

## CHAPTER - VIII

### Summary and Conclusion:

A wide variety of chemical compounds have been known to interact with DNA so as to induce certain structural alterations within the nucleic acid molecule. Such interactions affect the biological processes in which nucleic acids participate. In the present investigation an attempt has been made to understand the influence of size and shape of some phenothiazine dyes on the process of interaction of the dye molecules with polynucleotides as well as DNA and also on any possible dependence of size on specificity of binding. The study is primarily concerned with the interaction of Thionine, Tetramethyl Thionine, N,N-diethyl, N', N'-dimethyl Thionine and Tetraethyl Thionine with poly A, poly G, poly C, poly I, poly U and calf thymus DNA.

The introductory chapter contains a general survey on nucleotides and polynucleotides. The primary structure of biosynthetic polymers have been discussed in some detail in order to understand the binding processes more clearly. The discussions on solution properties of the ordered polynucleotide systems have been somewhat elaborate. Some of the results contained in the references have been checked to establish ideal conditions for the present work. The discussions

of the secondary structure of the ordered polynucleotides have been comparatively shorter but possibly sufficient to interpret the experimental results. General survey on DNA has been short because most of the information is widely acceptable. A short review of literature concerning the interaction of polynucleotides and DNA with dyes, drugs and metal ions has been incorporated at the end of the introductory chapter.

The synthesis, estimation and identification of the dyes have been included in Chapter III. The results of estimation of the dyes by two different methods have been compared.

Since aggregation of the dyes plays an important role in the binding processes of dyes on polyelectrolyte surfaces, the aggregation of the four dyes under study has been investigated. The dissociation constant, transition moment length and oscillator strength and angle of stacking of these dyes have been determined and tabulated. The aggregation tendency follows the order Thionine > MB > DEDMT > TET.

The spectral shifts which are observed with Thionine, MB, DEDMT and TET on addition of increasing concentration of poly A, poly U, poly I, poly G and poly C have been recorded in Chapter V. It has been observed that the band intensity of the shoulders, the so called  $\beta$ -band increases while

intensity of the monomer band,  $\alpha$  -band decreases as the ratio of polynucleotide phosphate  $[P]$  to dye  $[D]$  increases. The relative increase in the  $\beta$  -band follows the same order as that of aggregation of dyes. The changes in the  $\alpha/\beta$  band ratio are, however, not of the same magnitude. The  $\beta$  -bands of the dye-poly A and dye-poly I complexes correspond to the maxima of dimers of these dyes. But  $\beta$  -bands for the dye-poly U and dye-poly C complexes show a further blue shift indicating the formation of higher polymers. This observation is in conformity with the findings of other workers. The  $\beta$  -band for poly G-dye system does not show similar pattern of behaviour. The band goes on decreasing on addition of poly G. The  $\beta$  -band does not show any increase even around full coverage. This has been explained on the basis of the formation of charge-transfer complexes. The stacking parameters for binding of the dyes with single stranded polynucleotides also suggest aggregation of dyes on the polymer surfaces and their variation in the same order as in aqueous solution. Gradual decrease in the  $\beta$  -band on addition of increasing amount of poly G as opposed to high value of stacking parameter for the poly G-dye system indicates that the model of Wolf and Bradley might furnish misleading results where charge-transfer complexes are formed.

In Chapter VI the results of spectrophotometric experiments with doubly stranded polynucleotides and the four dyes have been recorded with a view to ascertain whether the secondary structure of the polynucleotides plays any specific role to its binding process with the dyes. The nature of changes in  $\beta$ -bands as well as the values of the stacking parameters indicate that the sequence of aggregation for the four dyes is maintained. Geometry of DEDMT and TET appears to inhibit aggregation of the dyes on polymer surface in these cases. This may be expected as the flexibility of the polynucleotides is less due to double helical structure by hydrogen bonding. The results also indicate that the influence of the purine or pyrimidine bases upon the formation of complex I or II is less than the influence of the shape and size of the interacting species with the double stranded polynucleotides. But the reverse is true for single stranded polynucleotides. But definite effect of the bases of double stranded polynucleotides is indicated in the spectra of the dyes where geometry of the dyes permit interaction of the dyes with the bases.

The spectra of the dyes on addition of DNA have been recorded in Chapter VII. It is observed that around full coverage of DNA sites i.e., when  $\frac{[\text{Phosphate}]}{[\text{Dye}]} = 1$  to 3, the dimer band ( $\beta$ -band) of the dyes increases while monomer band ( $\alpha$ -band) decreases. The relative increase in

the  $\beta$ -band follows the same order as that of aggregation of dyes in aqueous solution. It is, therefore, concluded that the dye-DNA interaction is accompanied with dye-dye interaction. When P/D ratio is high i.e., 30 to 40, a red shift of the  $\alpha$ -band results. This is attributed to the interaction of the dyes with DNA. In order to ascertain the process of dye-dye interaction on the polymer surface, stacking parameters have been determined. These are found to vary in the same order as aggregation of the dyes. The aggregation of dyes on polymer surface has been found to increase in the case of denatured DNA. This has been attributed to the fact that dye-dye interaction on the surface of the polymer increases because partially ordered chain could yield a single chirality in the bound molecules.

The aforesaid results have been discussed in the light of various models proposed to explain such interactions. In general, it has been concluded that the dyes are bound with DNA by intercalation of the dyes between DNA bases at low P/D and on the phosphate site also by intercalation at high P/D.

It has been observed that the dyes have specificity for poly G, where it forms probably charge-transfer complex in the excited state. The dyes also interact with poly A, poly I, poly C and poly U in a different way, which is manifested by increase in  $\beta$ -band as observed for exciton

like interaction. With double stranded polynucleotides also, these effects of the bases on the dye spectra have been observed in the case of dyes of smaller size and shape and not with the bulkier ones. It is, therefore, concluded that geometry of the molecule is an important factor for binding either by the process of intercalation or stacking. The bases present in DNA do not affect the dye spectra in the same way as the polynucleotides do. This is possibly due to the co-operating effect of the different bases.

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