ON THE INTERACTION OF OLIGOPEPTIDES CONTAINING AROMATIC AMINO ACIDS WITH DNA IN AQUEOUS SOLUTION

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1. Introduction

A great deal of work is being devoted to the interactions between proteins and nucleic acids. In this respect the specific interactions of aromatic amino acids with nucleic acid bases have been thoroughly investigated.

One group of workers studying the interactions with DNA in aqueous solution of simple dipeptides and tripeptides containing aromatic residues have come to the conclusion that such aromatic residues are able to stack with DNA bases by partial intercalation [1–4]. This mechanism would produce a bending of the DNA helix at the point of insertion with consequent decrease in viscosity of the solution. Moreover the stacking of a peptide's aromatic portion with DNA base pairs would produce an immobilization of this moiety with resulting broadening of p.m.r. peaks and their upfield shifts.

As a corollary of this a 'selective bookmark' recognition hypothesis has been advanced [2].

A second group of researchers studying the same interactions using p.m.r. fluorescence and CD techniques have come to similar conclusions, at least for the interaction of oligopeptides containing tryptophan, tyrosine and phenylalanine with single stranded polynucleotides [5–10].

In our previous study of the interactions of some aromatic dyes and antibiotics with DNA we have shown that, in most cases, where intercalation of the aromatic moiety between base pairs has been proved, the enthalpy of such interaction is large and negative, amounting to about minus 5–7 kcal/mole of drug bound [11–16]. From these results we have proposed a 'calorimetric criterion' which should give, in our opinion, strong indications of the molecular mechanism of the interaction.

This criterion is based on the following considerations: whenever intercalation occurs one $\pi - \pi$ interaction between base pairs is disrupted and replaced by two new $\pi - \pi$ interactions of the intercalating molecule with the sandwiching base pairs*. The net enthalpic balance of this process is negative due to the similar enthalpy values involved in each $\pi - \pi$ contact (at least when three fused-ring systems are concerned).

With these premises we have carried out calorimetric measurements on the interactions between DNA and L-Lys–L-Trp–L-Lys, L-Lys–L-Tyr–L-Lys, L-Lys–L-Tyr and L-Lys–L-Tyramide, respectively.

2. Experimental

The oligopeptides were purchased from Schwarz-Mann or from Cyclo Chemical Co. and used without further purification. All other experimental details have been described [11,12]. The solvent used was 10^{-3} M cacodilate buffer, pH 5.5, plus 10^{-3} M NaCl. The choice of pH was because the terminal NH_2 of each peptide has a pK value around 7.5, this value shifts to a higher one on interaction with DNA [9].

*This is a very simplified view of the process. Many other interactions (electrostatic, with the solvent etc.) are cancelled and reformed in this process but on the basis of our experimental evidence we are inclined to conclude that the major contribution to the single enthalpic term is due to $\pi - \pi$ interactions.

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At pH 5.5 the terminal NH_2 is charged both in the free and bound states. The concentrations used were about 2.5 mM for the oligopeptides and about 3 mM_P for calf thymus DNA. The volumes of the two solutions in the calorimeter mixing cell were chosen in order to have a final molar ratio, r, peptide/DNA < 0.15, i.e. a range of r values where binding is highest (of the order of 10^4 M^{-1} [2,8]). In all cases a binding value greater than 90% was obtained.

3. Results and discussion

The results of all measurements (corrected for heat of dilution) yielded a zero value of the enthalpy of interaction, with an uncertainty of ± 0.1 kcal/mole of bound peptide. One can roughly divide this enthalpy into two contributions: the first due to the electrostatic interaction between opposite charged groups and the second due to the interaction of the aromatic portion of the molecule. The electrostatic contribution plus all other interactions, due the aliphatic chains, can be estimated by measuring the enthalpy of binding of $(L\text{-}Lys)_3$ with DNA in the same solvent by reasonably assuming that the overall mode of interaction of the lysyl residues in $(L\text{-}Lys)_3$ is not much different from that in the oligopeptides containing aromatic moieties.

The ΔH obtained with $(L\text{-}Lys)_3$ was + 0.3 ± 0.1 kcal/mole of bound peptide, which introduces a small correction in the enthalpy of binding of the aromatic oligopeptides.

From these results and from the considerations mentioned above we are inclined to think that the interactions between aromatic oligopeptides and DNA occur without significant intercalation, even partial. The only way in fact to reconcile the intercalation hypothesis with our experimental data is to assume that with indolyl, and tyrosyl groups this mechanism would give rise to a net zero ΔH because of the smaller number of aromatic rings involved compared to the aromatic parts of the dyes and antibiotics used in our previous work. This would necessarily mean that the enthalpy required to disrupt a $\pi - \pi$ contact between two base pairs is in all peptides used *exactly* compensated by the new $\pi - \pi$ contacts created in the intercalation (partial or not) of indolyl and tyrosyl groups. We cannot exclude this but we think that, due also to the difference of these two

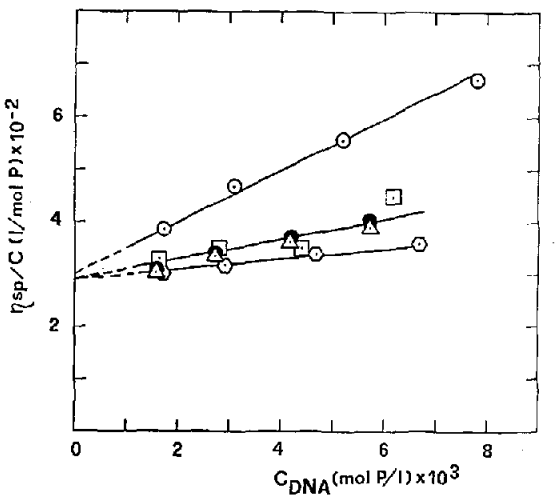

Fig.1. Reduced viscosity of DNA and of DNA-complexes with oligopeptides. Molar ratio oligopeptide/DNA = 0.1. Solvent: 10^{-3} M cacodilate buffer + 10^{-3} M NaCl, pH = 5.5. t = 25°C. (○) Free DNA. (⊞) DNA + L-Lys–L-Tyramide. (●) DNA + L-Lys–L-Tyr–L-Lys. (△) DNA + L-Lys–L-Trp–L-Lys. (⊙) DNA + $(L\text{-}Lys)_3$.

aromatic residues, this is highly unlikely. We therefore conclude that it is very likely that the aromatic parts of the peptides studied here do not stack or intercalate between base pairs, at least with DNA. In the case of single stranded polynucleotides, as reported by Hélène's group for poly-A, in fact, van 't Hoff plots obtained with the overall binding constant data [9] result in a ΔH value close to −7 kcal/mole for L-Lys–L-Trp–L-Lys, a value that would agree with a stacking hypothesis according to our criterion.

In addition to the calorimetric arguments against intercalation, we think that viscometric results are also not in agreement with this type of interaction. We in fact do not share the opinion of Gabbay's group that viscosity measurements on DNA–drug complexes made at finite concentrations cannot be extrapolated to zero concentration. If it is true that, having a binding constant of finite value, decreasing the concentration must lead to dissociation of the complex, nevertheless meaningful apparent intrinsic viscosity data are obtained if a range of concentrations of the complex is used where dissociation is negligible (it is possible to decrease the dissociation by diluting the complex in the viscometer with an oligopeptide solution at a concentration corresponding to the free oligopeptide con-

centration). This procedure has been proved successful for the intercalation of dyes or antibiotics with DNA. In these cases the intrinsic viscosities are notably higher than for free DNA.

We have measured the specific viscosity of solutions of sonicated DNA–oligopeptide complexes and the results are plotted as a function of DNA concentration in the figure. As can be seen linear plots are obtained for a given molar oligopeptide/DNA ratio. The linearity of the plots is direct evidence that no substantial dissociation of the complex occurs. In fact with a K value of 10^4 in the range 8–2 mM_P for DNA concentration ($r = 0.1$) the amount of binding changes from 98.7% to 95.3%. With a K value of 3×10^3 in the same range of concentration the amount of binding changes from 95% to 86%.

From the experimental data it appears clearly that, whereas the specific viscosities of all complexes are much lower than that of free DNA at any finite concentration of the latter, the apparent intrinsic viscosities differ only by a small amount from that of free DNA solution, the small difference being attributable, in our opinion, to an electrostatic effect exercised by the charged bound oligopeptides. The same effect can explain the difference in the Huggins constant between free DNA and complexed DNA and between the complexed DNAs. In fact the viscosity of the complex with $(L\text{-}Lys)_3$ is more affected with respect to DNA than that with other oligopeptides. The same marked effect on the specific viscosity has been reported by Gabbay's group for the case of L-Lys–L-Leu–L-Lys and NH_3^+-(CH_2)-NH_3^+ [1,2] bound to DNA. Using the Gabbay's reasoning one should conclude that these last compounds together with $(L\text{-}Lys)_3$ should also partially intercalate into DNA chains.

Finally the p.m.r. results obtained with DNA–aromatic oligopeptide complexes show, in our opinion, only the fact that aromatic groups are immobilized and experience a different environment compared to the free state in solution. This is not in contraddiction with different models of binding, other than intercalation, where the exposed portion of the DNA bases may play a role in terms of ring currents.

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