Volume 13 Number 1 1985 Nucleic Acids Research

The infrared spectrum and structure of the type I complex of silver and DNA

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Received 26 September 1984; Accepted 4 December 1984

ABSTRACT

Infrared spectroscopy was used to study films of the type I complex of Ag^+ and DNA as a function of hydration with the following conclusions. (1) Ag^+ binds to guanine residues but not to cytosine or thymine residues. (2) Cytosine becomes protonated as Ag^+ binds to guanine. (These conclusions confirm previous models.) (3) The type I complex remains in the B family of structures with slight modifications of the sugar-phosphate geometry. (4) This modified B structure remains stable at lower values of hydration for which pure DNA is in the A form. (5) Binding of Ag^+ to PO_2^-, O-P-O or the deoxyribose oxygen is excluded.

INTRODUCTION

Silver(I) ions form three distinct complexes with purified DNA depending on the pH and ratio (r) of Ag^+ bound to nucleotide residues.[1] At lower values of r and pH, cooperative binding yields an association product which has been called the type I complex to distinguish it from types II and III which are formed at higher pH and r values.[1,2] Here we report on IR studies of the type I complex (hereafter referred to as AgDNA-I).

AgDNA-I is of interest since the binding of relatively small amounts of Ag^+ may cause significant modifications of the B structural form of DNA.[3] Such cooperative switching of structures may be involved in gene regulation and may be related to the mechanisms of toxicity of heavy metal ions (e.g., $AgNO_3$ is a carcinogen and decreases the fidelity of DNA transcription[4]). Silver ions have also been used to probe the structure of DNA in filamentous viruses.[5]

Davidson and coworkers used UV spectroscopy, potentiometric titrations and sedimentation in pioneering studies of the silver-DNA complexes and drew the following conclusions.[1] AgDNA-I forms best below pH 6 and is fully formed when r = 1/5 for calf-thymus DNA (which is 42% C + G). No hydrogen ions are released during formation and AgDNA-I retains a double helical configuration. Silver(I) ions bind to the bases. Values of r at saturation for

calf thymus DNA and other DNAs lead these authors to suggest that Ag^+ binds to C-G base pairs. Chelation was proposed as a binding mechanism possibly involving the π electron clouds of guanine and cytosine bases.

Duane et al. extended these methods to synthetic polynucleotides and also measured thermal transitions.[2] They observed that AgDNA-I forms with poly (dC-dG) and saturates at r = 1/4. It was later suggested that for poly(dC-dG), Ag^+ binding is subject to the "nearest neighbor exclusion principle".[3] Duane et al. state that Ag^+ binds to the nitrogen atoms of the bases which produces a change in charge distribution similar to the protonation of cytosine.[2]

Optical detection of magnetic resonance (ODMR) has been used to measure triplet states induced by the binding of Ag^+ to the bases in AgDNA-I.[6] Only signals from guanine were observed which is clear evidence that Ag^+ binds solely to guanine in AgDNA-I.

Circular dichroism anisotrophy has confirmed that Ag^+ binds to the N7 atom of guanine, which is also the site for nonenzymatic methylation.[7] Comparison of the CD spectra for methylated, protonated and complexed DNA shows that Ag^+ binds to N7 of guanine and suggests that a hydrogen ion is transferred from N1 of guanine to N3 of cytosine as AgDNA-I is formed. (This binding scheme was also suggested by Bloomfield et al.[10]). N3 is the normal site of protonation of cytosine in the nucleotide and in the polynucleotide as known from IR spectroscopic studies.[8,9]

Electric dichroism (and its relaxation) have been used to study AgDNA-I but significant differences in the data and interpretations exist.[3,11,12] The relaxation data[3] are however very interesting and suggest cooperative switching between two structures of different lengths (i.e., AgDNA-I as compared with pure DNA).

The large body of past work on AgDNA-I has left several questions unanswered. These include: (1) Does proton transfer accompany the binding of Ag^+ to N7 of guanine? (2) Does the structure of the sugar-phosphate chains in DNA change when Ag^+ binds? (3) Can AgDNA-I have more than one structure? (4) What is the relative stability of such alternative structures?

The infrared absorption spectrum of hydrated films of DNA has been extensively studied.[13,14,15] Absorption bands arise from the vibrations within molecular subgroups and many assignments have been made.[16] The majority of past IR studies used linear dichroism to detect and distinguish between the A and B forms of DNA. However, many IR absorption bands are quite sensitive to changes in hydrogen bonding or to changes in the geometry of the sugar-

phosphate chain which has allowed the frequencies and intensities of several bands in the nonpolarized IR spectrum to be correlated with the A, B and disordered forms of DNA.[17,18,19] These "indicator bands" provide a method for the rapid detection of B and A forms of DNA (or modifications of these forms) without the need to produce highly oriented films of DNA and record spectra with polarized light.

In this paper we present the IR spectra of hydrated films of pure DNA and of AgDNA-I which allow the above questions to be answered.

MATERIALS AND METHODS

Calf thymus DNA was purchased from Sigma Co. (several lot numbers were used) and was deproteinized by shaking with chloroform containing 5% isoamyl alcohol followed by centrifugation and precipitation of the aqueous layer (0.6M NaCl) with ethanol. The precipitate was washed three times in 80% ethanol to remove residual NaCl. Aqueous solutions of this DNA gave no precipitate upon addition of $AgNO_3$.

Films for IR measurement were formed by mixing 0.10 mL of double distilled H_2O with a known mass (ca. 2 mg) of dry DNA on a silver chloride plate. To ensure a homogeneous solution, the sample was placed in an atmosphere of 100% r.h. for 24 to 48 hr. The pH of these solutions was approximately six. A film of pure DNA was obtained by spreading this solution and drying slowly at 75% or 85% r.h. Precautions were taken to avoid orienting the film during spreading and mixing.

To prepare DNA containing silver ions, the required volume of stock solution of $AgNO_3$ was added to the homogeneous, aqueous DNA solution. This was allowed to equilibrate for at least 24 hr at 100% r.h. with occasional stirring to ensure that a homogeneous complex formed throughout the solution. Films were prepared and dried as described above. If these precautions are not taken, crystals of $AgNO_3$ may be obtained. All completed films were examined for homogeneity and lack of crystals with a ten power magnifier or a 30 power microscope as needed.

The AgCl plate with the adhered film was mounted as one window in a hygrostatic cell which exposes the film to an atmosphere of constant r.h.[13,14] A saturated salt solution made with H_2O or D_2O was used to regulate r.h.[13,14] and thereby obtain DNA hydrated with H_2O or D_2O as desired.

After the required equilibration time at a given r.h. value (at least 24 hr), the IR spectrum was recorded for the film in such a way as to minimize the effects of beam heating. The beam was attenuated (ca. 50%) and

short spectral regions were scanned. No significant dehydration or changes in structure were observed under the conditions used (1.5 min in the beam, 20 min to recover).

IR spectra were measured with a Perkin-Elmer model 683 ratio recording spectrophotometer which was purged with dry nitrogen to reduce the interference of atmospheric water vapor. The spectrophotometer was operated at the widest slit option to ensure a maximum signal to noise ratio. The resulting spectral slit width was sufficient to resolve all bands in the DNA spectrum. The "noise filter" was set at four.

RESULTS

Infrared spectra were recorded as a function of hydration for films of pure DNA and of DNA containing one Ag^+ per five nucleotide residues (r = 0.2). For calf-thymus DNA, this is equivalent to one Ag^+ per guanine residue and corresponds to the fully formed AgDNA-I found in aqueous solutions.

Alternative hydration with H_2O and D_2O was used primarily to avoid spectral interference from the 1640 cm^{-1} band of adsorbed H_2O and the 1220 cm^{-1} band of adsorbed D_2O. The approximate level of hydration is indicated by the relative humidity (r.h.) of the ambient atmosphere. Hydration was checked spectroscopically by measuring the absorbance of the band near 3400 cm^{-1} (2500 cm^{-1}) due to adsorbed H_2O (D_2O). The addition of $AgNO_3$ to pure DNA (at r = 0.2) did not significantly change the absorption isotherm previously measured for pure DNA.[14]

Comparison of the IR spectra for highly hydrated DNA and AgDNA-I (Figure 1 curves A & B) shows clear differences. Changes in the bands between 1600 and 1700 cm^{-1} indicate that the binding of Ag^+ in AgDNA-I alters one or more of the vibrational modes of the bases. Since the vibrations of individual groups within a base are coupled (e.g., C=O and aromatic rings) and the resultant normal modes of vibration are further perturbed by varying strengths of interbase hydrogen bonding, a base by base analysis is difficult for bands above 1600 cm^{-1}. However several conclusions can be drawn from currently accepted assignments.[16] The decrease in relative absorbance near 1690 cm^{-1} for hydration with D_2O (and especially at 1714 cm^{-1} in DNA hydrated with H_2O) suggests that Ag^+ binds to guanine, thymine or to both. This is more clearly seen for curves C and D which were recorded for films at 75% r.h. The decrease in absorbance near 1646 cm^{-1} indicates that cytosine residues are perturbed in AgDNA-I.

Of particular interest is the weak but clear band near 1502 cm^{-1} in pure

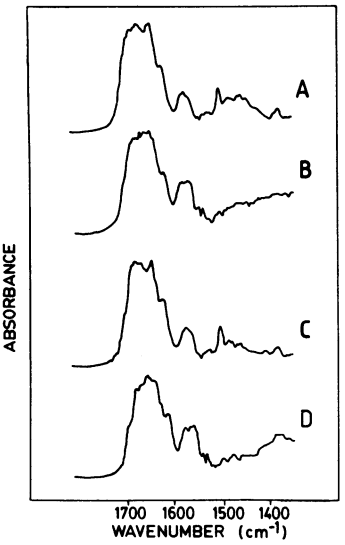


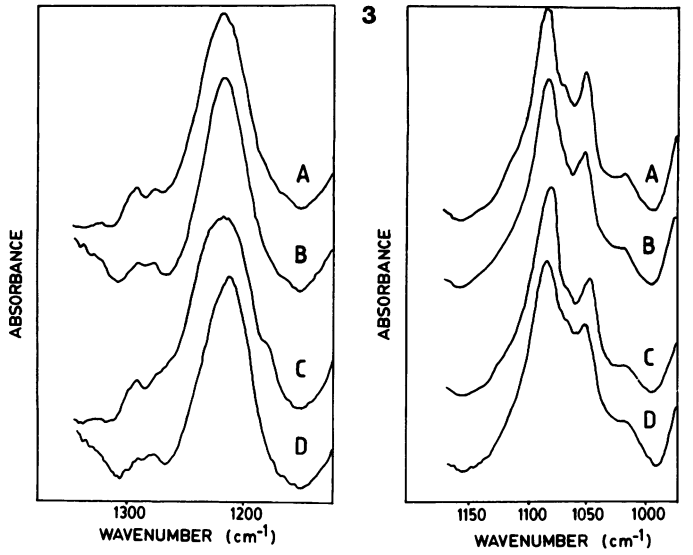
Figure 1. IR spectra of AgDNA-I and pure DNA hydrated with D_2O showing carbonyl and ring stretching vibrations. A, pure DNA, 94% r.h. B, AgDNA-I, 94% r.h. C, pure DNA, 75% r.h. D, AgDNA 75% r.h.

DNA (Figure 1 curves A and C) which is due to a ring mode of the neutral cytosine residues. This band essentially disappears in AgDNA-I (Figure 1 curves B & D) which strongly suggests that the cytosine residues are protonated at N3 in AgDNA-I.[8,9] The disappearance of the 1502 cm^{-1} band upon protonation of N3 of cytidine[8] and polycytidylic acid[9] is well known. The direct binding of Ag^+ to cytosine residues can be ruled out since this would produce a band near 1535 cm^{-1} which is not observed.[20] Therefore we interpret the absence of bands near 1535 and 1502 cm^{-1} in AgDNA-I as conclusive evidence for the transfer of a proton from N1 of guanine to N3 of cytosine upon complex formation as previously proposed.[7,10]

Similarly, if we assume that the binding of Ag^+ to thymidine produces the same spectral changes as occur in uridine, the lack of a new band near 1545 cm^{-1} rules out the binding of Ag^+ to thymine residues in AgDNA-I.[20]

New bands which are less easily interpreted occur near 1584 and 1545 cm^{-1} in the spectra of AgDNA-I at both hydration levels. These are on either side of the distinct band in pure DNA (ca. 1570 cm^{-1}).

The region from 1350 to 1150 cm^{-1} contains the strong band due to the antisymmetric PO_2^- stretching mode near 1220 cm^{-1} in addition to several weaker bands which indicate the A or B families or DNA structures (see Table 1). The spectra of pure DNA and AgDNA-I (both at 94% r.h.) are very similar in this region (Figure 2 curves A and B). This shows directly that Ag^+ does not bind to the PO_2^- portion of the phosphodiester groups (i.e.,



Figures 2 and 3. IR spectra of AgDNA-I and pure DNA hydrated with H_2O showing bands from the sugar-phosphate groups. Curves are labeled as in Figure 1.

the frequency and extinction coefficient of the antisymmetric stretching band are the same for both samples). Furthermore AgDNA-I remains in the B family of DNA structures (i.e., no bands indicating the A structural form appear and the band at 1220 cm^{-1} does not shift to higher frequencies).

These conclusions are reinforced by comparing the spectra measured at 75% r.h. Pure DNA (Figure 2 curve C) shows increased absorbance near 1275 cm^{-1} and a distinct shoulder near 1185 cm^{-1} which indicate that much of the DNA in the film has assumed the A form. AgDNA-I at 75% r.h. (curve D) gives neither of these bands and has the same spectrum at 75% and 94% r.h. This demonstrates that AgDNA-I remains in the B family of structures at levels of hydration which change pure DNA to the A form.

These conclusions are confirmed and extended by consideration of Figure 3. The strong band near 1088 cm^{-1}, due to the symmetric stretch of PO_2^-, is unpreturbed in AgDNA-I which further rules out participation of this most negatively charged portion of the phosphate group in binding Ag^+. However, the shoulder near 1070 cm^{-1} in curve A, is reduced in curve B which suggests some minor changes in the ribose-phosphate geometry. The relative constancy of other bands in this region (which arise from the ribose group) suggest that direct binding of Ag^+ to the ribose oxygen atom does not occur.

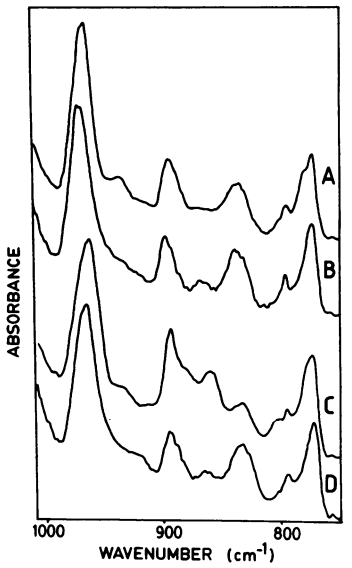


Figure 4. IR spectra of AgDNA-I and pure DNA hydrated with D_2O showing other conformationally sensitive bands. Curves are labeled as in Figure 1.

The region below 1000 cm^{-1} contains several important diagnostic bands and one strong band (at 968 cm^{-1}) which is little changed by the B to A structural transition. The 968 cm^{-1} band, which is due to a stretching vibration of the P-O single bonds, is similar in frequency and intensity for all spectra in Figure 4 which again confirms the lack of involvement of the phosphate group in the binding of Ag^+. Pure DNA at 94% r.h. (curve A) shows a clear band at 835 cm^{-1} and no bands at 860 or 880 cm^{-1} which is the pattern for pure B-family structures (Table 1). AgDNA-I at 94% r.h. gives a very similar spectrum (Figure 4, curve B) although a small band is observed

TABLE 1
Indicator bands for the A and B forms of DNA

B DNA	A DNA
1280 w	1275 m,sh
1220 s	1230 s
	1185 m,sh
1070 m,sh	
	885 w,sh
	860 w
835 w	

Bands at 1280, 1275 and 1070 cm^{-1} are proposed in this work and in reference 23. The other bands are from references 17 and 18.

near 860 cm^{-1} and the weak band near 930 cm^{-1} in pure DNA is reduced. We again conclude that highly hydrated AgDNA-I remains almost completely in the B-family of structures but that some change in the sugar phosphate geometry occurs.

Comparing the same region for hydration at 75% r.h. (Figure 4, curves C and D) confirms previous conclusions. Pure DNA shows a major band at 860 and a shoulder at 880 cm^{-1} which indicate A form. However, a weakened band remains at 835 cm^{-1} indicating that some B form still exists in the film of pure DNA when hydrated at 75% r.h. We believe that the incomplete transition from B to A form is due to a lack of equilibrium as has been discussed elsewhere.[14,21]

The spectrum of AgDNA-I between 1000 and 700 cm^{-1} at lower values of hydration (Figure 4, curve D) is quite different from the spectrum of pure DNA (curve C) but is essentially identical to the spectrum of highly hydrated AgDNA-I (curve B). This confirms that complex formation has stabilized the modified B helical form at levels of hydration that would cause the B to A transition in pure DNA. This stabilization persists to 65% r.h.

As the hydration of pure DNA is further reduced by lowering the r.h. below 60%, the A helical structure becomes randomized as base stacking and pairing diminish due to the removal of hydrophobic stabilization.[14,21,22] We therefore further dehydrated AgDNA-I in order to observe the effect of Ag^+ on the transition to the disordered form. The results (spectra not shown here) clearly demonstrate that order decreases with dehydration (below 60% r.h.) but that the cytosine groups remain partially protonated down to 33% r.h. AgDNA-I becomes disordered at lower hydrations without passing through the A form. It is likely that as the bases are displaced from the helical array, the positively charged cytosine residues approach the PO_2^- groups (which bear the remaining hydration). This would provide an electrostatic stabilization for the denatured structure. At very low hydration levels (ca. one or two H_2O per PO_2^-) the absorbance of the 1502 cm^{-1} band of cytosone increases which suggests that some fraction of the silver ions has dissociated from guanine residues

DISCUSSION

The conclusions presented above confirm and extend past work and give stronger evidence for previous speculations on the scheme of binding of Ag^+ in AgDNA-I. Since the binding of a subgroup of DNA (as a ligand) to Ag^+ would strongly perturb the covalent bonding of the group, major changes in

the vibrational spectrum of the ligand would occur. Therefore IR spectra provide clear evidence for both binding and lack of binding to a given group, whereas UV and CD spectra cannot exclude binding to groups not represented by absorption bands (e.g., phosphate and deoxyribose). Furthermore, IR bands from individual bases (and their Ag^+ adducts) may be resolved or partially resolved so that binding (or lack of it) to certain bases may be examined. IR absorption bands are also modified (in frequency and extinction coefficient) by changes in conformation and by hydrogen binding but such changes can be distinguished from those due to complex formation by the magnitude and known characteristics for the structural changes.

An example is the clear band (due to a ring mode) from neutral cytosine residues at 1502 cm^{-1} which is observed in dilute solutions of cytidine and in DNA and is unpreturbed by changes in hydration and structure.[8,13,14,16,20] Complex formation with Ag^+ causes this band to vanish and be replaced by a band near 1535 cm^{-1}. Similarly, binding of Ag^+ to guanine and uracil residues produce changes in ring vibrational modes (and the resulting IR bands) which are much larger than changes due to variations in hydrogen bonding or environment.

The spectra presented here therefore clearly demonstrate that Ag^+ binds to the DNA bases which confirms results from UV spectra.[1,2] This binding was restricted to guanine residues which confirms ODMR and CD results.[6,7] In addition, binding of Ag^+ to the sugar-phosphate chain was excluded.

Two major conclusions from our work deserve some discussion. (1) The spectra conclusively show that cytosine residues are protonated in AgDNA-I as has been hypothesized from less direct evidence.[7,10] (2) For highly hydrated films, the structures of AgDNA-I and pure DNA are quite similar. Both are B-family structures and AgDNA-I has some modifications in the sugar-phosphate geometry. It is important to note that our spectra cannot exclude changes in hydrogen bonding or orientation of the bases of the magnitude discussed by Dattagupta and Crothers[3] since the spectral changes so produced would be overwhelmed by those due to complex formation and protonation. (3) The B-type structure of AgDNA-I is stable at much lower values of hydration than is the case for pure DNA. As hydration is decreased, AgDNA-I never assumes an A-form structure but instead denatures from the B form. The extent to which this behavior is due to thermodynamics (changes in ΔG^o) or to kinetics (changes in ΔG^+) is unclear but it is possible that the binding of Ag^+ to DNA *in vivo* and *in vitro* could lock regions of DNA into the modified B form and thereby interfere with normal transcription, translation and/or regulation.

ACKNOWLEDGEMENT

The authors thank the University of Rhode Island for financial support.

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