INTERACTION BETWEEN INOSITOL HEXAPHOSPHATE AND CARBOBENZOXY PEPTIDE: A MODEL FOR NUCLEIC ACID – NONHISTONE CHROMOSOMAL PROTEIN INTERACTION

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The nonhistone chromosomal (NHC) and acid proteins such as T4-gene 32 protein, DNA polymerase, *E. coli* lac repressor, etc. which carry out various structural, enzymatic and regulatory roles are anionic at physiological pH. They are rich in tryptophan and bind strongly to DNA [1–3]. To understand how negatively charged proteins can interact with DNA we report here a model study. The interaction between N-carbobenzoxy (CBZ) peptides of different amino acids and inositol hexaphosphate* (IHP, Na salt) has been studied by solubility measurements [5] at pH 6.3 where both the molecules would be in the anionic form. The phosphate groups of IHP would resemble the phosphates of nucleic acid exposed to solvent [6]. Our study indicates that the interaction between two negatively charged molecules can take place through metal ion.

The solubility of the majority of CBZ derivatives show linear increase with the increase in IHP concentration (Figure 1) indicating a 1:1 complexation [5, 7] between the reactants within the concentration range studied. The most remarkable effect is seen with CBZTrp where > 15 fold increase in solubility takes place in 5×10^{-3} M IHP. The solubility of tryptophan and N-acetyl-tryptophan amide (ATRA) are not effected as has been observed with α and ϵ-CBZLys peptides.

To understand the mechanism of interaction, the effect of temperature on CBZTrp solubility in IHP solution (Figure 2) has been studied. This shows the exothermic nature of interaction between the compounds. Apparent association constants, Ka, between peptides (P) and IHP (I) assuming a direct equilibrium $P + I \rightleftharpoons P.I$ are obtained [5] from slopes of linear plots of Figures 1 and 2 and are presented in the legends there. The values of enthalpy, ΔH obtained from van't Hoff Plot (Figure 2 inset) is -5.1 Kcal/mole ($5–41°$ range) and the values of free energy ΔG and entropy ΔS are -4.8 Kcal/mole and -3 e.u. respectively at 29° for the reaction between CBZTrp and IHP indicating the polar nature of the interaction. The addition of electrolyte e.g. 0.2 M NaCl reduces the extent of interaction (Figure 1) and suggests an ionic interaction between the peptides and IHP. Whether the absence of any favourable interaction of N–α and N–ϵ Lys derivatives and tryptophan in IHP solution is due to the overall net zero charge (as in ATRA) at the

* Choice of this organic phosphate arose from our study to understand the mechanism (to be published) of the alteration [4] in the quaternary structure of proteins induced by IHP.

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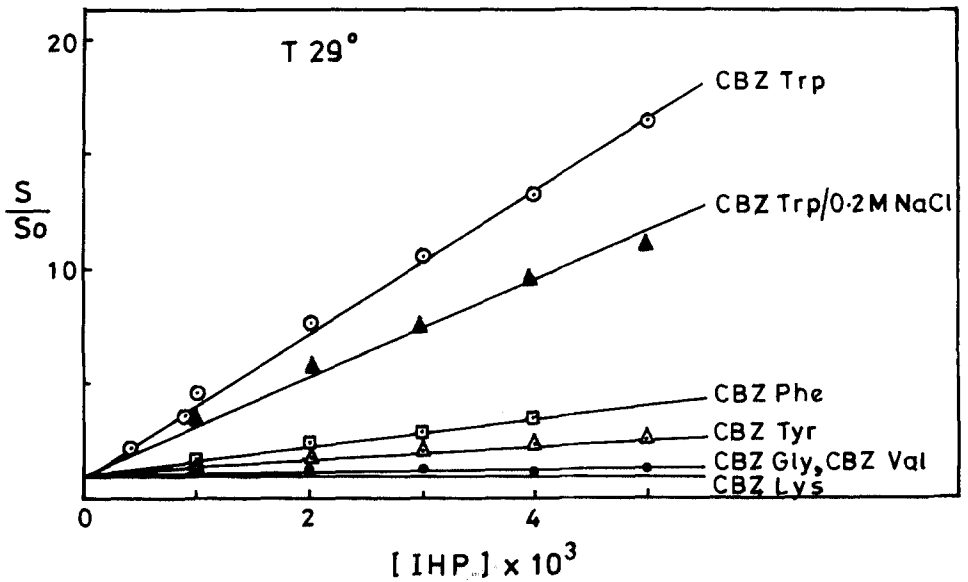

Fig. 1 The effect of IHP (Na salt, Sigma) on the solubility of the CBZ peptides (L-derivatives, Sigma). The solutes were equilibrated in stoppered tubes in an incubator shaker (New Brunswick Scientific Co.) at 200 oscillation/min at 29 ± 0.1 and 40.5 ± 0.1° for 48 hrs; the equilibration at 5 ± 0.2° was carried out by rotating the tubes end-over-end at 35–40 rpm for 7 days in a thermostatic water-bath kept in a cold room. Attainment of equilibration was checked by measuring the concentrations of the solutes at various intervals of time. Filtration of the equilibrated solution and the sampling of the aliquots were carried out near the temperature of equilibration. S_0 in moles/litre for the CBZ peptides at 29° are: Trp 5.36×10^{-4}, Phe 3.3×10^{-3}, Tyr 7.5×10^{-3}, Val 6.0×10^{-3}, Gly 4.6×10^{-2}. Solubility values were calculated using the following molar extinction coefficients of the derivatives; Trp 5600, Phe 200, Tyr 1300, Val and Gly 180. The values of Ka in M^{-1} are: Trp 3150, Phe 715, Tyr 285 Val and Gly 80. ATRA (Cyclo) and tryptophan (Sigma) like CBZLys (α and ϵ) do not show any change in their solubility in IHP solution.

experimental pH or the inability of $^+NH_3$ groups to compete with either Na^+ or H^+ ions for the phosphates sites is not certain.

Association of negatively charged IHP and CBZ peptides through simple electrostatic interaction is not possible. Hydrophobic association of the peptides with the CH-surface of IHP overcoming the electrostatic repulsion is not reflected from the negative entropy of reaction. Moreover, Tyr and Val derivatives should have shown higher increase in the solubility than Phe and Gly derivatives respectively from hydrophobic consideration [5].

Na^+ ion has been suggested to form a diffuse and highly mobile ionic cloud around the lattice of negatively charged DNA phosphate and other phosphates [6, 8]. The increased solubility of the peptides in IHP solution may result from the sharing of this diffused ion cloud by the carboxyl and phosphate groups. In addition, coordination of Na^+ between IHP and peptide as in the coordination of Na^+ to uracil 0(2) and adenosyl 0(3) and phosphate oxygen atoms in the compound adenosyl-3′5′-uridine phosphate [9], is also possible. The strong interaction between CBZTrp and IHP probably results, in addition to the interaction described above, from the donation of lone pair electrons of the indole N

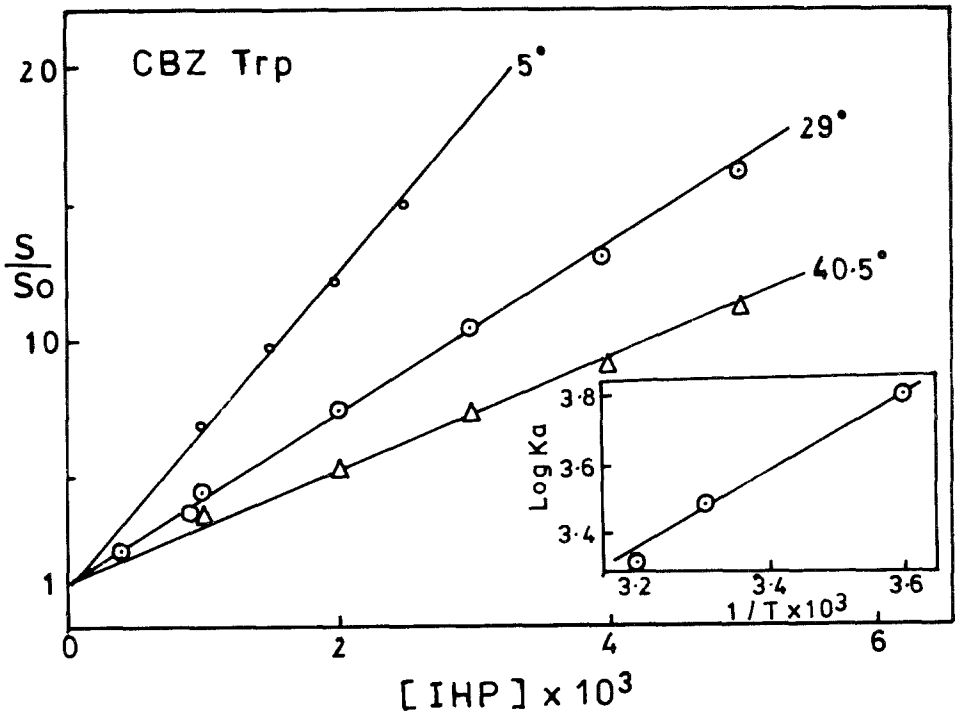

Fig. 2 Effect of temperature on the solubility of CBZTrp in IHP solution. S_0 at 5 and 40.5° are 2.3 and 7.6 x 10^{-4} M respectively, Ka values are 6250 and 2100 respectively. The free energy, ΔG, of the interaction at temperature T, has been calculated using the equation, $\Delta G = -RT \ln Ka$. *Inset.* van't Hoff's plot for the determination of enthalpy, ΔH of the reaction. The entropy of the interaction, ΔS, was calculated from $\Delta S = -(\Delta G - \Delta H)/T$.

to the low energy d orbital of phosphorus [10]. The possibility of tryptophan group interacting with the P atom of DNA has been observed from pmr measurements [11]. Comparison of the results of CBZTrp, ATRA and tryptophan in IHP would suggest that the proximity of an anion e.g. COO^- group is necessary for any favourable interaction of the peptides with the phosphate groups. We are not sure whether interaction of the diffused Na^+ ion cloud with the aromatic rings is responsible for the relatively favourable interaction of Phe and Tyr derivatives compared to CBZVal with IHP.

We suggest that anionic NHC and acid proteins interact with the phosphate groups of nucleic acid through metal ion** as is involved in CBZ peptide-IHP interaction. The negatively charged cellulose phosphate has been found to bind [12] anionic *E. coli* RNA polymerase at pH 7.9 indicating the role of phosphate groups in the interaction. Our results also suggest that the presence of tryptophan residue in an overall anionic environment would bind strongly to the phosphate groups. The involvement of tryptophan residue of anionic F-32 gene protein in binding to DNA and other polynucleotides has

** Mg^{+2} can also participate in this type of binding because of its better coordinating ability and formation of diffused ionic cloud [6] around phosphate group.

been shown [13]. The binding takes place at the sugar-phosphate bond and is independent of the nature of bases and sugar moeities [14]. Our results also show that it is not necessary to envisage the involvement of a 'positive patch' of anionic proteins to bind to nucleic acid [15, 16]. Further, the binding of peptide residues with a positive charge in the vicinity of aromatic groups and particularly tryptophan has been found to induce considerable increase in the stability of the nucleic acid [17, 18], contrary to the destabilisation of double helix observed in the presence of the 32-protein. Support for our suggestion of involvement of tryptophan group in anionic environment is obtained from a recent model building study [19] which suggests that the binding site of anionic protein prealbumin with the major grove of DNA is rich in ionic side chains and tryptophan 41 is involved in the binding. The immediate environment of this tryptophan is anionic [20] e.g., -Asp-Asp-Thr-$\overset{(40)}{\text{Trp}}$-Gln- and can possibly bind to the DNA phosphates by the mechanism suggested here. Similarly the binding [21] of anionic TMV protein to the core RNA may involve the -Ala-$\overset{(17)}{\text{Trp}}$-Ala-Asp- region [22] of the protein.

The present study, in addition to the understanding of the interaction between phosphate groups with anionic peptides/proteins, probably also indicates that in DNA-protein recognition process [2, 6, 23] perhaps primary recognition of the proteins would involve the phosphate groups of DNA. The results may also suggest that metal ion mediated interaction between similarly charged (anionic) molecules probably contributed to the association and aggregation of the (poly-) nucleotides with specific (poly-) peptides during early period of evolution.

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