Solution structural studies of the Ag(I)-DNA complex

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ABSTRACT

We report equilibrium dialysis and electric dichroism studies of the two strong complexes (I and II) of silver ion with DNA. Cooperative conversion of DNA to the stronger type I complex results in a 9% length decrease, and a structure in which intercalated ethidium is perpendicular to the helix axis. Upon addition of more Ag^+ to form the type II complex, the DNA length reverts to its original value and bound ethidium once again becomes tilted from the plane perpendicular to the helix axis. In both type I and type II Ag (I) - DNA complexes, ethidium binding is mildly cooperative. We interpret the results in terms of a sequence of silver-induced cooperative switches of DNA from its B-form structure with propeller twisted base pairs to a structure with flat base pairs in the type I complex, and back again to propellered base pairs in the type II complex.

INTRODUCTION

Switching of DNA structure between different conformations is a subject of particular current interest. The products of two such transitions are now known in molecular detail: The long established cooperative transition to the A-form induced by ethanol (1) or low humidity, and the switch to the Z-form (2,3), first discovered as a cooperative solution process by Pohl and Jovin (4,5). Other apparently highly cooperative transitions include the distamycin-induced conversion of several DNAs to forms characterized by higher affinity for the drug (6,7), but the structural basis for this effect is unknown.

Transitions such as B to A or Z are cooperative because the boundary between the two structures, whatever its nature, has a high free energy. Other DNA conformational changes, such as are induced by binding of standard intercalators, appear non-cooperative because the modified structure is compatible with the adjacent B-form helix. Compatible structures intermix readily along the helix, whereas incompatible struc-

tures do not; the latter can therefore be responsible for long range switching of DNA conformation.

In this paper we call renewed attention to the two highly cooperative transitions induced in double helical DNA by Ag^+ binding. Leaving aside the unlikely possibility that Ag^+ ions interact directly when bound, the observed cooperativity of binding implies that the first complex formed (I) is structurally incompatible with B-DNA, and that complex forms I and II are mutually incompatible. Our solution studies provide enough information to allow us to propose a tentative model designed to help provoke further detailed structural studies of the complex. The fundamental question to be answered is the general origin of long range allosteric effects in DNA, of which the Ag^+-DNA complex provides an interesting example.

Interaction of silver ion with nucleic acids has been studied extensively (8-16). It has been shown that binding is accompanied by a large UV absorption change and liberation of protons at certain binding ratios. Potentiometric and spectrophotometric titration indicate that the binding is highly cooperative and at least three modes of binding can be distinguished in the case of DNA (8,12,14). The shape of the binding isotherm is highly pH dependent. Although the binding of Ag(I) to DNA is cooperative and follows the neighbor exclusion mechanism, hydrodynamic studies (12) indicate that the double helical structure remains intact in the complex. Ag(I) has been shown to induce ordered structure in poly U and poly I (16) and Ag(I) also shows cooperative binding to poly A and poly C (14). Although the site of Ag(I) interaction on purines and pyrimdines has been postulated (12,14) the detailed structure of the complex remains uncertain. It has been proposed that N3, N7 and N1 (12,14) of purines react with Ag(I). Since N1 of adenine is involved in hydrogen bonding, Ag(I) binding at this site must involve disruption of the H-bond and possible alteration of the structure of the double helix. Since hydrodynamic measurements (12) indicate that the helix remains intact, the structural distortion by Ag(I) must involve changes of helical parameters such as base tilt angle, rotation per residue or pitch.

Recently, it has been shown that the structure of DNA in solution is different from that in fibers (17,18,19). We have shown that the base twist angle in solution is about 73° with respect to the helix axis (17), consistent with Levitt's (20) theoretical calculation and model, in which the twist is accommodated by a propellered conformation of the two bases

in the pair. A change in base twist angle in such a conformation could conceivably propagate a cooperative structural transition without any appreciable effect on the length of the double helix.

Linear dichroism in an electric field can be used to monitor changes in transition moment angle relative to the orientation axis. Since in DNA the UV absorption band (240 - 300 nm) is primarily due to π-π^* transitions, the corresponding transition moments are in the plane of the bases. Hence, the transition moment angle reflects the base twist or tilt angle. Moreover, the rise time of the dichroism signal can be used to determine the rotational diffusion coefficient, which is highly sensitive to length changes.

In the present communication we show that Ag(I) ion interaction causes a change of base orientation angle in DNA and has a long range effect on the binding of ethidium bromide, an intercalating ligand.

MATERIALS AND METHODS:

Silver acetate (Fisher Scientific Co.) was used as the source of Ag(I). DNA (calf thymus) was purchased from Sigma Chemical Co. and purified and fractionated as described elsewhere (17). Electric dichroism and equilibrium dialysis measurements were done in 2.5 mM sodium cacodylate buffer adjusted to pH 6.3±0.05 with acetic acid. Dithizone, acetic acid, chloroform, and Na-cacodylate were obtained from Fisher Scientific Co. Ethidium bromide was purchased from Sigma Chemical Co. Dialysis membranes were boiled in 0.1M sodium carbonate followed by boiling in buffer, cooled and extensively washed with cacodylate buffer.

Equilibrium dialysis experiments were done by placing DNA solutions ($\sim$1 ml) of known concentration in dialysis bags; each was immersed in a large volume of buffer containing variable concentration of ligand. The dialysis was done at room temperature, 23° ± 3°C, with continuous stirring on a magnetic stirrer. After about 60 hours the concentrations of ligand inside and outside the bag were determined. The method of estimation of ethidium has been described elsewhere (21). Dithizone (diphenylthiocarbazone) was used as indicator for Ag(I). The procedure is similar to that used for Hg(II) and 3.6-bis-(acetatomercurymethyl)-dioxane (23): 2 ml dithizone solution (2.5 mg per 250 ml chloroform) and 0.7 ml glacial acetic acid were taken in a teflon stoppered glass test tube. To it x ml experimental solution containing Ag(I) and (2 - $\underline{x}$) ml buffer solution were added. The tube was stoppered, shaken well and then cooled

in ice, and centrifuged for 20' in a tabletop centrifuge. A part of the chloroform phase was removed and the absorption spectrum in the visible region was measured against the corresponding reference solution which was prepared from pure chloroform instead of dithizone solution. A standard curve was constructed with known amounts of Ag(I). It was found that DNA does not have any measurable effect on the estimation. The change in absorbance per mole of silver is maximum at 595 nm but ΔOD/mole of Ag^+ deviates from the constant value when the amount of Ag^+ is higher than 0.08 μ mol in the $\underline{x}$ ml sample. The difference in absorbance was unity at 595 nm (1 cm path length) when 0.0781 μ mol of Ag(I) were taken as the sample. Since the percentage error is higher in the estimation of free Ag(I), the dithizone estimated values were compared with the weighed amount present in the dialysate. Each estimation was repeated three times and the average value was used for the calculation of r (bound Ag(I)/base pair).

Electric dichroism is a form of linear dichroism in which electrically and optically anisotropic molecules are oriented in an electric field. The dichroism amplitude ρ is related to the transition moment angle (α) by equation (1)

$$\frac{A_{\parallel} - A_{\perp}}{A} = \rho_{\phi} = \frac{3}{2} (3 \cos^2 \alpha - 1)\phi \qquad (1)$$

$A_{\parallel}$ and $A_{\perp}$ are the absorption of the sample when incident light is parallel and perpendicular to the field axis respectively, and A is the absorption of an unoriented sample. ϕ is the fractional orientation and α is the angle between the transition moment and the helix axis. A detailed description of the method has been presented elsewhere (17,22).

The relaxation time (τ_c) for the rise of dichroism is related to the size of the rod shaped DNA by the Broersma relationship (24).

$$\tau_c = \frac{1}{6D_r} \text{ and } \quad D_r = \frac{3kT}{\pi\eta L^3} [\ln \frac{L}{b} - 1.57] \qquad (2)$$

where k is the Boltzmann constant, T the temperature in Kelvin, η is the solvent viscosity and L and b are the length and radius of DNA.

RESULTS

Dialysis equilibrium confirms the existence of two strong Ag(I) binding modes. Figure 1 shows the binding isotherm found for Ag(I)

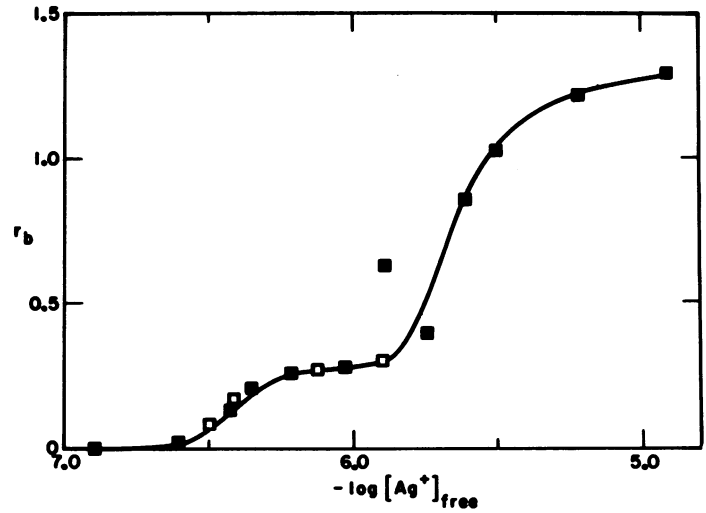

Figure 1: Binding of silver (I) to calf thymus DNA. □ and ■ dialysis in presence and absence of ethidium bromide, respectively. Buffer = 2.5 mM Na-cacodylate pH = 6.3, temperature = 23 ± 3°C.

interacting with calf thymus DNA in 2.5 mM sodium cacodylate buffer adjusted to pH 6.3 with acetic acid, 23°C. The biphasic binding curve confirms earlier spectrophotometric and potentiometric titrations (8,12, 14). Differences in absolute binding affinity between our results and those of Daune et al. (14), are probably due to differences in conditions such as ionic strength, pH, and nature of the anions present. The first binding step (I), which saturates at 1 Ag^+ per two base pairs for poly(dG-dC) (14), differs from the weaker second binding step (II) (saturation at 1 Ag^+ per base pair) in that type I binding involves no proton release, whereas in type II binding approximately 1 H^+ is displaced per Ag^+ bound (8,12).

Implications of cooperative binding. The cooperative binding isotherm shown in Figure 1 reveals two transitions, each only 2 to 3 times broader than the alkaline melting transition of double helical DNA (25). Hence the transitions are highly cooperative, and by analogy with DNA melting the average length of a helix section of one type in the transition is probably of the order of 100 base pairs. All GC containing DNAs undergo the first transition to complex I (14), so only very long (100bp)

AT sequences could be immune. Since such satellites do not exist in calf thymus DNA, we conclude that the cooperative binding transitions must involve the whole molecule, not just special sequences.

Earlier hydrodynamic studies (12) indicated that DNA remains rigid in the Ag^+ complex, implying that the double helix remains intact. Hence the cooperativity of binding cannot arise because of coupling to the helix-coil melting transition, which, in contrast, is probably the case for binding (12). Therefore Ag^+ binding must induce two distinguishable conformational switches of double helical DNA. The remainder of our experiments focus on characterization of the two new states induced by type I and type II Ag^+ binding, using electric dichroism, rotational relaxation, and drug binding studies on short rod-like fragments of calf thymus DNA.

The rotational correlation time indicates that the length of rod shaped DNA is altered due to type I Ag(I) binding. The relaxation time measured from the rise time of electric dichroism was used to calculate the rotational diffusion coefficient and thus the length of the DNA by equation (9). The hydrodynamic radius of the DNA helix was assumed to be 13 Å. The effect of Ag^+ on the length of DNA is presented in figure (2) Ag^+ binding causes reduction in the hydrodynamic length by about 9% at r = 0.4 per base pair (type I binding). Above this binding

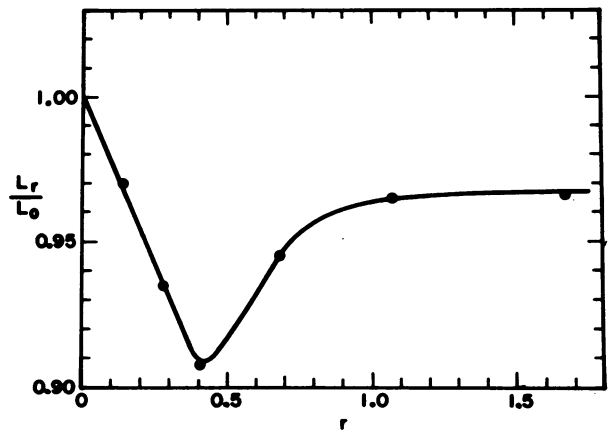

Figure 2. Effect of Ag^+ on the length of rod shaped DNA. Lengths were calculated from the rise time of electric dichroism. The pH and ionic strength are the same as in Figure 1; temperature = 11°C.

ratio the length starts to increase and reaches a constant value at about r = 1.0 (type II binding). The plateau value is about 97% of the original length.

Silver binding alters DNA dichroism. In figure (3) a typical field extrapolation plot is shown at 245 nm and 265 nm for r_{Ag+}=0.133. The theoretical basis for the plot has been described earlier (17). It can be seen from the figure that the dichroism values at perfect orientation (ρ_∞) are -1.45 and -1.0 at 245 and 265 nm respectively, corresponding to α of 83.9° and 70.5° respectively. The dichroism spectra are shown in figure (4). The measured tilt angles in the type I binding range are dependent on wavelength, with values dropping continuously from 240 nm to 275-280 nm, then reaching a constant value. The type II complex differs in the limiting 240 nm dichroism, but also shows a pronounced wavelength dependence. In contrast, for DNA the transition moment angle remains the same throughout the absorption band. The variation of α with the amount of Ag^+ bound is shown in figure (5). At 265 nm α decreases from 71.5° to about 68° at r =1.0, and then to 66° at r = 1.67. At 245 nm α first increases from 71.5° to about 84° at about r = 0.25 (type I binding) then drops to 74° at r = 0.7 (type II complex). At r = 1.67 α is about 70°.

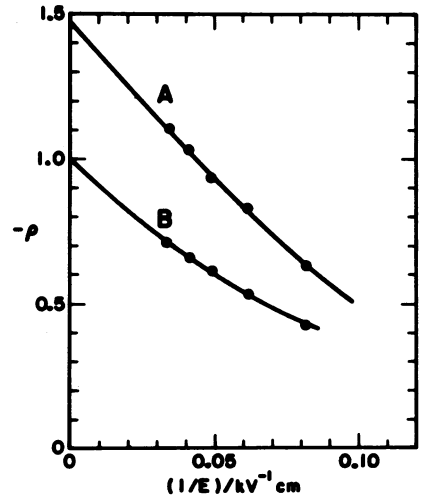

Figure 3. Extrapolation of the reduced dichroism to infinite field. The experimental conditions are the same as in Figure 2. A and B use dichroism values measured at 245 nm and at 265 nm respectively; r_{Ag+} = 0.133.

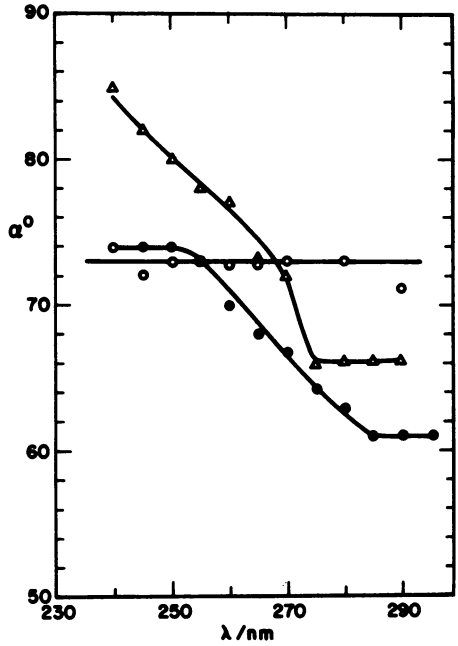

Figure 4. Dichroism spectra of Ag^+ DNA complex. ● r_{Ag+} = 1.06; △ r_{Ag+} = 0.133; ○ r_{Ag+} = 0. Experimental conditions as in Figure 2.

The change in α at 245 nm is remarkable in the sense that one silver per 4-7 base pairs is enough to change the measured $-\rho_\infty$ by 50%, increasing it to virtually the maximum limiting value of 1.5.

Base tilting versus out-of-plane transitions. The results in Figures 3-5 indicate that the UV transition moments of the type I and II Ag^+ DNA complexes differ in their orientation relative to the helix axis. This could arise from two possible sources: (a) the DNA bases could be tilted from the plane perpendicular to the helix axis, and the transition moments may not be parallel to each other. The 245 nm transition must lie in a direction perpendicular the helix axis, but the longer wavelength transitions could move toward the predominant tilt direction of the bases. Alternatively, (b) the longer wavelength transitions could contain out-of-plane contributions due to perturbation by the metal ion of the base electronic structure.

Use of bound ethidium as a probe. In order to test whether the high reduced negative dichroism at 245 nm is indicative of a 90° angle of the

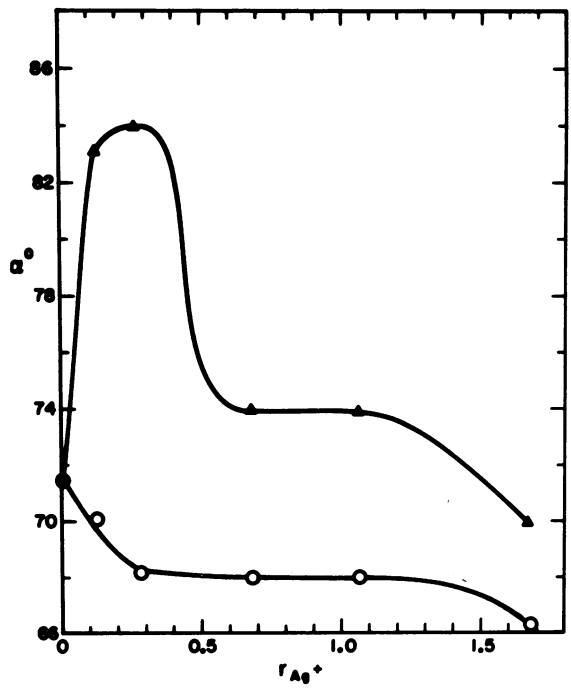

Figure 5. Variation of orientation angle of transition moments of the DNA bases with silver. ▲ , 245 nm; ○ ,265 nm.

base plane or not we used ethidium bromide as a probe. We have shown previously (21) that the intercalated phenanthridine chromophore does not lie at a 90° angle relative to the helix axis, but rather the chromophore long axis is tilted by about 23° relative to the plane perpendicular to the helix axis, and the chromophore short axis is twisted by about 10° with respect to the same plane. Figure (6) illustrates a typical field extrapolation plot for EB-DNA complex in presence of Ag^+, showing that at r_{Ag+}= 0.175 and r_{EB}= 0.075 the short molecular axis 520 nm transition moment for EB is nearly perpendicular to the helix axis. Exactly the same result is found for the long axis transition moment at 320 nm. These values indicate that the EB chromophore is virtually perpendicular to the helix axis when r_{Ag+} = 0.175 (type I complex).

High r_{Ag+} and r_{EB} both reduce the tilt angle. In Figure (7) the effect on the 520 nm transition moment of EB due to addition of different amounts of Ag^+ at fixed amounts of EB (r_{EB}= 0.075) and different amounts

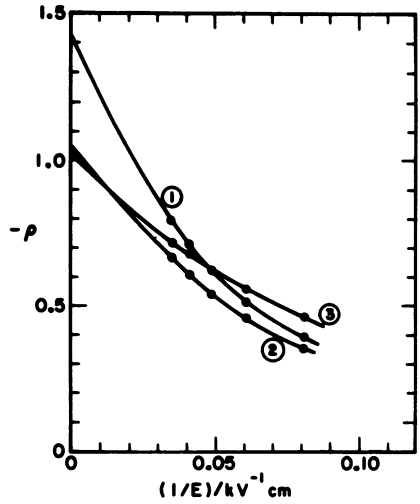

Figure 6. Field extrapolation of the dichroism values of ethidium bromide with varying silver ion. λ=520 nm. 1, 2 and 3 are for $r_{Ag(I)}$= 0.075, 0.46 and 0 respectively. r_{EB}= 0.1 in all cases.

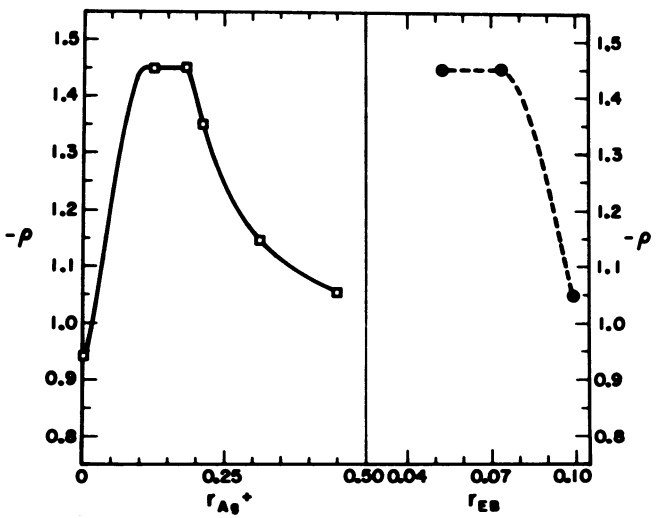

Figure 7. Variation of the dichroism of ethidium at 520 nm with r_{Ag+} at constant r_{EB} and with r_{EB} at constant r_{Ag+} right, r_{Ag+} 0.175; left, r_{EB} = 0.075.

of EB at fixed Ag^+ (r_{Ag+}= 0.175) are shown. The data clearly indicate that at very low r_{EB} (up to 0.08) all the chromophores are nearly perpendicular to the helix axis, whereas at higher binding ratios the chromophore is tilted. Similarly, at higher r_{Ag+} the chromophore deviates from perpendicularity.

Presence of out-of-plane transitions. We conclude that in the type I complex the EB chromophore, with both its long and short axis perpendicular to the helix axis, must have its molecular plane also perpendicular to the helix axis. By inference, assuming that the chromophore will follow the orientation of adjacent base pairs, we tentatively conclude that the base pairs in the type I complex must be perpendicular to the helix axis. Therefore, the long wavelength transitions in the type I complex must include a significant out of plane contribution (alternative b). Ding and Allen (26) reached the same conclusion on the basis of the dichroism of the Ag^+ - DNA complex at fixed values of the field, although the logic they followed does not seem to have included the possibility that the DNA bases could be tilted in the complex.

In the type II complex, the ethidium dichroism reverts to values close to those found for uncomplexed DNA. Therefore it seems likely that the base pairs are twisted or tilted in this complex form, as is consistent with the smaller 245 nm dichroism amplitude compared with the type I complex.

Cooperative binding of eithidium to the Ag^+ -DNA complex. We used equilibrium dialysis to measure the binding of EB to the Ag^+- DNA complex at various Ag^+/DNA ratios, with the results shown in Figure 8. The extent of silver binding r_{Ag+}= 0.083, remains effectively constant up to r_{EB}= 0.4. Cooperativity in the binding reaction is more immediately evident in the Scatchard plots of the binding isotherm, as illustrated in Figure 9. Assumption of a neighbor exclusion range of 2 base pairs, and a preference (compared to isolated site binding) by a factor of about 5 for binding at a site located the minimum distance from another bound ethidium, allows one to calculate the shape of the full binding isotherm. Clearly, the presence of bound Ag^+ affects the binding of EB by DNA.

Possible structural models. Our overall conclusion is that Ag^+ binding in the type I complex converts DNA from its normal solution conformation with propeller twisted base pairs (17) to a structure in which intercalated ethidium is perpendicular to the helix axis. The helix length decreases by about 9% (0.3 Å per base pair) in this process,

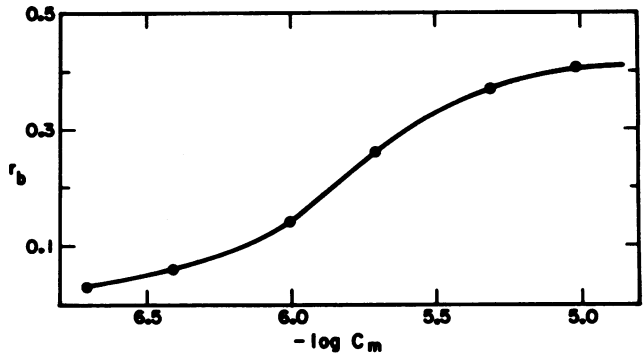

Figure 8. Binding of ethidium bromide in the presence of Ag(I), r_{Ag+} = 0.083. Other conditions are as in Figure 1.

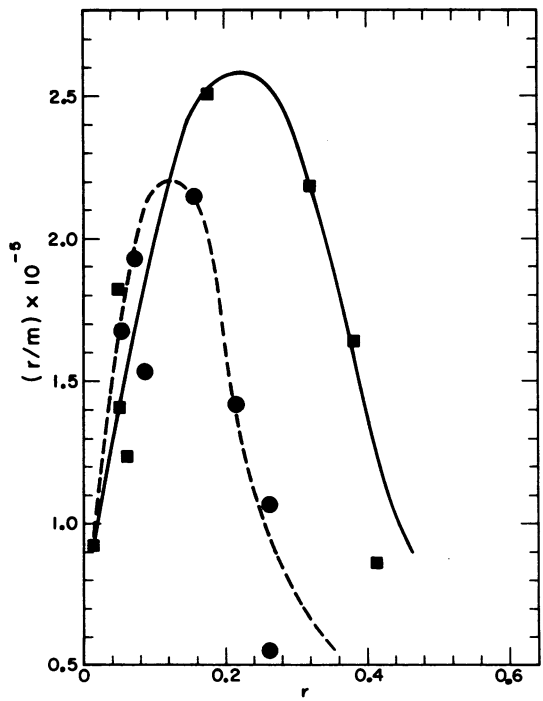

Figure 9. Scatchard plot for binding of ethidium bromide to calf thymus DNA in presence of Ag(I). m is the free EB concentration. ■, $r_{Ag(I)}$ = 0.173; ● $r_{Ag(I)}$ = 0.083. Other conditions are as in Figure 1.

and we infer that the base pairs are probably flattened from their propellered conformation, and are perpendicular to the helix axis. We found only a slight increase of the sedimentation coefficient of super-helical closed circular DNA, upon Ag^+ binding so any change in the helix winding must be small. As more Ag^+ is added to form the type II complex, intercalated drugs, and probably the bases, become tilted from the plane perpendicular to the helix axis, and the helix length reverts to its B-form value.

The results we have obtained are consistent with models which have been proposed for the Ag^+-DNA complex. Bloomfield et al. (27) suggested that in the type I complex the Ag^+ ion is chelated to the guanine N7 and keto oxygen (Figure 10), producing a tautomeric shift in G and transfer of the proton to C. The net positive charge on the base pair which results from the lack of proton release could be primarily responsible for the length decrease because of the reduction in net electrostatic repulsive forces along the helix. Furthermore, the chelated Ag^+ ion could increase the strength of the H-bond donated by the cytosine amino group because of the residual negative charge on the guanine enol oxygen. (The positive Ag^+ ion is shared between N7 and the enol oxygen.) A stronger H-bond provides a driving force to flatten the base pair in order to reduce the bond length to a minimum. One disadvantageous feature of the model in Figure 10 is the lack of the usual linearity of Ag(I) two-ligand complexes.

In type II binding we believe that base pair propeller twisting reverts toward its normal state in the solution form of DNA. This is

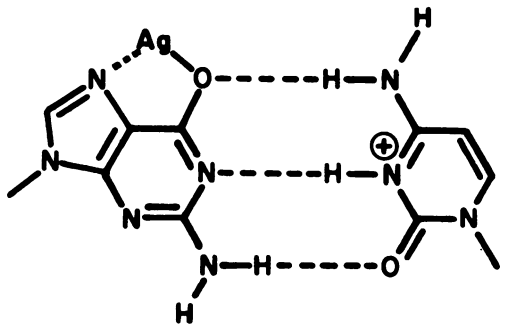

Figure 10. Proposed model for type I Ag^+ - DNA complex with a G-C base pair.

consistent with replacement of H^+ by Ag^+ in an H-bond, either along an N-H---O bond or the N-H ---N bond as proposed by Jensen and Davidson (1). One might expect some lengthening of the H-bond from this substitution, and hence possibly a net weakening of the base - base H-bonds. It follows that there should be an increase in propeller twist as the energy gain from stacking interactions takes precedence over the H-bonding interaction (13).

Thus in summary we tentatively interpret the cooperative binding of silver as due to an initial switch from the B-form with propeller twisted base pairs to the type I complex with flat base pairs, followed by a switch back again to twisted base pairs in the type II complex. A corollary of this interpretation is that there is a significant positive free energy associated with the boundaries between propellered and non-propellered states, since otherwise the transition would not be cooperative.

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