Nucleic Acid–Metal Interactions: V. The Effect of Silver(I) on the Structures of A- and B-DNA Forms

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Synopsis

The interaction of silver(I) with DNA has been studied with uv LD in aqueous solution and in a humid anisotropic poly(vinyl alcohol) host (B-DNA) and also in 80% ethanolic solution (A-DNA). Addition of silver ions has a pronounced effect on the dichroic spectra of DNA, indicating that the DNA structure is significantly altered. By correlation with calculated reduced LD spectra, using intensities and moments of the corresponding electronic transitions of the DNA bases, the experimental spectra of DNA at high silver content may be interpreted in terms of tilt and roll angles of the bases in the double helix. In ethanolic A-DNA solution there is a pronounced decrease in the orientation by flow of DNA, suggesting that the complexation of DNA to silver may be accompanied by the formation of compact tertiary structures.

INTRODUCTION

The interaction of silver(I) ions with DNA in solution is known[1] to involve the formation of coordination complexes with the heterocyclic bases of DNA, accompanied by significant changes in the nucleic acid structure, as inferred from the results of flow dichroism,[2,3] CD,[4] and electric dichroism[5,6] measurements. From these and other physical studies including potentiometric, sedimentation, viscosity,[7–9] and nmr[10] and ir[11–13] spectroscopic measurements there is strong evidence that silver(I) ions form several distinct complexes with DNA, depending on the extent of binding and on the specific conditions of the medium. However, the structural details of silver(I)–DNA complexes have remained largely unexplored in spite of their possible biological relevance as models for heavy-metal toxicity and as explanation for the reported carcinogenic activity of silver(I) salts.[14] In a previous report[3] the possible structural implications of the results of flow dichroism measurements of DNA solutions in the presence of silver(I) ions were referred to. However, an attempt to interpret quantitatively the observations was postponed pending the completion of a project concerned with the

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determination of the magnitude and direction of electronic transition moments in the nucleic acid bases when complexed to silver ions.[15-17]

The flow dichroism technique for studying the structure and interactions of DNA exploits the very stable orientation that can be achieved by mild stationary flow gradients that do not perturb the local DNA structure and the high sensitivity of the measuring technique using phase-modulated polarization.[18-20] Correlation with calculated spectra, using moments and band shapes of the electronic transitions of the DNA bases determined for the isolated bases in an anisotropic host in sufficient dilution to avoid exciton interactions, can then lead to information about the structural organization of the bases within the DNA molecules.[21-23]

In this work we present the results of flow dichroism measurements of both aqueous and ethanolic solutions of DNA in the presence of silver(I) ions, augmented by CD data and by LD obtained in stretched poly(vinyl alcohol) (PVA) films. Consideration of the combined results will enable us to draw some quantitative conclusions concerning the conformational changes of DNA due to the binding of silver ions.

EXPERIMENTAL

Calf thymus DNA was purchased from Sigma Chemical Company (type I). Aqueous solutions were prepared either in pH 7.2 phosphate buffer of ionic strength 0.04 mol dm^{-3} or in 0.1 mol dm^{-3} sodium perchlorate at pH 5.8; there were no observable differences in the results of measurements on the two types of solutions. Solutions in ethanol were prepared as described by Ivanov et al.,[24,25] the ethanol was 80% w/v, and the solutions were 6×10^{-4} mol dm^{-3} with respect to sodium perchlorate. The preparation of PVA films incorporating DNA has been described before.[21] In order to prepare the corresponding silver complexes, calculated amounts of silver solution were spread evenly over the DNA-containing films and placed for one hour into a 100% relative humidity chamber before measurements. The temperature when not explicitly stated was 25°C. Before stretching a film, two small dots were marked on the film in the projected direction of orientation. The stretch ratio was defined as the final distance between the dots divided by the initial distance.

CD and LD spectra were determined by the use of a JASCO-J500 spectropolarimeter. The use of this instrument for the determination of LD has been described before;[26,27] the dichroic absorbances corrected for background, $A_{\parallel}$ and $A_{\perp}$, are obtained by the use of incident light polarized parallel and perpendicular, respectively, to the direction of flow or stretch in the case of films. Quantities related to these absorbances are the absorbance of the isotropic solution A, the LD and the

reduced linear dichroism (LD_r) defined by

$$LD = A_{\parallel} - A_{\perp}$$

$$LD_r = LD/A$$

In addition one has the following relation for the case of stretched films (uniaxial orientation):

$$3A = A_{\parallel} + 2A_{\perp}$$

RESULTS

LD in Solution

Figure 1(a) shows the isotropic absorption spectra A, the LD and the LD_r curves for a series of silver-containing DNA solutions at pH 7.2. The quantity X is defined as the molar ratio of silver(I) ions to DNA phosphates in solution. The LD was measured at a constant shear gradient of 1980 s^{-1}; the shape of the LD_r was the same at the very much lower gradient of 100 s^{-1}. One may thus assert that the local structure of DNA is not measurably distorted by the forces of shear under these conditions.

The results may be seen to conform to previous findings[8,9,28] that there are at least two types of silver(I) binding to DNA. At low values of $X(<0.15)$, which corresponds to "binding type I,"[9,28] there is relatively little change in the spectra compared with the corresponding pure DNA solutions. However, as the relative silver concentration increases to the region of "binding type II," the observed spectra change significantly; the B-form spectrum of DNA changes into a LD_r spectrum with a positive peak near 238 nm. Saturation is observed at $X \sim 0.5$. We also found that DNA–silver solutions at a higher ionic strength, 0.1 mol dm^{-3} sodium perchlorate, and at pH 5.8 behave identically.

Figure 1(b) shows the isotropic absorbance spectra and the reduced LD_r of DNA in 80% ethanol solution with a series of added silver(I) ions. We found that in order to ensure reproducible results in this case, measurements at relatively low values of X were best carried out at the low shear rate of 150 s^{-1} and 8°C. At higher silver concentrations ($X > 0.25$) such precautions were not necessary; the shape of the LD_r spectrum was not affected by increasing rates of shear, at least up to a shear gradient of 1980 s^{-1}. This observation will be significant in discussing the structure of A-DNA/silver ion complex taken together with the simultaneous significant decrease in the magnitude of the negative reduced dichroism of ethanolic DNA solution characteristic of the A-form of DNA.[21]

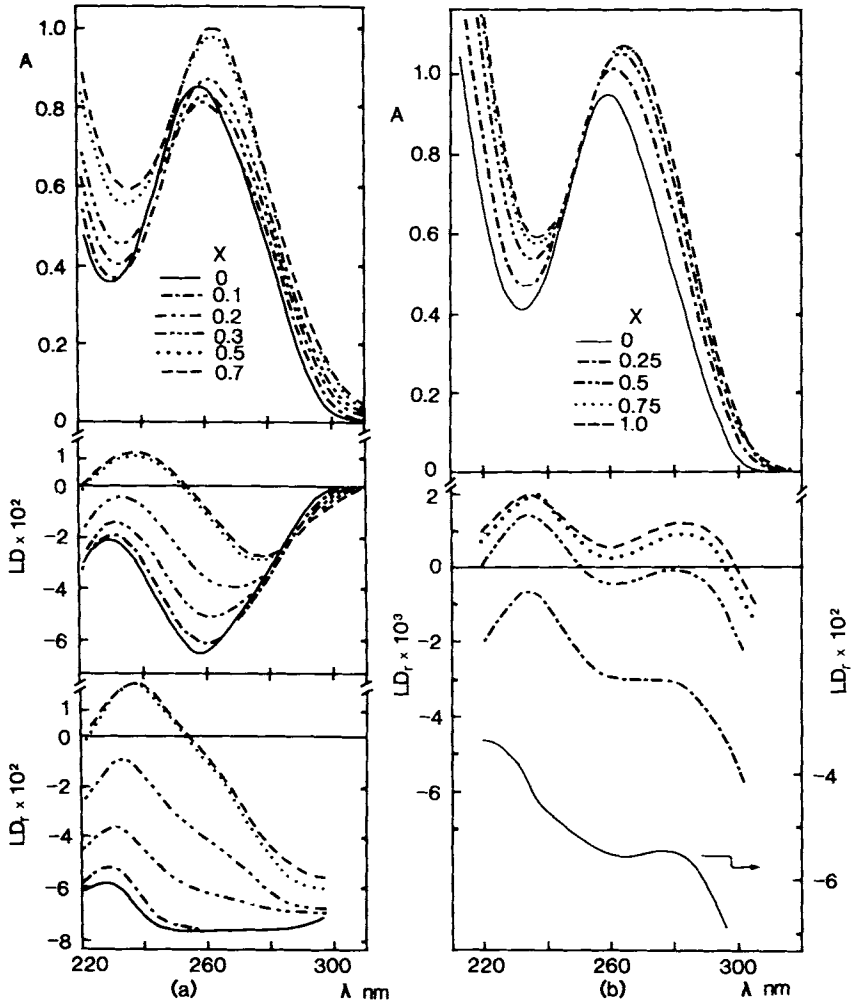

Fig. 1. (a) A, LD, and LD_r of DNA–silver(I) ion aqueous solutions (containing 0.040 mol dm^{-3} $NaClO_4$) at pH 7.2. Ratios of $X = [Ag^+]/[P]$ where P stands for DNA–phosphorus are indicated. Flow gradient $G = 1980\ s^{-1}$. (b) A and LD_r of DNA–silver(I) ion ethanolic solutions (containing 6×10^{-4} mol dm^{-3} $NaClO_4$); the values of X are indicated. Flow gradient $G = 150\ s^{-1}$. Temperature = 8°C.

CD in Solution

Figure 2(a) shows the CD of DNA–silver(I) solutions at various values of X. It may be seen that the spectrum characteristic of the B-form of DNA in the presence of silver ions undergoes a very significant change upon the addition of silver, as has already been reported by Walter and Luck.[29]

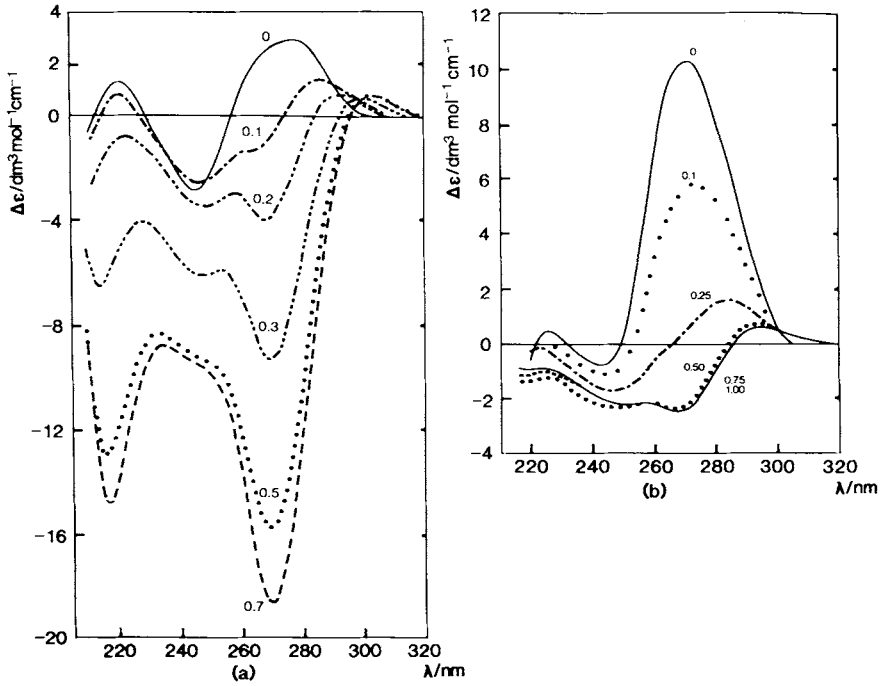

Fig. 2. (a) CD of DNA–silver(I) ion aqueous solutions at values of X indicated. (b) CD of DNA–silver(I) ion ethanolic solutions at values of X indicated.

Figure 2(b) shows that the characteristic CD of the A-form of DNA in the absence of silver ions in 80% ethanol solution changes upon addition of silver into a CD spectrum of much reduced magnitude and of negative sign below about 280 nm.

LD in Stretched Films

Figure 3 shows the isotropic absorbances and the LD_r of DNA in stretched PVA films in the absence and in the presence of silver ions at 100% relative humidity. This figure is to be compared with the results shown in Fig. 1(a) referring to the corresponding aqueous solutions. It may be seen that the shapes of the relevant spectra in aqueous solution and in PVA film are essentially identical.

Transition Moments and Component Bands

The calculation of the LD_r of DNA has been described;[22] it requires as input the transition moment direction, and the position, intensity, and shape of the absorption envelope contributed by each relevant electronic transition. The input parameters describing these spectral

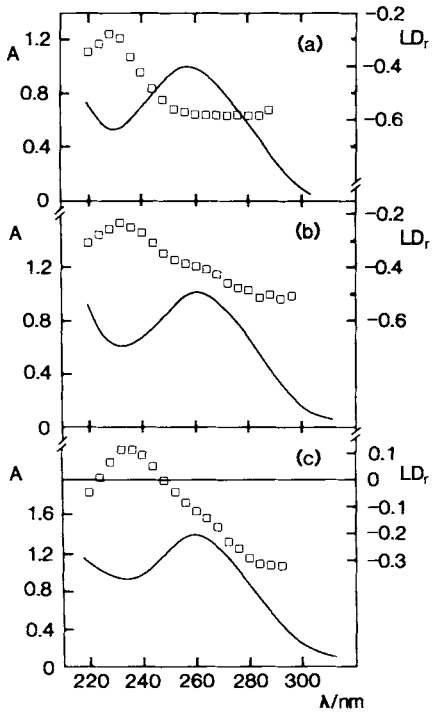

Fig. 3. Isotropic absorbance (solid lines) and reduced LD (□) of DNA–silver(I) ion complexes in PVA matrix of 100% relative humidity and at a stretch ratio of 4.3. (a) No Ag^+ added, (b) $X = 0.25$, (c) $X = 0.5$.

properties will be different for DNA in the presence and in the absence of silver(I) ions, in accordance with the changes observed in solution of the spectra of the bases and their derivatives upon complex formation with silver.[8,9,15–17,28] We note that exciton interactions between the bases that produce significant effects on observed spectra exists only when the bases are stacked.[30] Since Ag(I) forms linear complexes, such stacking of bases is not at all likely to take place and thus exciton effects on the spectra of even polymeric silver complexes of purines and pyrimidines[31] introduce no complications to the interpretation of their absorption spectra.

There is insufficient information for detailed models of the binding of silver(I) ions to DNA and polynucleotides but the evidence is increasing in the case of purines in neutral solution in favor of the involvement of both the N1 and N7 positions in the complexing.[10,32,33] Our results on the effect of silver(I) ions in solutions of guanosine[15] and adenine[16] are consistent with these conclusions. In Fig. 4(a, b) we show the absorption spectra above 220 nm of the adenine–silver and guanosine–silver 1:1 complexes, and their decomposition into Gaus-

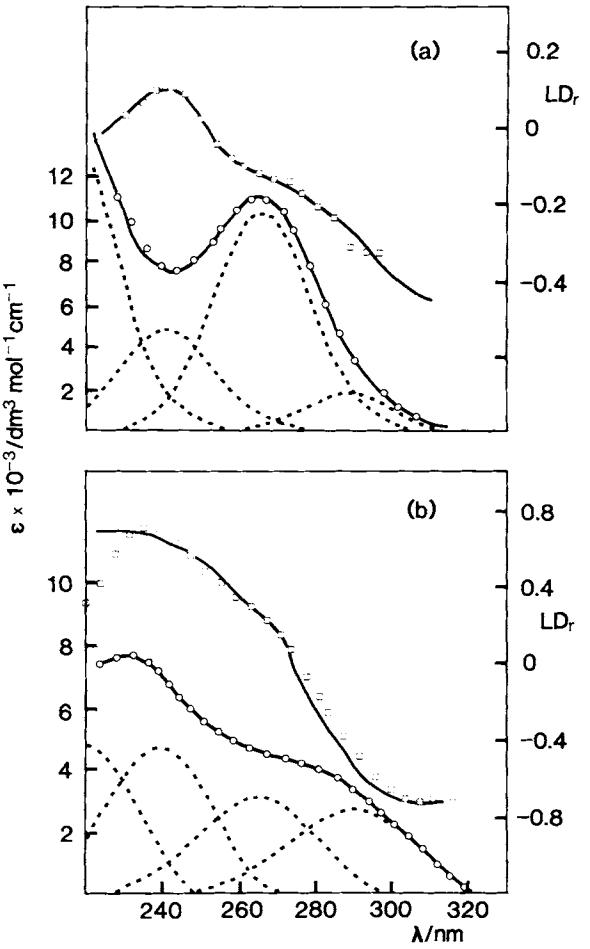

Fig. 4. Isotropic absorbance (○) and LD_r (□) in stretched PVA matrix at a stretch ratio of 4.3. Broken lines refer to the absorption envelopes of individual transitions; solid lines are calculated spectra based on parameters listed in Table I. (a) adenine–silver(I), (b) guanosine–silver(I).

sian bands, each of which is described by

$$\varepsilon_i(\lambda) = \varepsilon_{i,\max} \exp\{-(\lambda - \lambda_{i,\max})^2/\Delta_i^2\} \quad (1)$$

where for each transition i, $\varepsilon_{i,\max}$ is the maximum intensity, $\lambda_{i,\max}$ is the corresponding wavelength, and $\Delta_i^2 = b_i^2/ln\ 2$ where $2b_i$ is the bandwidth at $\varepsilon_{i,\max}/2$. We note that the spectrum of the guanosine–silver complex was previously described in terms of harmonic *progressions*. However, a single Gaussian band on the wavelength scale approximates a harmonic progression on the wave-number scale; an approximation that reduces the number of fitting parameters and that was considered to be satisfactory for our purposes in this instance.

Also shown in these figures are the LD_r of the silver complexes in stretched PVA films and the fitting of these curves to the formula[22]

$$LD_r(\lambda) = \sum \varepsilon_i(\lambda)(LD_r)_i / \sum \varepsilon_i(\lambda) \quad (2)$$

where $\varepsilon_i(\lambda)$ has been defined above and $(LD_r)_i$ is the value of the reduced dichroism of each *pure* transition i. The parameters arising from the fit of Eqs. (1) and (2) are shown in Table I.

As shown in the Appendix the $(LD_r)_i$ values may be related to transition-moment directions. The transition moments for these (π^*,π) transitions are in the plane of the bases and the directions are specified by the angle ξ_i, also shown in Table I; ξ_i are the angles measured counterclockwise relative to the N3-C6 lines for purines and the N1-C4 lines for pyrimidine.[34]

The corresponding parameters for the pyrimidines shown in Table I have been obtained previously.[17]

LD_r of DNA–Silver Complexes

The contribution to the LD_r of each transition i in the absence of the others, $(LD_r)_i$, is given by[22]

$$(LD_r)_i = 1.5\, S \{3(-\sin \delta_i \sin \theta_X + \cos \delta_i \cos \theta_X \sin \theta_Y)^2 - 1\} \quad (3a)$$

TABLE I
Characteristic Parameters of the Transitions in DNA Bases in the Presence of Silver(I) Ions

		$\lambda_{i,max}$ (nm)	$\varepsilon_{i,max}$ ($dm^3mol^{-1}cm^{-1}$)	Δ_i (nm)	$(LD_r)_i$	ξ_i (°)
Adenine–silver	I	290	1700	16	−0.45	−30 ± 15
	II	268	10200	18	−0.13	5 ± 9
	III	242	4500	18	0.22	−90 ± 20
Guanosine–silver	I	291	2800	23	−0.72	−14 ± 20
	II	265	3200	22	0.40	25 ± 20
	III	239	4700	19	+0.72	−74 ± 20
Thymine–silver	I	294	1700	22	0.40	−90 ± 25
	II	269	6000	20	0.22	−67 ± 10
	III	242	2600	18	0.05	11 ± 6
Cytosine–silver	I	295	2200	19	−0.07	−44 ± 14
	II	274	5600	20	0.39	27 ± 25
	III	241	4500	20	0.11	5 ± 15
7-Methylguanine–silver	I	289	6000	20	0.45	9 ± 12
	II	249	6300	28	0.18	−79 ± 12
(π^*, n)		233	250	13	—	—

for transitions in the plane of the DNA bases and by[21]

$$(LD_r)_i = 1.5\ S\ (3\cos^2\theta_X \cos^2\theta_Y - 1) \tag{3b}$$

for transitions perpendicular to the bases.

The (X,Y,Z) coordinate system implied by these equations may be defined in terms of a DNA double helix with its axis in the Z-direction and the base pairs in the XY plane; the X-direction corresponds to the pseudo-dyad axis of the base pairs and Y, perpendicular to Z and X, is in the plane of the base pairs.[22] If the base pairs are not in the XY plane their relative geometry is determined by rotations about the X and Y axes, through the angles θ_X and θ_Y. The rotation θ_X has been referred to as a tilt[22,35] but θ_Y has been called both a twist[22] and a roll.[35] Of the remaining symbols, S is an orientation factor and the δ_i is the transition-moment direction relative to the X-axis, given by 198° + ξ_i for purines and 219° + ξ_i for pyrimidines.[22]

The reduced dichroism as a function of wavelength is then given by an equation similar to Eq. (2)

$$LD_r(\lambda) = \sum A_i(\lambda)(LD_r)_i / \sum A_i(\lambda) \tag{4}$$

with

$$A_i(\lambda) = \varepsilon_i(\lambda) \cdot F_i \tag{5}$$

where F_i is the fractional base content of the DNA type studied; for calf thymus DNA this is 0.29 for adenine and thymine, and 0.21 for guanine and cystosine. Equation (4) together with Eq. (3) may then be used to describe the experimental LD_r as a function of wavelength with three fitting parameters, S, θ_X, and θ_Y for each curve. In Fig. 5(a) we show the fit of the "saturation" LD_r curve of the B-form of DNA in the presence of silver(I) ions and the values of the best-fit parameters are given in Table II. Also included in the figure is the corresponding absorption spectrum generated by the same set of transitions, $\Sigma A_i(\lambda)$.

In Fig. 5(b) we show two separate "best-fit" results for the saturation LD_r curve of the A-form of DNA in the presence of silver(I) ions. One of these employed the same set of input parameters as was used in the fit of the B-form; the second, much improved fit, involved the replacement of the parameters of the guanosine–silver spectrum by those of the 7-methylguanine-silver spectrum[15] listed in Table I. The relevant fitting parameters are shown in Table II.

We note that in conformity with previous calculations of the reduced dichroism of DNA solutions we included here an (π^*,n) transition[22] perpendicular to the plane of the purine and pyrimidine bases, centered at 233 nm, as shown by the last entry in Table I; omission of such a transition does not significantly affect the results of the calculations in these instances.

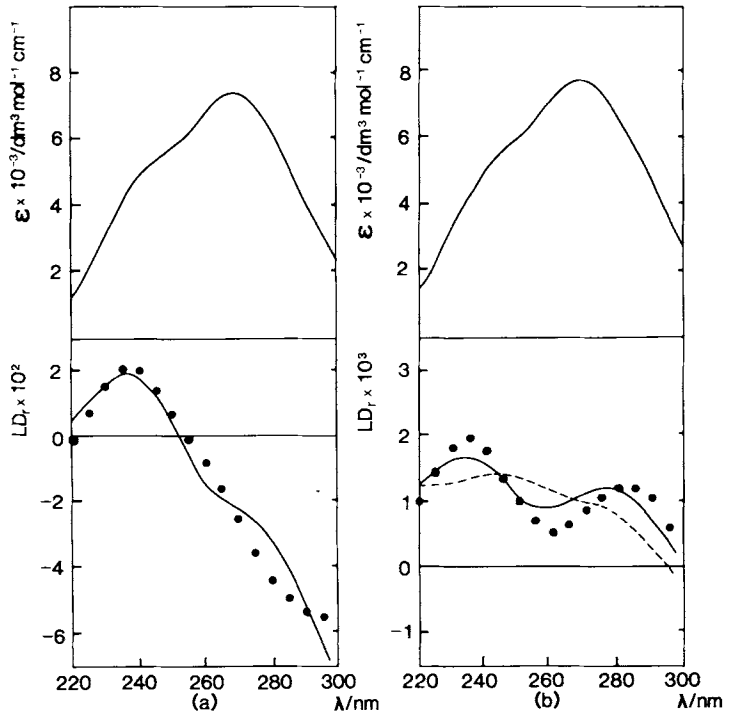

Fig. 5. Simulated LD_r and A of DNA–silver(I) solutions shown by solid lines. Experimental data are represented by circles. (a) Aqueous solution; (b) 80% ethanolic solution, broken line: input spectra as for aqueous solution, solid line: guanosine–Ag^+ spectrum replaced by 7-methylguanine–silver spectrum.

DISCUSSION

B-DNA

Comparison of Fig. 3 with Fig. 1(a) shows that there is no significant difference between the LD_r spectra of aqueous solutions and of stretched PVA films. Incorporated in a PVA matrix, the movement and reorien-

TABLE II
Parameters of Fit of LD_r Spectra

	θ_X	θ_Y	S
B-DNA	−2.1[a]	4.0[a]	0.052
B-DNA/Ag^+	47 ± 5	−24 ± 10	0.095 ± 0.009
A-DNA	19.3[a]	−3.2[a]	0.041
A-DNA/Ag^+ (guanosine)	(63)	(−89)	(1.3 ± 0.9) × 10^{-3}
A-DNA/Ag^+ (7-methylguanine)	44 ± 10	−43 ± 10	(5.8 ± 1.9) × 10^{-3}

[a] Ref. 22.

tation of the DNA helix is likely to be very much more restricted than in solution. We thus conclude that in the aqueous solution of B-DNA the spectral changes upon addition of silver reflect alterations in the DNA secondary structure and are unlikely to be the result of an overall change in orientation caused by the formation of aggregates, such as ψ-DNA, for example.[36] A similar conclusion has been arrived at previously from a consideration of the results of flow CD measurements.[32]

Because of the difficulty in unequivocally separating from the observed spectra at relatively low silver(I) concentrations the individual contributions due to "type I" and "type II" complexes,[8,9,28] quantitative examination of the results was restricted to the spectra obtained at high silver to DNA-base ratios. The very minor change of the LD_r spectrum upon addition of small amounts of silver ions suggests that DNA in its type I complex with silver remains in a B-like conformation. The very large increase in the CD signal in solutions of high silver to DNA-base ratios—compared with B-DNA itself, seen in Fig. 2(a)—suggests that the planes of the base pairs must deviate substantially from being approximately normal to the helix axis[32]; this conclusion is quantified by the fit of the corresponding LD_r curve shown in Fig. 5(a) and with the relevant fitting parameters included in Table II. The very different wavelength dependence of the LD_r of aqueous DNA in the absence and in the presence of silver(I) ions is a consequence of differences in both the tilt (θ_X), and the twist or roll (θ_Y), of the base pairs as shown in Table II. At the same time, from a comparison of the corresponding values of *S*, the orientation parameter, one infers that the silver complex is rather more stiff and hence easier to be oriented by the flow lines than B-DNA itself.

The simulated LD_r curve was obtained using the set of transition moments given in Table I. Altering the set of δ_i values for these calculations within the limits of their precision had very little influence on the fitting parameters θ_X, θ_Y, and S in this instance, and we consider, therefore, the confidence limits of these quantities shown in the table as realistic.

Although the present results do not allow firm conclusions to be drawn of a detailed structure of the DNA–silver complex, they provide a basis to speculate about the role of the silver ions and why they have such a drastic effect on the local DNA structure. It is known that humid PVA can keep DNA firmly in duplex form even at otherwise denaturing conditions,[14,27] and similarly, there are indications that the DNA–silver complex can adopt its solution structure in PVA. Also, the persistent hydrodynamic behavior of the DNA–silver complex, compared to B-DNA, strongly favors a rigid duplex DNA structure in an extended (not compact) form. At low binding ratios there is evidence suggesting that guanine is the main target of silver(I) association,[17,20] but no details are known about the silver distribution at higher binding ratios. The apparent saturation limit ($X \sim 0.5$) corresponds to a 1:1 ratio of

silver to base pairs; however, it cannot be excluded that the silver may be unevenly distributed. In such a case our quantitative interpretation, which assumes identical conformations of the bases, has to be reconsidered. Also, our apparent tilt and roll angles, which formally take the base pairs as planar entities, may have to include the possibility that the bases are propeller-twisted or bent relative to each other. It is suggestive to imagine that coordination of silver(I) ions in the interior of DNA makes the base pairs "swell," and their reorientation into a strongly tilted conformation is the consequence of a restricted envelope defined by the double-helical ribophosphate backbones.

A-DNA

Figure 5(b) shows that the fit of the LD_r curve of ethanolic DNA at high silver(I) to DNA-base ratios is not very satisfactory by the use of the same set of spectroscopic parameters that proved to give acceptable results for aqueous solutions. However, as shown by the smooth curve in Fig. 5(b), the replacement of the spectrum of the guanosine–silver complex by that of the 7-methylguanine–silver complex in the calculations results in a very substantially improved simulation of the experimental LD_r spectrum. It is not possible at this stage to decide if the above result is entirely fortuitous or if it indicates that the N7 position of guanine is a binding site for silver in aqueous but not in ethanolic solution.

The parameters of fit for both simulated LD_r curves are shown in Table II. Importantly, both fits resulted in drastically reduced values of S compared with A-DNA; the ability to orient the molecules by shear gradient is severely curtailed upon complexation of silver(I) ions. The implication is that in this instance profound alterations in the tertiary structure of A-DNA take place as a result of complexing with silver with the formation of relatively compact structures not easily oriented by hydrodynamic forces. This conclusion is corroborated by the significant decrease of the shear dependence of the reduced dichroism of A-DNA solution upon addition of silver referred to before. In the absence of a structural model for the complex in solution, the fitting angles θ_X and θ_Y shown in Table II cannot be interpreted; in any event, the precision of one pair of these angles due to the very small value of S is extremely low, and for this reason these are shown in brackets in Table II.

CONCLUSIONS

We regard as one of the most important aspects of this work the demonstration of the ease with which LD_r data may lead to important molecular structural information, even in such complex systems as

metal-coordinated DNA in solution. However, precisely because of the complexity of the system, we were not able to arrive at a complete and definitive description and interpretation of the systems under consideration. Thus, for example, in spite of the knowledge acquired from the study of model systems, precise knowledge of the spectra of the bases complexed to silver(I) *in* DNA is lacking; inded, the postulated transitions shown in Table I, when summed, conform to the experimentally found red-shift of the absorption spectrum, but the shape and exact position of the spectrum is not faithfully reproduced.

Under these circumstances it was not considered meaningful to include in the calculations the effect of exciton interactions between the transition moments of the bases, as has been done before in studies of the reduced dichroism of metal-free DNA solutions.[21,22] Incorporation of exciton interactions modifies the results of calculations that neglect such interactions, but it does not significantly change the overall shape and magnitude of calculated curves in this instance. The neglect of exciton interactions in this work is thus consistent with our judgement that we should be content to attempt to reproduce through our calculations the general features of the experimental data rather than to aim at a precise fit to theory.

APPENDIX

The structure of the guanosine–silver complex is twisted, as clearly evidenced by the existence of measurable CD in solution[15]; a similarly twisted conformation of the adenosine–silver complex in solution is highly likely.[16] Nevertheless, for the purposes of this work, we shall make the approximation that the orientation axes of the complexes and the relevant transition moments may be taken to be coplanar and that the following formula may be applied:

$$(LD_r)_i = 3(S_{yy} \sin^2\alpha_i + S_{zz} \cos^2\alpha_i) \qquad (A1)$$

where z is the direction of the orientation axis, y is perpendicular to z in the plane of the complex, α_i the angles between the transition moments and the z-axis, and S_{yy} and S_{zz} are orientation factors.[15,16] The procedure followed will be illustrated *via* the guanosine–silver complex.

The values of S_{yy} and S_{zz} are not known exactly; however, their limits have been established[15]; specifically

$$S_{yy} \leq -0.25 \quad \text{and} \quad S_{zz} \geq 0.5$$

Substitution of these limiting values into Eq. (A1) together with the $(LD_r)_i$ shown in Table I provides the angles shown in column 2 of Table

TABLE AI
Transition Moment Directions in Guanosine–Silver(I) Complex

	$\lvert\alpha_i\rvert$(°)	ξ_i(°)
Transition 1	84	−14 or −26
Transition 2	45	25
Transition 3	36	−74
Orientation axis	0	70

AI. Based on SCFMO calculations,[15] one anticipates transitions labeled 2 and 3 to correlate with the two lowest energy transitions in guanosine that subtend an angle of 69°. The results conform to this expectation since

$$|\alpha_2 \pm \alpha_3| = 81° \text{ or } 9°$$

If we now equally apportion the difference of 12° between the directions of moment for transitions 2 and 3 of the silver complex, then the angles in the next column are obtained, which are now given relative to the C4-C5 bond measured toward the C6 atom. The direction of the orientation axis is also shown in the same column in the same system: it subtends an angle of 50° with the transition moment of the NH_2 stretching vibration, an angle smaller than would have been anticipated from the results of ir LD measurements.[15] The discrepancy may be attributed to the approximations involved in this analysis both with respect to the coplanarity of the transition moments and to their correlation with the transition moments of guanosine; correspondingly, the confidence limits of the angles calculated are relatively large, as shown in Table I. It is not possible to distinguish between the two alternative transition-moment directions for transition 1; calculations using the smaller value gave a marginally better fit and this is the angle transferred to Table I.

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