INTERACTION BETWEEN PROFLAVINE AND DEOXYRIBONUCLEIC ACID INFLUENCE OF DNA BASE COMPOSITION

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SUMMARY : Interaction between proflavine and DNAs of various base compositions has been studied by viscosimetry and relaxation techniques, in the strong binding region. The increase of the contour length of DNA depends strongly of its base composition, being larger with A.T rich DNA than with G.C rich DNA. Kinetic studies show mainly two relaxation times with calf thymus DNA-proflavine complex and only one with Micrococcus lysodeikticus DNA-proflavine complex. It is concluded that the location of the strongly bound dye depends on the nature of the bases involved in the binding site. The binding is more external in G.C rich than in A.T rich regions.

Introduction :

Numerous studies on proflavine binding to nucleic acids have led to the proposal that most of the bound dye molecules are intercalated between consecutive base pairs at moderate ionic strength and large polymer to dye ratios [1]. A good evidence for the intercalation model has been obtained from the finding that proflavine binding increases the contour length of rodlike macromolecules (e.g. sonicated DNA [2], poly A. poly U [3]). However, the length increase was found to be slightly smaller than that expected from the assumption of total intercalation of all bound molecules. This can be interpreted by the existence of a small amount of externally bound dye molecules which do not contribute to the increase in molecular length [3, 4]. Comparing the effect of proflavine binding on Micrococcus lysodeikticus DNA and chemically methylated Micrococcus lysodeikticus DNA [5] we observed large differences in the molecular length increase. We have therefore

investigated the binding of proflavine to DNAs of various base compositions by viscosity and temperature-jump relaxation techniques.

Material and Methods :

Clostridium perfringens DNA (70 % A.T), Calf thymus DNA (58 % A.T), Serratia marcescens DNA (42 % A.T) and Micrococcus lysodeikticus DNA (28 % A.T) were prepared as already described [6]. The method of Dr MULLER (personal communication) was also used to prepare samples of M. Lysodeikticus DNA. All samples were treated several times with phenol.

Viscosity measurements were performed with a Ubbelhode semi-microviscometer n° 50. The flow times of the solvent and the solutions were respectively around 300 sec and 325 sec. The reduced viscosity was assumed to be equal to the intrinsic viscosity $[\eta]$. Experiments were carried out by adding increasing amounts of proflavine to DNA solutions of known concentrations. The amount of bound proflavine was determined from absorption spectra with the assumption that the spectrum of the strongly bound dye do not depend on the base composition of DNA. The concentrations of the DNA solutions were deduced from their absorbances at 260 nm. The values of the extinction coefficients were taken from ref. [7]. However we adopted $\varepsilon_{260} = 6\,700$ for Cl. perfringens DNA.

DNAs were degraded with a MSE ultrasonicator. Prior irradiation, the sample was flushed with nitrogen. During irradiation the sample was cooled by circulation of cold water. The total time of sonication was about 4 minutes, alternating one minute of sonication with one minute of nitrogen bubling. The sedimentation constants s_{20} of sonicated DNA samples were found between 7 and 8.2 Swedberg in standard sodium citrate.

The kinetic studies were performed with a Temperature-Jump apparatus purchased from Messanlagen Studiengesellschaft mbH (Göttingen). The chemical relaxation process was followed by recording at a fixed wavelength (430 nm) the time dependent variation of the optical transmission of the solution after a fast rise of temperature. In order to improve the signal-to-noise ratio a R 2 E multichannel signal averager was used (memoscope RE 10).

Results :

1 - Viscosity -

For rodlike macromolecules, COHEN and EISENBERG [2] have

derived a relation between the relative contour length L/Lo and the relative intrinsic viscosity $[\eta]/[\eta_o]$

$$\frac{L}{Lo} = \left(\frac{[\eta]}{[\eta_o]}\right)^{1/3}$$

In this formula L and Lo are the contour lengths respectively in presence or in absence of bound proflavine, $[\eta]$ and $[\eta_o]$ are the corresponding intrinsic viscosities.

Using this formula and assuming that the reduced viscosity is equal to the intrinsic viscosity, the results obtained with different DNAs are plotted in fig. 1. In all cases, the relationship bet-

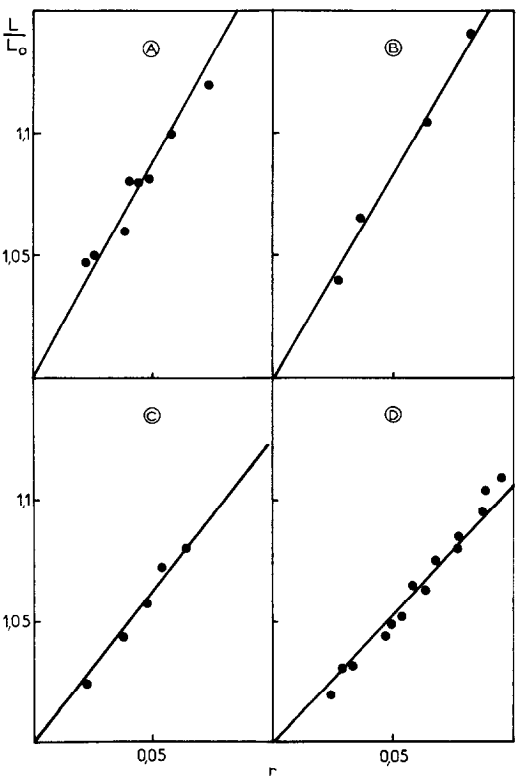

Figure 1 :

Variation of L/Lo as a function of r. Solvent : 0.1 M NaCl, 0.01 M AcNa, 0.1 mM EDTA, pH 5, T = 20 °C.

Ⓐ Cl. perfringens DNA, Ⓑ Calf thymus DNA, Ⓒ S. marcescens DNA, Ⓓ M. lysodeikticus DNA.

ween r (number of bound proflavine molecules per nucleotide) and the relative contour length was

$$L/Lo = 1 + \alpha r \quad ,$$

but the slope α depends on the G.C content of the DNA. The results obtained with calf thymus DNA are in good agreement with those already published [2] if one takes into account the fact that the experimental conditions are slightly different. The main result is that the slopes are very different for A.T rich DNA and G.C rich DNA-proflavine complexes.

Since our experiments were carried out at pH 5, we have checked that the observed effect was not related to the state of protonation of the DNAs. Experiments performed at pH 7 on M. lysodeikticus DNA-proflavine complex gave identical results.

The salt effect was also studied. As shown in fig. 2, the variation of α is almost independent of the salt concentration within experimental accuracy. The approximation that the reduced viscosity is equal to the intrinsic viscosity can lead to some error. However this error is certainly small. In the case of pure DNA, the Huggins's constant is small ((12) and unpublished results). It is possible that this constant depends on the amount of bound proflavine. Preliminary results indicate that this change can be neglected in a first approximation.

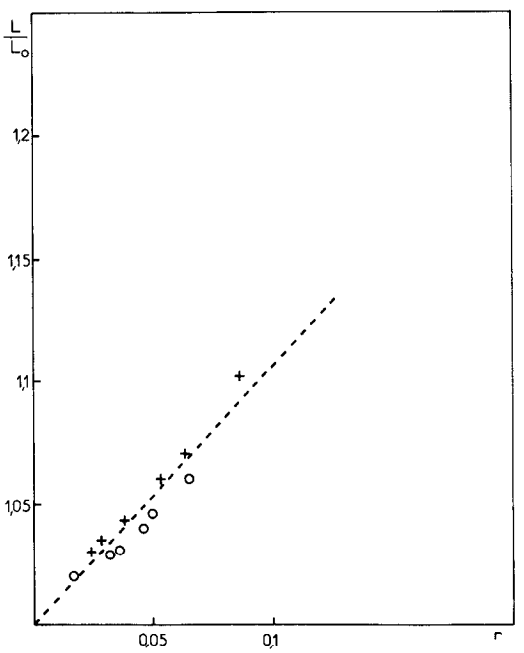

Figure 2 :

L/Lo as a function of r. M. lysodeikticus-proflavine complex, pH 5, T = 20 °C. — — — 0.1 M NaCl ; + 0.01 M NaCl ; O 1 M NaCl.

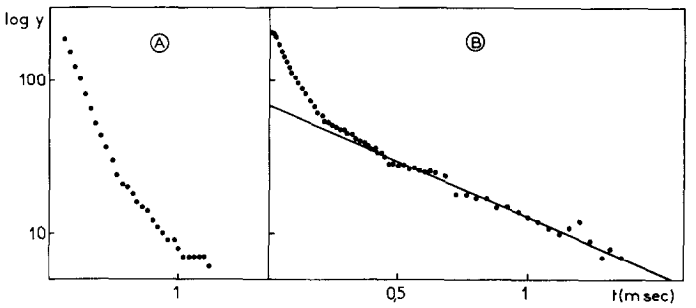

Figure 3 :

Typical semi logarithmic analysis of a Temperature-jump experiment.

(A) M. lysodeikticus DNA. Same solvent as in fig. 1, T = 16 °C, $(\bar{C}_D + \bar{C}_F)$ = 1.235 mM, r = 0.036, Risetime = 0.1 ms.

(B) Calf thymus DNA. Same solvent as in fig. 1, T = 16 °C, $(\bar{C}_D + \bar{C}_F)$ = 1.25 mM, r = 0.037, Risetime = 50 µs.

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2 - Kinetic studies

These studies were performed on sonicated calf thymus and M. lysodeikticus DNAs. With calf thymus DNA we found mainly two relaxation times differing by about one order of magnitude. The concentration dependences of these relaxation times (fig. 3) agree with the results obtained by LI and CROTHERS [4] and with the following mechanism proposed by these authors

$$P + DNA \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} (P)_{out} \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} (P)_{in}$$

where P is the free proflavine, $(P)_{out}$ and $(P)_{in}$ are respectively the outside bound and intercalated forms of the dye. The faster relaxation time τ_1 is associated to the first step of this scheme [8] and therefore

$$\frac{1}{\tau_1} = k_{12} \ (\bar{C}_D + \bar{C}_F) + k_{21}$$

$$\frac{1}{\tau_2} = k_{32} + k_{23} \ \frac{(\bar{C}_D + \bar{C}_F)}{\frac{1}{K_{12}} + (\bar{C}_D + \bar{C}_F)}$$

TABLE I

	k_{12} $M^{-1} \times sec^{-1}$	k_{21} sec^{-1}	k_{23} sec^{-1}	k_{32} sec^{-1}	K_{12} M^{-1}	K_{23}	K_{ap} M^{-1}
M. Lysodeikticus (1)	0.72×10^7	0.3×10^3			2.4×10^4	0.25	3×10^4 *
Calf thymus (2)	0.56×10^7	0.57×10^4	1.54×10^3	1.6×10^2	4.4×10^3	9.6	4.4×10^4 4×10^4 *
Calf thymus (3)	0.8×10^7	3.8×10^3	2.1×10^3	1.5×10^2	2.1×10^3	14	3.4×10^4

Solvent for (1) and (2) : 0.1 M NaCl, 10^{-2} M Na acétate pH 5, 10^{-4} M EDTA, T = 16 °C.

" " (3) : 0.2 M NaCl, pH 6.9, T = 15 °C, taken from ref. (4).

* Apparent equilibrium constants determined according to the procedure of LI and CROTHERS (absorption at 430 nm) - (ref. 4).

Polymer concentrations are expressed in nucleotides equivalent per liter.

In these equations, $\bar{C}_F$ is the equilibrium concentration of free dye, $\bar{C}_D$ is the equilibrium concentration of free binding sites and K_{12} is the equilibrium constant for outside binding.

The rate and equilibrium constants obtained with our sample are given in Table I. These values are slightly different from those obtained by LI and CROTHERS but this can be explained by differences in pH and ionic strength.

Relaxation kinetics on M. lysodeikticus DNA-proflavine systems show essentially one measurable relaxation time τ_1. A slower relaxation effect is present but its corresponding amplitude is too small to allow any proper analysis. The reciprocal of the measurable relaxation time varies linearly with $(\bar{C}_D + \bar{C}_F)$ in the same concentration range as for calf thymus DNA-proflavine systems (fig. 3). Rate and equilibrium constants are given in Table I. K_{23} was obtained from the assumption that the non-resolvable relaxation effect corresponds to the

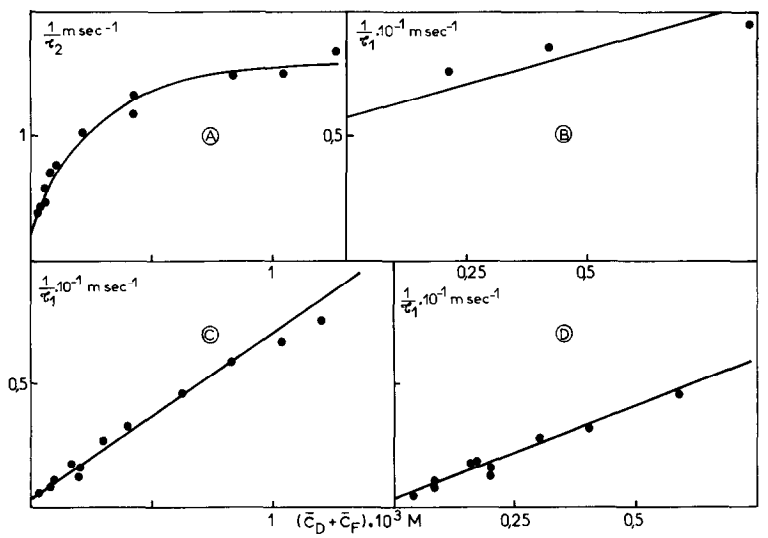

Figure 4 :

(A) Variation of the reciprocal of the slower relaxation time τ_2 with $(\bar{C}_D + \bar{C}_F)$. Calf thymus DNA, $[Na^+]$ = 0.1 M, pH 5, r = 0.037, T = 16 °C.

(B) Variation of the reciprocal of the fast relaxation time τ_1 with $(\bar{C}_D + \bar{C}_F)$. Calf thymus DNA, $[Na^+]$ = 0.1 M, pH 5, r = 0.037, T = 16 °C.

(C) and (D) Variation of the reciprocal of the fast relaxation time τ_1 with $(\bar{C}_D + \bar{C}_F)$. M. lysodeikticus DNA, $[Na^+]$ = 0.1 M, pH 5, r = 0.036, T = 16 °C.

second step of the aforementioned mechanism and using the relation $K_{ap} = K_{12}(1 + K_{23})$ between the apparent equilibrium constant K_{ap} (measured from equilibrium spectrophotometric data (ref. 4)) and the constant K_{12} obtained from relaxation data. It is interesting to note that the values obtained for k_{12} are of the same order of magnitude for calf thymus and M. lysodeikticus DNAs while they are very different for k_{21}. In addition, K_{12} and K_{23} show great differences between calf thymus and M. lysodeikticus DNA.

Discussion :

All experiments reported in this work were performed in the strong binding region because of the large polymer to dye ratios which were used ($D/P > 10$). For all DNAs a well-defined isosbestic point was observed at 455 nm between the absorption spectra of free and bound proflavine. Our viscosity and kinetic results reveal differences between DNAs of various base compositions.

Theoretical considerations predict that the DNA molecule is lengthened by 3.35 Å per intercalated dye molecule. If all bound molecules were completely intercalated the slope of the L/Lo versus r plot should be 2. Slightly smaller values obtained with calf thymus DNA and poly A. poly U have been interpreted by the existence of the so called "outside" bound form which would not increase the viscosity of the polymer [3]. However, in the case of ethidium bromide - DNA complex, although a slope smaller than 2 was found, it was claimed that the outside binding was negligible [9].

In the hypothesis of non-negligible outside binding, the percentage of the outside bound form can be obtained either from thermodynamic data or from viscosity data [3]. For calf thymus DNA the values obtained using both methods are small and agree within experimental accuracy. In the case of M. lysodeikticus DNA, thermodynamic and viscosity data gave respectively 75 % and 50 % of outside bound dye. Some comments have to be made in order to explain this apparent discrepancy :

i) $(P)_{out}$ may contribute to some extent to the increase of the length of DNA. The calculated percentage of $(P)_{out}$ obtained by ignoring this effect is therefore underestimated.

ii) Viscosity and T-jump experiments have been carried out at slightly different temperatures (20 °C and 16 °C respectively).

iii) There can be a large uncertainty in K_{23} whose value was not deduced directly from relaxation experiments.

In any event, thermodynamic and viscosity data indicate a large percentage of the $(P)_{out}$ form in the case of M. lysodeikticus DNA-proflavine complexes. Since the percentage of $(P)_{out}$ is much larger with G.C rich DNA than with A.T rich DNA, we conclude that the location of the strongly bound dye depends on the nature of the base pairs involved in the binding site and that the bound dye is more external in G.C rich than in A.T rich regions.

On the other hand, the analysis of the binding isotherms between proflavine and M. lysodeikticus according to Scatchard [10] is in agreement with the nearest neighbor exclusion model [11, 12]. Moreover, the value obtained for K_{12} agrees with that expected for intercalation.

Thus far, we cannot say whether $(P)_{out}$ corresponds to a partly intercalated form or to some other form of the interacting dye.

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