrease in Rg, radius of gyration. Organic diamines such as putrescene and cadaverine, which usually accompany DNA in vivo, show a different behaviour : they are bound stoichiometrically, probably to the phosphate residues. Thus, within this stoichiometric range they are far more effective than the monovalent ions. In addition, aliphatic diamines and especially other relatively large organic molecules may exert other effects on DNA structure not duplicated by the inorganic ions.

The Secondary Structure of the Ordered Polynucleotides:

The first example of the elucidation of the secondary structure of an ordered polynucleotide is furnished by the doubly stranded helical model for natural DNA. The X-ray diffraction pattern of DNA was recognised to have many features consistent with a helical structure (52-55). At low relative humidities the sodium salt of DNA crystallizes in the A form. This is very regular and crystalline. In the A form the bases are not strictly perpendicular to the helical axis, being tilted about 25^{o} to that axis (56). At higher relative humidities ($>70\%$) B form exists. The degree of crystallinity is less than for the A form. There is quite strong reflection on the meridian at 3.4 $\mathring{A}$. The fibre axis repeat is 34.6 $\mathring{A}$ with ten residues per turn. In the model of

Wilkins and co-workers, the nucleotide pairs are normal to the fibre and are closer to the helical axis than in the case of A form.

Solution Properties of DNA:

The basic key to an understanding of the solution properties of DNA has been provided by the Watson-Crick structure. The available evidence, while overwhemligly in favour of this fundamental picture of the DNA molecule still does not exclude the possibility that localised deviations from this structure may occur. Studies of the solution properties of DNA are also complicated by the high degree polydispersity shown by most of the samples examined so far.

If the intact DNA molecule had the form of an essentially perfect Watson-Crick double helix, it can be confidently predicted that its configuration would be that of a thin, rigid rod. For a thin rod the radius of gyration is equal to $L \times \sqrt{12}$. Even a superficial examination of the light scattering data reveals that the observed lengths are much to small, by a factor of 3 to 5, to be consistent with this model. To reconcile this degree of coiling with the known structure it is necessary to postulate that DNA consists of ordered doubly stranded helical sections separated by regions of disorder which are sufficiently numerous to endow the molecule with some degree of flexibility.

Electron microscopic observation of DNA has generally revealed extensions which are much to great to be consistent with this model and which in fact approach those of rigid rod rather closely. In all probability, however, the apparent inconsistency is not real may well reflect some degree of straitening of the DNA molecule upon drying (56,57).

One of the most extensively studied features of DNA is its capacity to undergo a process which, by analogy with globular proteins, has been referred to as denaturation. This process which occurs at extremes (58-67) of pH, temperature, or at very low ionic strength, is always reflected by a decrease in hypochromism. The properties of denatured DNA are consistent with a molecule which approaches the properties of a true random coil more closely than does native DNA.

In view of the stringent matching requirements of the Watson-Crick structure it is obvious that any extensive rupture of bonding will be almost impossible to reverse completely.

(B) Interaction of molecules and ions with polynucleotides

(1) Interaction with metals:

The available information regarding the binding of ions and small molecules by polynucleotides include hydrogen ion titration curves, the binding of metallic cations and the

binding of hydrocarbons and dyes. Though in the case of hydration it has not been possible to identify clearly the sites of water binding, somewhat less hesitant statements may be offered concerning interactions with small ions, definite conclusions as to the role of both cations and anions with respect to nucleic acid structure and function are lacking though. The interpretation of binding data for nucleotides is rather simplified for linear character and well established chemical composition. Eisinger and co-workers (68) have shown that purine nucleotides, poly A and poly I which may form ordered structures are precipitated by Mg, whereas pyrimidine polynucleotides, poly C and poly U, which do not form ordered structure are not precipitated. Thus, it appears that although Mg is not bound to the bases, the nature of the bases and the secondary structure of the polynucleotides are not without influence on the stability of the complexes with this ion. Sander and Ts'o (69) have found that the intrinsic constant for Mg^{++} binding by nucleic acids in neutral sodium phosphate buffer follow the order tRNA > poly A, poly A plus poly U, poly I plus poly C, native DNA, stabilised stored denatured DNA > freshly denatured DNA, poly U, poly C $\gg$ monomers. Clearly conformation and the nature of nucleic acid determines binding, although binding itself is to the phosphate group.

Complexes of Ag^+ with DNA, natural and synthetic polynucleotides have been studied by Jensen and Davidson (70), by Daune et al (71) and Wilhelm and Daune (72).

Silver forms complexes by interaction with the purine and/or, pyrimidine bases, rather than by interaction with the phosphates. The binding is Complex and many types of binding have been considered. The strength of binding increases with GC - content. Denatured DNA binds more strongly than does native DNA.

Various di-and trivalent metal ions are able to bring about the depolymerization of polynucleotides and RNA at neutral pH-Zn (II) being most active amongst the metals of the first transition series.

(ii) Interaction with hydrocarbons and dyes:

In order to compare the reactivity of DNA, RNA or polynucleotides to our chemical agents, the generalisation to the effect that the reactivity of bases or of the internucleotide linkages in polynucleotides will only differ from those of the corresponding monomeric systems in rate is commonly made. The generalization is introduced because it provides the justification for the view that a study of the chemistry of monomeric species, either it is a base or a nucleoside or a nucleotide, is a necessary and valid approach to the chemistry of the same residue in a polymeric system.

Carcenogenic 7-bromo methyl benz [a] anthracene reacts faster with DNA than with mononucleotides (73). In synthetic polynucleotides, 3,4-benzpyrene is linked preferentially to purine nucleotides with poly G being the most reactive.

Dyes are of special cytological interest because in addition to exhibiting the usual metachromatic effect, it has been found by several workers to possess the capacity of differentiating DNA with RNA. Dyes of the acridine series, such as proflavine and acridine orange when bound to heparin, isotactic or atactic polystyrene sulphonate, DNA, RNA and polynucleotides show metachromacy and optical activity. Most of the polymerizable dyes which exhibit the metachromatic effect are planer molecules. In solution, at least two and sometimes three distinct absorption bands, whose relative hights depend upon concentration, have been observed in each case. In the order of decreasing wave length, they have been referred to as the α , β and γ bands. The α -band is characteristic of the monomer and decreases in magnitude with increasing concentration.

At low nucleotide : dye mole ratios the dye-nucleotide shows a bathochromic shift and decreasing fluorescence intensity. This Complex has been referred to as Complex I by many authors (74-77). When nucleotide : dye mole ratio is high,

a small hypsochromic shift for most of the dyes is observed. This is attributed to Complex II.

The detailed nature of the visible spectrum of Complex I varies from system to system and in the case of polymers of a single nucleotide it is dependent on the nature of the purine and pyrimidine bases. In the case of the pyrimidine polynucleotides the shoulder is broader and the maximum is displaced to lower wavelengths compared to the purine nucleotides poly A and poly I as well as natural DNA and RNA.

The extent of formation of Complex I and Complex II are sensitive to such experimental parameters as pH, ionic strength, temperature and the concentration of organic solvent. At neutral pH and ionic strength of 0.5 the formation of Complex I by poly A proceeds almost to completion, while the interaction with poly U, native DNA or formaldehyde treated poly A is greatly reduced. In general, it appears that increased helical content tends to lower the stability of Complex I. Thus, thermal denaturation of DNA, which largely disrupts its ordered helical structure, greatly increases the extent of formation of Complex I at high ionic strengths.

In the case of poly A and poly U the nucleotide : dye mole ratios at which the formation of Complex II becomes

prevalent are so large that only a few dye molecules can be bound per polynucleotide chain under these conditions. It is very probable that Complex II represents the contribution of dye molecules bonded in an isolated manner without nearest neighbours and that Complex I reflects that existence of linear clusters of bound dye with important nearest neighbour interactions. This hypothesis is reinforced by the similarity of the phenomena accompanying the formation of Complex I to those produced by the association of free dyes. For dye-polynucleotide interactions the results are in accord with the models proposed by Lerman (78) Muller and Crothers (79), and Schmechel and Crothers (80) found a result from the kinetic and hydrodynamic studies of the Complex proflavine with poly A plus poly U system in support of the above models.

Interaction of Metal Ions and Organic Molecules with DNA

I. Interaction with metal ions:

Nucleic acids, at neutral pH, are characterized by the high electrostatic potential residing on the phosphate residues. The bases, hydrogen bonded or free, do not carry electrostatic charges at neutral pH. The binding of the counter ions to the phosphate group leads to effective reduction of the electrostatic potential and is non-specific in nature. In nucleic acid literature, site binding and specific binding are

not clearly distinguished. It seems appropriate to define site binding, as opposed to ion-cloud interaction, whenever an unpair is formed from which the exchange of the counter ion with the counter ions in the bulk of the solution is considerably slowed down. Specific binding should be restricted to the very strong site binding, in which the specific nature of the counter ion carries a significant role. Another type of binding is by chelation to the bases, and finally it is possible to have chelation by metal ions between the bases and the phosphate groups.

At low ionic strength the metal cations (Mg^{++}, Zn^{++}, Co^{++}, Fe^{++} and Cu^{++}) may form a bridge between the phosphate ion and a purine site (presumably N-7) on the base (81). According to Eichhorn (82) Cu^{++}, Cd^{++} and Pb^{++} reduce the thermal stability of DNA in solution, while in the presence of Mg^{++}, Ca^{++}, Ba^{++}, Mn^{++}, Co^{++}, Ni^{++} and Zn^{++} the heat stability of DNA rises. Kuznetsov et al (83) found that stabilisation of DNA structure is related to the affinity of the DNA for various alkali metal ions and decreases in the order $Li^{+} > Na^{+} > Rb^{+} > K^{+} > NH_4^{+} > Cs^{+}$ at 0.03 ionic strength. The interpretation which is related to the hydration energy of the counter ions is complicated by the fact that at different ionic strengths, reversal of order may occur. There may be a correlation with the fine structure of DNA in solution, as

evident from changes in the number of base pair per turn in the Watson-Crick helix with change in ionic environment (84).

Lyons and Kotin (85) have studied the interaction of Mg^{++} with DNA. They concluded on the basis of the absence of shift in the ultraviolet absorption and the proton magnetic resonance spectrum that Mg^{++} is essentially bound to phosphate sites and not to the bases.

Schreiber and Daune (86) conclude from spectrophotometry, melting profiles and hydrodynamic techniques that at least two types of sites are available for copper. The first one, where Cu^{++} is chelating N-7 of purines is observed only at low ionic strength and destabilises the double helix. The second exists mainly at higher ionic strength and could be attributed to two successive guanine residues in the same strand as was found for Fe^{++}, Mn^{++} and Co^{++}. Metal ions may, according to their ability to influence DNA structure be placed in the sequence $Mg^{++} < Co^{++} < Ni^{++} < Mn^{++} < Zn^{++} < Cd^{++} < Cu^{++}$.

II. Interaction with hydrocarbons and dyes:

Studies on interaction of polynuclear hydrocarbons with DNA by flow dichroism in vitro (87) reveals that 3, 4-benzpyrene, pyrene, phenanthrene and 4-nitroquinoline 1-oxide oriented parallel to the direction of flow, whereas tetracene, pentacene and coronene produced a perpendicular orientation. The amount of binding and carcinogenicity were

found to be related. Lerman (88) proposed an intercalation mechanism resulting into expectation that actinomycin D, acridine dyes and methylene blue were oriented parallel to the DNA bases. Green and McCarter (89) found in measuring the characteristic changes in intensity of the polarized fluorescence that the hydrocarbons have a definite orientation with respect to the DNA helix axis. Isenberg et al (90) and Craig and Isenberg (91) proposed a model which states that, except the larger molecules, hydrocarbons small enough to intercalate into DNA are found to bind to DNA, which were found valid in all cases. Denatured DNA interacts to a somewhat greater extent than native DNA, whereas poly A in neutral pH remains completely inactive towards hydrocarbons tested. Under identical conditions, carcinogenic hydrocarbons (e.g. 3,4-benzpyrene) are much more reactive than their non carcinogenic isomers or analogs (e.g. 1, 2-benzpyrene) (92,93).

The covalent binding to the extent of one hydrocarbon molecule for every 100 and 270 DNA nucleotides were observed using benz $\lceil a \rfloor$ pyrene and pyrene when exposed to γ-irradiation. The template function of the DNA was inhibited as well (94). Cavalieri and Calvin (95) believe that the Chemical reactivity and presumably the carcinogenic activity induced in aromatic hydrocarbons by hydroxylating enzymes may be due

to the generation of electrophilic centres in those. Circular dichroism and melting curve analysis showed that modified bases are shifted outside the double helix, while the fixed carcinogen is inserted and viscosity and light-scatterling studies indicated that the fixation of the carcinogen induces hinge points in the DNA molecule (96).

In distinction to the neutral hydrocarbons which are bound in rather low amounts, the cationic heterocyclic dyes, antibiotics and their derivatives are bound to a much higher extent. At low ionic strength binding on the outside of the nucleic acid is favoured and one-to-one Complex (with respect to phosphate groups) may be achieved (97), whereas at higher values of ionic strength intercalation (Complex II) appears to be favoured and proceeds up to an apparent limit of one ligand molecule per two base pairs. This is due to the fact that the sites close to an already occupied site - the nearest neighbours - are strongly deactivated.

Muller and Crothers (98) believe that the actinomycin chromophore is intercalated between the base pairs in the DNA Complex and that binding can occur adjacent to any GC pair, but binding at a given site produces a distortion of the helix that greatly disfavours binding of another actinomycin closer than six base pairs away. This intercalation model differs from the hydrogen bonded 'outside bonding' model proposed by

Hamilton et al (99) because of the fact that the latter is not consistent with the experimental data. Gurskii (100), on the other hand, does not accept the intercalation method on stereo-chemical grounds. Wang (101) showed that actinomycin D unwinds the DNA helix by an angle identical to unwinding angle of ethidium which supports the intercalating model of Muller and Crothers. Wawra et al (102) studied sonicated Na-DNA complexes with actinomine by small angle X-ray scattering and found that for short DNA fragments an average of one actinomine molecule was bound per 7.1 base pairs, and the DNA is extended by 18%. This corroborates the intercalation hypothesis. Paoletti and LePecq (103) believe, from an analysis of resonance energy transfer between ethidium bromide molecules bound to nucleic acids, that the change of torsion of the DNA helix caused by intercalation, winds, rather than unwinds the DNA helix, contrary to what is generally proposed (104,105). They propose a modified intercalation model which is tested by determining the amounts of various drugs necessary to relax the supercoiled DNA (106). This model proposes winding equal to 12^{o} per ethidium bromide, 8^{o}-9^{o} for proflavine and quinacrine, 7^{o} for methoxyellipticine and only 4^{o} for daunomycin. It has been found (107) that acridine orange shows stronger intercalation in AT rich rather than GC rich DNA. Comparison with the complexes of proflavine with double stranded polynucleotides, poly A plus poly U, poly I plus poly C and poly

G plus poly C confirmed that strong binding sites correspond to AT rich regions of DNA. The binding of acridine orange to DNA results in an increase of the quantum yield of fluorescence of the bound dye with respect to the free dyes, whereas the binding of proflavine under the same conditions results in a large decrease of quantum yield (108-111).

The red spectral shift of the visible spectrum of the dye-DNA Complex is usually attributed to the interaction of the heterocyclic ring system of the bound dyes with the purine and pyrimidine bases of DNA (112,113), which has been substantiated by a study of fluorescence spectra of the Complex formed between proflavine and DNA (114). It has been shown by Thomas et al (115) that 2 to 3% of the base pairs from sites where the dye is strongly bound (Complex II referred to previously) and fluoresces normally while in the other set of sites the binding constant is weaker and the fluorescence is completely quenched. Strong binding sites correspond to AT rich region while quenched site corresponds to neighbouring guanine of the DNA molecule.

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