Silver(I) Complexes with DNA and RNA Studied by Fourier Transform Infrared Spectroscopy and Capillary Electrophoresis

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ABSTRACT Ag(I) is a strong nucleic acids binder and forms several complexes with DNA such as types I, II, and III. However, the details of the binding mode of silver(I) in the Ag-polynucleotides remains unknown. Therefore, it was of interest to examine the binding of Ag(I) with calf-thymus DNA and bakers yeast RNA in aqueous solutions at pH 7.1–6.6 with constant concentration of DNA or RNA and various concentrations of Ag(I). Fourier transform infrared spectroscopy and capillary electrophoresis were used to analyze the Ag(I) binding mode, the binding constant, and the polynucleotides' structural changes in the Ag-DNA and Ag-RNA complexes. The spectroscopic results showed that in the type I complex formed with DNA, Ag(I) binds to guanine N7 at low cation concentration ($r = 1/80$) and adenine N7 site at higher concentrations ($r = 1/20$ to 1/10), but not to the backbone phosphate group. At $r = 1/2$, type II complexes formed with DNA in which Ag(I) binds to the G-C and A-T base pairs. On the other hand, Ag(I) binds to the guanine N7 atom but not to the adenine and the backbone phosphate group in the Ag-RNA complexes. Although a minor alteration of the sugar-phosphate geometry was observed, DNA remained in the B-family structure, whereas RNA retained its A conformation. Scatchard analysis following capillary electrophoresis showed two binding sites for the Ag-DNA complexes with $K_1 = 8.3 \times 10^4\ M^{-1}$ for the guanine and $K_2 = 1.5 \times 10^4\ M^{-1}$ for the adenine bases. On the other hand, Ag-RNA adducts showed one binding site with $K = 1.5 \times 10^5\ M^{-1}$ for the guanine bases.

INTRODUCTION

Recently, the antibacterial properties of the Ag(I) salts have been reviewed (Guo and Sadler, 1999). Some of the Ag(I)-containing DNA complexes exhibit antibacterial activity (Kitamura et al., 1997; Yamada et al., 1999). Extensive studies on the interaction of silver(I) ion with nucleic acids have been reported (Jensen and Davidson, 1966; Eichhorn et al., 1967; Dove and Davidson, 1962; Yamane and Davidson, 1962a,b; Daune et al., 1966; Izatt et al., 1971; Arya and Yang, 1975; Shin and Eichhorn, 1980; Dattagupta and Crothers, 1981; DiRico et al., 1985).

It has been shown that the Ag(I) binding is accompanied by UV absorption changes and the liberation of proton at certain binding ratios. Spectrophotometric and potentiometric titration showed that at least three modes of binding could be distinguished in the case of Ag-DNA complexes (Izatt et al., 1971). At lower ratios of Ag/nucleotide ($r < 0.2$), type I complex is formed with the guanine N7 atom as a major binding site, whereas at $r = 0.2–0.5$, type II complexes are formed with A-T or G-C base pairs as main binding targets accompanied by proton liberation. Finally, at $r > 0.5$, type III occurs when the major binding sites in the type I and type II complexes are saturated. It has been reported that the silver-DNA complexation induced the 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 (Dattagupta and Crothers, 1981). When the type II complex was formed, the flat base pairs back again to propeller base pairs. However, Ag(I) showed one major binding site in the Ag-RNA complexes (Arya and Yang, 1975).

UV spectroscopy, potentiometric titration, and sedimentation studies in the pioneer work of Jensen and Davidson (1966) showed that Ag binding in the type I complex occurs at G-C base pairs. Using poly-dGC and poly-dAT oligonucleotides, it was suggested that a second complex with DNA is formed in which Ag(I) binds to A-T base pairs following the first complex formation, in which Ag(I) binds to G-C base pairs during the type I complexation (Daune et al., 1966). However, the binding modes of silver ion in the Ag-DNA adducts are different from other metal-polynucleotide complexes (Duguid et al., 1993, 1995; Loprete and Hartman, 1993; Tajmir-Riahi et al., 1993; Alex and Dupuis, 1989; Keller and Hartman, 1986a,b; DiRico et al., 1985; Dattagupta and Crothers, 1981). Transition and nontransition metal ions bind DNA through the backbone phosphate group at low cation concentration, whereas base binding occurs as metal ion concentration increases (Langlais et al., 1990; Tajmir-Riahi et al., 1993). The Ag(I) shows strong interaction for base binding with no affinity toward the backbone phosphate group at low or high cation concentration (DiRico et al., 1985; Dattagupta and Crothers, 1981).

Based on these reports, it can be speculated that type I complexation with DNA involves not only G-C base pairs but also A-T bases. On the other hand, IR spectroscopic study of the type I complex formed from mixture containing Ag(I) and calf-thymus DNA at a molar ratio of 1/5 revealed that Ag binds to guanine but not to adenine, thymine,

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cytosine, and the backbone phosphate groups (DiRico et al., 1985). Thus, it remains unclear whether or not Ag binds the A-T base pairs in the type I complexes and what is the mode of Ag binding in type II complexation. Therefore, it was of interest to address the above questions by examining the Ag binding mode to DNA and RNA, using Fourier transform infrared (FTIR) spectroscopy and capillary electrophoresis.

We now report the FTIR spectroscopic and capillary electrophoresis results on the Ag(I) complexes with calf-thymus DNA and bakers yeast RNA, using various cation/polynucleotide (phosphate) ratios from 1/80 to 1/2 (for infrared measurements) and 1/640 to 1/4 (for capillary electrophoresis) with a final DNA or RNA concentration of 12.5 mM for infrared and 1.25 mM for capillary electrophoresis. Structural characterizations regarding Ag-binding sites, apparent binding constant, and the effects of cation complexation on the biopolymer secondary structure are provided here.

MATERIALS AND METHODS

Materials

Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical Co. (St. Louis, MO) and was deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. Bakers yeast RNA sodium salt was purchased from Sigma and used as supplied. $AgNO_3$ and $AgClO_4$ salts were from Aldrich Chemical Co. (Milwaukee, WI). Other chemicals were of reagent grade.

Preparation of stock solutions

Sodium-DNA or sodium-RNA were dissolved to 1% w/w (25 mM DNA (phosphate)) in 50 mM $NaClO_4$ and 1 mM sodium cacodylate (pH 7.1) at 5°C (we avoided using NaCl solution for polynucleotide preparation, due to a strong Ag-Cl interaction) for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The solutions of $AgNO_3$ were prepared in distilled water and added drop-wise to the polynucleotide solutions to attain the desired Ag/polynucleotide(P) molar ratios of 1:640, 1:320, 1:160, 1:80, 1:40, 1:20, 1:10, 1:8, and 1:4 (for capillary electrophoresis) and 1:80 to 1:2 (for infrared measurements) at a final DNA(phosphate) concentration of 12.5 mM for infrared and 1.25 mM for capillary electrophoresis. The pH values of stock solutions were between 7.1 and 6.6. The infrared spectra and the capillary electrophoresis of Ag-DNA and Ag-RNA complexes were recorded after incubation of the mixtures of polynucleotide and silver solutions for 2 h.

FTIR spectra

Infrared spectra were recorded on a Bomem DA3–0.02 FTIR spectrometer equipped with a nitrogen-cooled HgCdTe detector and KBr beam splitter. The solution spectra were taken using specially designed AgBr cells with path lengths of 50 μm and resolution of 2–4 cm^{-1} and 100–500 scans. Each set of infrared spectra was taken (three times) on three identical samples with the same polynucleotides and metal ion concentrations. The water subtraction was carried out with 50 mM $NaClO_4$ solution used as a reference at pH 6.5–7.1 (Alex and Dupuis, 1989). A good subtraction was achieved as shown by a flat baseline around 2200 cm^{-1} where the water combination mode is located. This method is a rough estimate but removes the water content in a satisfactory way. The spectra were smoothed with the Savitzy-Golay procedure (Alex and Dupuis, 1989). The intensity ratio variations of several DNA and RNA in-plane vibrations related to A-T, A-U, and G-C base pairs as well as the backbone PO_2 stretching were measured (with respect to the reference bands at 968 for DNA and 862 cm^{-1} for RNA) as a function of Ag cation concentrations with an error of $\pm$3%. The vibrations at 968 cm^{-1} (DNA) and 862 cm^{-1} (RNA) are due to the sugar C-C and sugar-phosphate stretching modes and show no spectral changes upon Ag complexation. These intensity ratios were used to determine the Ag bindings to the DNA and RNA bases or the backbone phosphate groups. Similar intensity ratio variations were used to determine the metal ion binding to DNA bases and the backbone phosphate group (Arakawa et al., 2000; Neault and Tajmir-Riahi, 1999).

Capillary electrophoresis

A P/ACE System MDQ (Beckman) with a photodiode array detector was used to determine the Ag-DNA and Ag-RNA binding constants. Uncoated fused silica capillary of 75 μm i.d. and 57 cm length was used. The capillary was initially conditioned by washing with 1 N sodium hydroxide for 30 min, followed by a 15-min wash with 0.1 M sodium hydroxide. Then it was extensively rinsed with deionized water and 20 mM $NaClO_4$ before use. Samples were injected using a voltage injection at 10 kV for 5 s. Electrophoresis was carried out at a voltage of 25 kV for 10 min using normal polarity. All runs were carried out at 25°C in a run buffer of 20 mM $NaClO_4$ (pH 7.1–6.6). The electropherograms were monitored at 260 nm. Stock solutions of Ag (6.25 mM) were prepared in deionized water. The capillary inlet and outlet vials were replenished after every run. The Ag-binding experiments were performed in a sample buffer of 4 mM $NaClO_4$ using constant concentration of polynucleotides and various concentrations of Ag cation. Polynucleotides were dissolved in 8 mM $NaClO_4$ at a polynucleotide phosphate (P) concentration of 2.5 mM. The stock solutions of Ag were added to polynucleotide solutions to attain desired Ag(I)/DNA(P) molar ratios of 1:640, 1:320, 1:160, 1:80, 1:40, 1:20, 1:10, 1:8, and 1:4. Each sample was allowed to equilibrate for 30 min before injection.

Data analysis

The apparent binding constants for the Ag-DNA complexes and Ag-RNA were determined by the capillary electrophoresis using Scatchard analysis (Klotz and Huston, 1971; Klotz, 1982). The average number (R_f) of Ag cation bound per one binding site of the polynucleotides was determined from the change of the peak height due to the presence of the Ag by the equation

$$R_f = (h - h_0)/(h_s - h_0), \quad (1)$$

where h is the change in the peak height measured for any added Ag cation concentration, and h_0 and h_s correspond to the peak heights of the free polynucleotides and Ag saturated polynucleotides, respectively. Using the equation for binding constant:

$$K_b = [\text{Ag-polynucleotides}]/[\text{polynucleotides}][\text{Ag}], \quad (2)$$

the experimental Ag binding constant K_b was then computed by fitting the experimental values of R_f and Ag concentrations to the following equation:

$$R_f = K_b[\text{Ag}]/(1 + K_b[\text{Ag}]) \quad (3)$$

The last equation gives a convenient form for Scatchard analysis:

$$R_f/[\text{Ag}] = K_b - K_b R_f \quad (4)$$

In recent years, capillary electrophoresis has been widely used to determine the binding constants of DNA-protein and metal-protein complexes

(Foulds and Etzkom, 1998; Guszczynski and Copeland, 1998; Xian et al., 1996; Li and Martin, 1998).

RESULTS AND DISCUSSION

Interaction of Ag(I) with DNA studied by FTIR

To examine the interaction of Ag(I) with calf-thymus DNA, the infrared spectra of Ag-DNA adducts with various molar ratios of the cation/DNA (phosphate) were recorded. The spectral changes (intensity and shifting) of several prominent DNA in-plane vibrations at 1717 cm^{-1} (G and T), 1663 cm^{-1} (T, G, A, and C), 1609 cm^{-1} (A and C), 1492 cm^{-1} (C and G), and 1222 cm^{-1} (PO_2 asymmetric stretch) (Alex and Dupuis, 1989; Keller and Hartman, 1986a,b; Spiro, 1987; Loprete and Hartman, 1993; Starikov et al., 1991; Brahms et al., 1974; Prescot et al. 1984; Taillandier et al., 1985) were then monitored (Fig. 1). At a low concentration of Ag ($r = 1/80$), the intensities of the bands at 1717 and 1663 cm^{-1} increased by 20% and 10%, respectively, whereas the bands at 1609, 1492, and 1222 cm^{-1} exhibited no major intensity changes upon Ag-DNA complexation (Fig. 2). The band at 1717 cm^{-1} shifted toward a lower frequency at 1712 cm^{-1}, whereas the bands at 1663, 1609, 1492, and 1222 cm^{-1} did not show such a spectral shifting (Fig. 1). It has been suggested that the IR spectral changes in the region 1700–1500 cm^{-1} are associated with the formation of cation-DNA complexes via the guanine N7 atom (Alex and Dupuis, 1989). It has also been reported that the Ag(I) cation binds preferentially to poly dG-dC rather than poly dA-dT (Daune et al., 1966). Thus, the major spectral changes observed for the guanine band at 1717 cm^{-1} (shifting and intensity variations) are due to the Ag binding to the guanine of the G-C base pairs. It is known that Ag forms at least three different types of complexes with DNA (Izatt et al., 1971). Up to $r = 0.2$, the type I complex is formed with little or no proton release. It was also suggested that in the type I complex Ag is chelated to the N7 and O6 of guanine bases (Jensen and Davidson, 1966). As the result, the guanine N-1 proton is transferred to cytosine N-3 (Bloomfield et al., 1974). This reaction is characterized by the infrared spectral changes of the cytosine band at 1502 cm^{-1} in D_2O solution, which essentially disappears when type I complex formed (DiRico et al., 1985). Our infrared results showed no major spectral changes for the cytosine bands at 1529 and 1492 cm^{-1} in the Ag(I)-DNA complexes formed at low cation concentrations (Figs. 1 and 2, $r = 1/80$ and 1/40). These results suggest that Ag(I) binds only to the guanine N7 rather than being chelated between N7 and O6 of guanine with no proton transfer. Similar spectral changes (intensity increase and downward shifting) were observed for the band at 1717 cm^{-1} where Cr(III) cation was coordinated to the guanine N-7 atom of the G-C base pairs (Arakawa et al., 2000).

At $r = 1/40$, reduction of intensity of the band at 1717 cm^{-1} was observed (Fig. 2). This is due to a minor helix

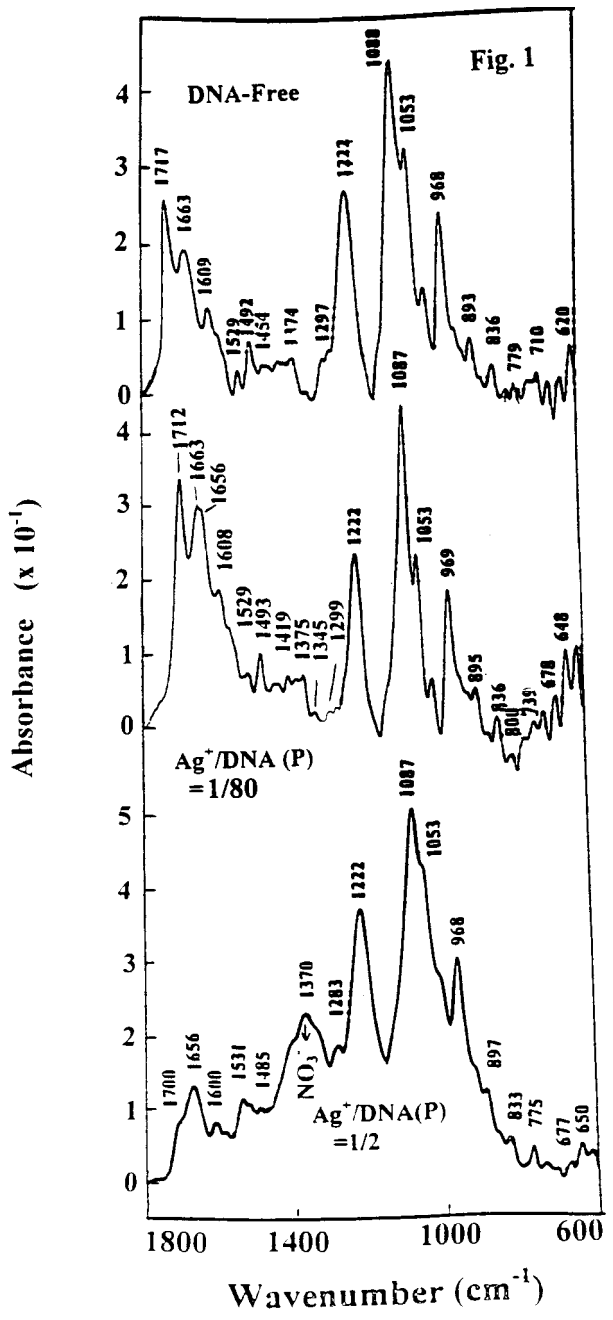

FIGURE 1 FTIR spectra of free DNA and Ag-DNA complexes formed at Ag/DNA(P) ratios of 1:80 and 1:2 in the region of 1800–600 cm^{-1}.

stabilization induced by Ag cation complexation. Similar spectral changes (reduced intensity) were observed when the DNA duplex was stabilized partially by cation complexation (Tajmir-Riahi et al., 1993).

As silver concentration increased ($r = 1/20$ to $r = 1/10$), the intensity of the adenine band at 1609 cm^{-1} increased by 20% and 80%, respectively (relative to free DNA) (Fig. 2), whereas the other bands at 1717, 1663, 1492, and 1222 cm^{-1} showed minor reduction of the intensity upon Ag

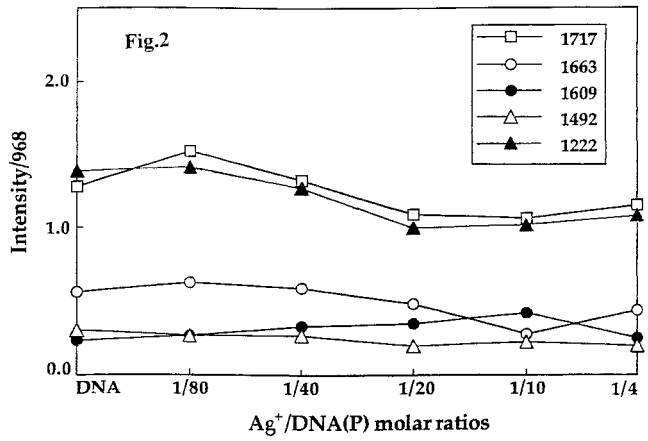

FIGURE 2 Intensity ratio variations for several DNA in-plane vibrations at 1717 cm^{-1} (G and T), 1663 cm^{-1} (T, G, A, and C), 1609 cm^{-1} (A), 1492 cm^{-1} (C), and 1222 cm^{-1} (PO_2 stretch) at Ag/DNA(P) ratios of 1:80, 1:40, 1:20, 1:10, and 1:4.

interaction (Fig. 2). In addition, the adenine band at 1609 cm^{-1} shifted toward a lower frequency at 1600 cm^{-1} (Fig. 1). These spectral changes are due to Ag cation binding to adenine bases at higher concentrations ($r = 1/20$ and 1/10). It has been reported that in the type II complexation, Ag is inter-chelated between adenine N1 and thymine N3 when Ag(I)/DNA phosphate ratios are 0.2–0.5 (Izatt et al., 1971). Our infrared results showed an increase in the intensity of the adenine band at 1608 cm^{-1} when the Ag(I)/DNA phosphate molar ratios are increased to 0.05 and 0.1 (Fig. 2). However, no increase in the intensity of the thymine band at 1663 cm^{-1} was observed at these concentrations (Fig. 2). Thus, the Ag-DNA adduct formed may not be due to the type II complexation, and the Ag cation most likely binds to the adenine N7 atom of the A-T base pairs, which is related to the type I complex formation. These results suggest that the type I binding of Ag-DNA complexation occurs at two steps with the guanine N7 atom as a primary target at low cation content ($r = 1/80$) and the adenine N7 as the secondary binding site at higher Ag concentrations ($r = 1/20$ and 1/10).

At $r = 1/4$, intensity of the adenine band at 1609 cm^{-1} decreased to almost the same level as of the free DNA (Fig. 2), which can be attributed to a partial helix stabilization as a result of cation-adenine complexation. Similar behaviors were observed when Ag-guanine binding occurred (at $r = 1/80$). It is important to note that at $r = 1/4$, the cytosine band at 1529 cm^{-1} lost intensity and shifted toward a higher frequency at 1531 cm^{-1}, whereas the band at 1492 cm^{-1} related to the cytosine and guanine modes lost intensity and shifted toward a lower frequency at 1488 cm^{-1} (spectrum not shown). Similarly, the guanine band at 1717 shifted to 1708 cm^{-1} upon Ag cation interaction. The observed spectral changes can be attributed to the protonation of cytosine N3 (DiRico et al., 1985) and cation chelation through the N7 and O6 group of guanine bases at this concentration.

At $r = 1/2$, major spectral changes occurred for DNA vibrations at 1717, 1663, 1609, 1529, 1492, and 1222 cm^{-1}. The intensity of these vibrations was markedly decreased upon Ag cation interaction (Fig. 2, $r = 1/2$). The bands at 1717 (G), 1663 (T), and 1609 (A) cm^{-1} shifted toward lower frequencies at 1700, 1656, and 1600 cm^{-1}, respectively (Fig. 1, $r = 1/2$). The cytosine band at 1529 cm^{-1} shifted toward a higher frequency at 1531 cm^{-1}, whereas the band at 1492 cm^{-1} (C and G) appears as a weak absorption at 1485 cm^{-1} (Fig. 1, $r = 1/2$). The phosphate band at 1222 cm^{-1} exhibited no shifting in the spectra of Ag-DNA adducts (Fig. 1, $r = 1/2$). The observed spectral changes are due to Ag binding to the G-C and the A-T base. It has been suggested that at the Ag(I)/DNA phosphate ratios of 0.2–0.5, Ag is inter-chelated between guanine N1 and cytosine N3 atoms as well as adenine N1 and thymine N3 atoms to form type II complexes (Izatt et al., 1971). Our infrared spectroscopic results are consistent with the formation of the type II Ag-DNA complexes because the vibrational frequencies related to the guanine, adenine, thymine, and cytosine residues are markedly altered (Fig. 1, $r = 1/2$). However, the asymmetric and symmetric stretching vibrations of the phosphate group at 1222 cm^{-1} and 1088 cm^{-1} exhibited no major spectral changes upon Ag interaction, which is indicative of no participation of the backbone phosphate group in the Ag-DNA complexation (Fig. 1). A strong band at 1370 cm^{-1} is due to the ν_3 of the ionic NO_3^- group (Nakamoto, 1963) in the spectrum of the Ag-DNA adduct and not arising from DNA vibrations (Fig. 1, $r = 1/2$).

The Ag complexation did not alter DNA conformation. It is known that in a B-to-A transition, the B-DNA maker band at 836 cm^{-1} (phosphodiester mode) shifts toward a lower frequency at ~810 cm^{-1} and a new band appears at 885 cm^{-1} and 860 cm^{-1}. Similarly, the band at 1717 cm^{-1} (G) appears at 1700 cm^{-1}, whereas the band at 1222 cm^{-1} shifts toward a higher frequency at 1240 cm^{-1} (Loprete and Hartman, 1993; Taillandier et al., 1985; Tajmir-Riahi et al., 1995). In a B-to-Z conformational change, the sugar-phosphate band at 836 cm^{-1} appears at 800–780 cm^{-1}, and the band at 1717 cm^{-1} displaces to 1690 cm^{-1}, whereas the phosphate band at 1222 cm^{-1} shifts to 1216 cm^{-1} (Loprete and Hartman, 1993; Taillandier et al., 1985; Tajmir-Riahi et al., 1995; Brahms et al., 1974; Neault and Tajmir-Riahi, 1999). Because such spectral changes did not occur for infrared maker bands of Ag-DNA complexes (Fig. 1), the DNA remains the B-conformation in the presence of the Ag cations. However, the shift of the band at 836 cm^{-1} to 833 cm^{-1} and the loss of intensity of the sugar-phosphate band at 1053 cm^{-1} can be related to minor alterations of the sugar-phosphate geometry upon Ag interaction (Fig. 1, $r = 1/2$).

Interaction of Ag(I) with RNA studied by FTIR

The infrared spectra of Ag-RNA adducts with various molar ratios of Ag/RNA(phosphate) were recorded. The spectral

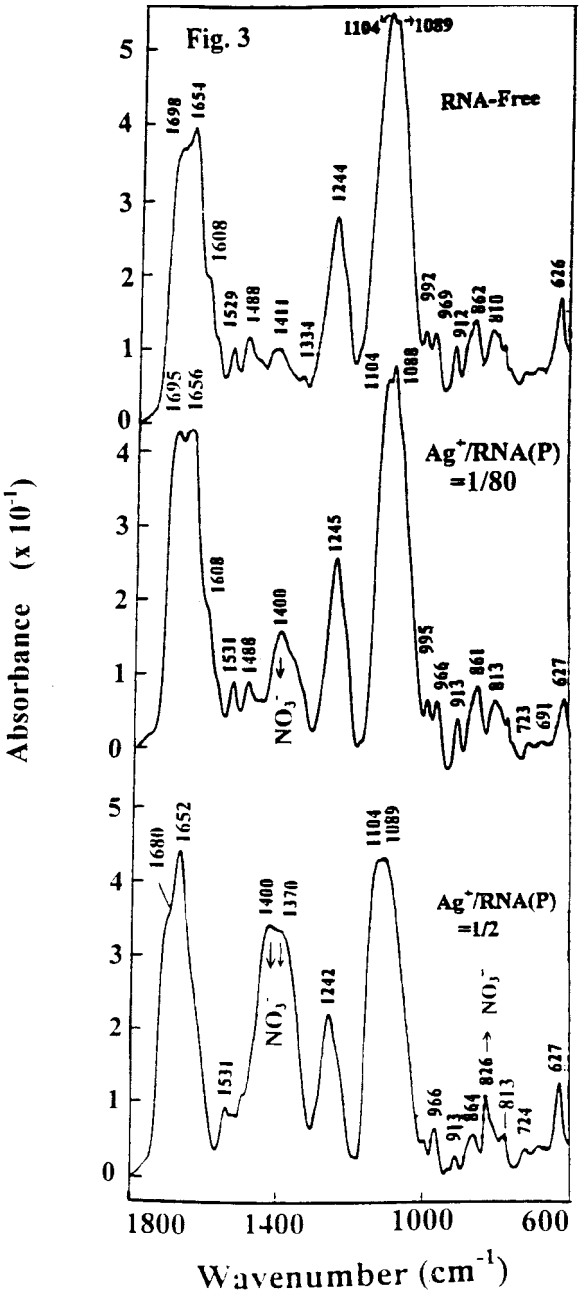

FIGURE 3 FTIR spectra of free RNA and Ag-RNA complexes formed at Ag/RNA(P) ratios of 1:80 and 1:2 in the regions of 1800–600 cm^{-1}.

changes (intensity and shifting) of several RNAs in plane vibrations at 1698 cm^{-1} (G and U), 1654 cm^{-1} (U, G, A, and C), 1608 cm^{-1} (A), 1488 cm^{-1} (C), and 1244 cm^{-1} (PO_2 asymmetric stretch) (Spiro, 1987; Loprete and Hartman, 1993; Starikov et al., 1991; Tsuboi, 1969; Chen and Thomas, 1974; Thomas et al., 1973) were monitored (Fig. 3). At $r = 1/80$, the intensity of the bands at 1698 (G) and 1654 cm^{-1} (U) increased by 20% and 15%, respectively (relative to the free RNA), whereas the bands at 1608 (A), 1488 (C), and 1244 cm^{-1} (PO_2) showed no major intensity

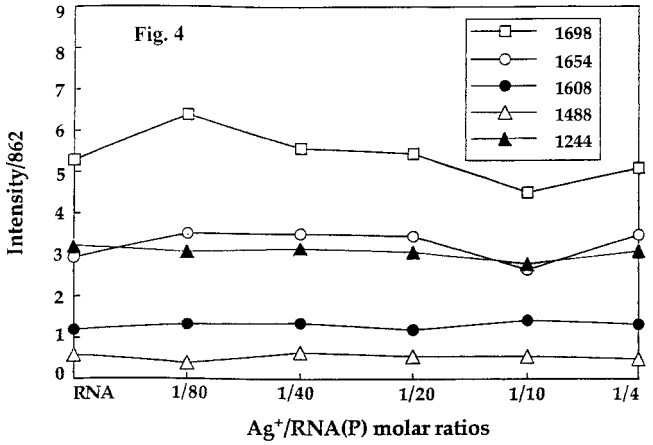

FIGURE 4 Intensity ratio variations for several RNA in-plane vibrations at 1698 cm^{-1} (G and U), 1654 cm^{-1} (U, G, A, and C), 1608 cm^{-1} (A), 1488 cm^{-1} (C), and 1244 cm^{-1} (PO_2 stretch) at Ag/RNA(P) ratios of 1/80, 1/40, 1/20, 1/10, and 1/4.

variations upon Ag cation interaction (Fig. 4). The band at 1698 cm^{-1} (mainly G) shifted toward a lower frequency at 1695 cm^{-1}, but the band at 1654 cm^{-1} (mainly U) exhibited minor upward shifting to 1556 cm^{-1} upon Ag-RNA complexation (Fig. 3, $r = 1/80$). The observed spectral changes for the guanine band at 1698 cm^{-1} (shifting toward a lower frequency with increase in intensity) are due to the Ag binding to guanine bases. The spectral changes for the uridine band at 1654 cm^{-1} (minor upward shifting and intensity variations) are not due to the Ag-uridine interaction. It is also known that Ag does not react with poly-U (Izatt et al., 1971). Because the adenine band at 1609 cm^{-1} and the phosphate band at 1244 cm^{-1} showed no spectral changes (Figs. 3 and 4), the Ag cation binding to adenine bases or the backbone PO_2 groups is not included here.

As the cation concentration increased ($r = 1/40$ to 1/10), reduction of the intensity of the band at 1698 cm^{-1} was observed, but the other DNA vibrational frequencies showed no major spectral changes upon silver cation interaction (Fig. 4).

At $r = 1/4$, a minor intensity increase was observed for the guanine at 1698 cm^{-1} and uridine at 1654 cm^{-1}, whereas the bands at 1609 (A), 1488 (C), and 1244 cm^{-1} (PO_2 stretch) showed no intensity variations upon Ag-RNA complexation (Fig. 4). However, at $r = 1/2$ a major shift of the guanine band at 1698 to 1680 cm^{-1} ($\Delta\nu = 18$ cm^{-1}) was observed in the spectra of the Ag-RNA complexes (Fig. 3). The major spectral changes for the guanine band at 1698 cm^{-1} are due to the Ag-DNA interaction via guanine N7 and O6. The adenine band at 1609 cm^{-1} overlapped by the strong uridine vibration at 1652 cm^{-1} (Fig. 3). It should be noted that the two strong bands at 1400 and 1370 cm^{-1} as well as a sharp band with medium intensity at 826 cm^{-1} are due to the ionic NO_3^- vibrational frequencies (Nakamoto, 1963) and not arising from RNA vibrations (Fig. 3, $r = 1/2$).

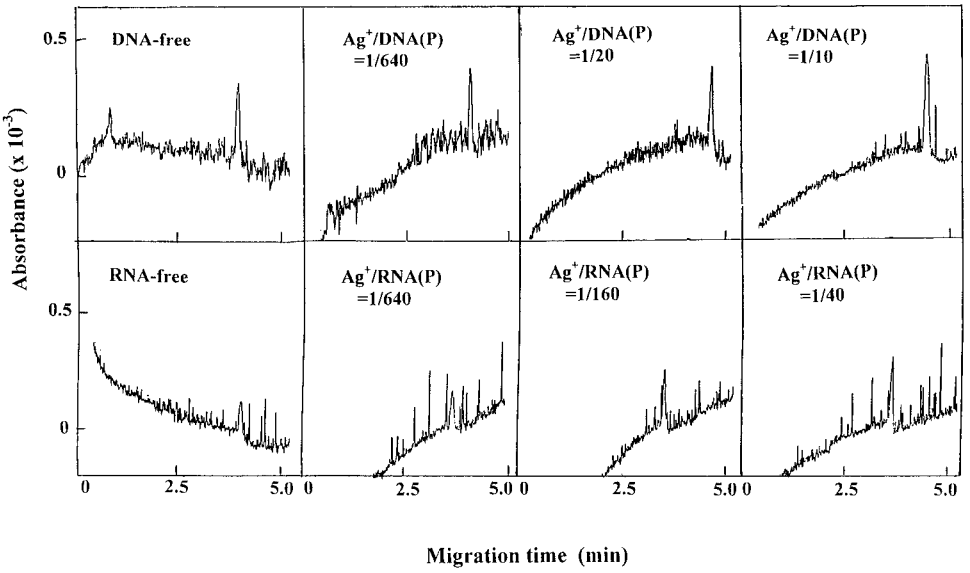

FIGURE 5 Electropherograms showing free DNA (*upper panel*) and mixtures containing Ag/DNA(P) molar ratios of 1:640, 1:20, and 1/10 and free RNA (*lower panel*) and mixtures containing Ag/RNA(P) molar ratios of 1:640, 1:160, and 1:40 at 260 nm. The capillary electrophoresis was performed at 25 kV in a run buffer of 20 mM $NaClO_4$.

Because the marker infrared bands for A-RNA conformation at 810 and 862 cm^{-1} (due to ribose-phosphate vibrations), 1698 cm^{-1} (guanine), and 1244 cm^{-1} (PO_2 stretch) (Taillandier et al., 1985; Brahms et al., 1974; Tajmir-Riahi et al., 1995; Neault and Tajmir-Riahi, 1999) did not show major shifting in the spectra of the Ag-RNA complexes (Fig. 3), RNA remains in the A-family structure in the Ag-RNA adducts.

Interaction of Ag(I) with DNA and RNA studied by capillary electrophoresis

The binding of Ag to DNA and RNA were further studied by capillary electrophoresis. Mixtures containing various concentrations of Ag and a constant concentration of DNA or RNA were subjected to capillary electrophoresis equipped with uncoated silica capillary (75 μm, 57 cm) at 25 kV. The electropherogram was monitored at 260 nm in 20 mM $NaClO_4$ at 25°C. As shown in Fig. 5, the peaks related to the Ag-DNA (*upper panel*) and Ag-RNA (*lower panel*) emerged at the migration times of 4.13–5.30 min. The peak heights of the complexes gradually increased as the molar ratio of Ag/polynucleotides increased. The maximum increase of the peak heights were at the molar ratios of 1/8 for DNA and 1/40 for RNA complexes (Fig. 6). These results indicate that the maximum Ag cation bindings to DNA and RNA occurred at the molar ratios of 1/8 and 1/40, respectively. Furthermore, the Ag-DNA binding occurs at two steps because the increase of the peak height reached a plateau at the Ag/DNA(P) ratios of 1/80 and 1/10 (Fig. 6). Thus, it seems there are two major binding sites for silver cation in the Ag-DNA and one binding for the Ag-RNA adducts. Scatchard analyses were carried out, using the data of R_f (the number of Ag bound per one binding site) and R_f/concentration of Ag cation. Fig. 7 shows the Scatchard plots for the Ag-DNA (Fig. 7 *A*) and Ag-RNA (Fig. 7 *B*). The slope for the Ag-DNA adduct is biphasic, indicating the presence of two distinct cation-binding sites with the apparent binding constants of $K_1 = 8.3 \times 10^4\ M^{-1}$ and $K_2 = 1.5 \times 10^4\ M^{-1}$ (Fig. 7 *A*). This is consistent with our infrared results that showed major spectral changes for the guanine and adenine bases at Ag/polynucleotide(P) molar

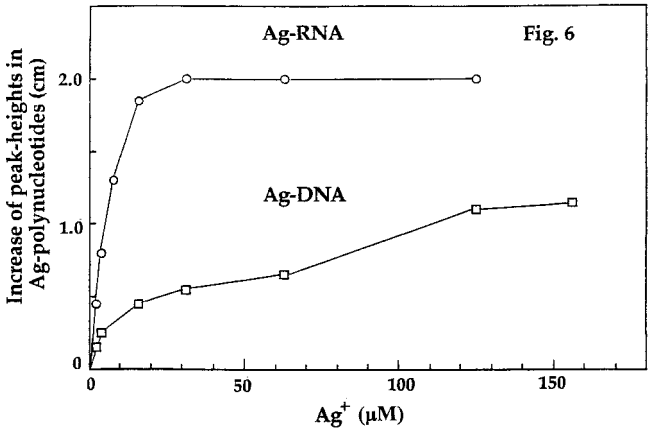

FIGURE 6 Plots for Ag concentrations versus increase of peak heights in Ag-polynucleotide complexes. Incubations of constant concentration of DNA or RNA (1.25 mM phosphate) with various concentrations of Ag were carried out in 4 mM $NaClO_4$, and the mixtures were subjected to capillary electrophoresis. The increase of peak heights in Ag-DNA and Ag-RNA was determined by subtracting the peak height of the free DNA or RNA from each Ag-polynucleotide.

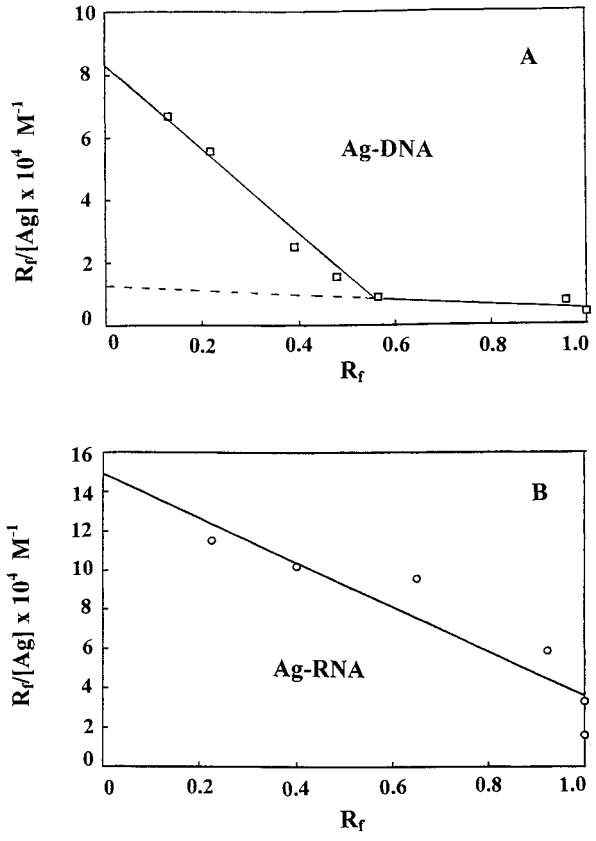

FIGURE 7 Scatchard plots for Ag-DNA (*A*) and Ag-RNA (*B*).

ratios of 1/80 and 1/10, respectively. Therefore, it can be suggested that guanine is the stronger binding site, whereas adenine acts as a weaker target in the Ag-DNA complexation. It should be noted that the binding constants for the type II Ag-DNA complexes could not be calculated because a decrease in the peak height of the Ag-DNA complex was observed ($r = 1/4$) as a result of Ag-DNA precipitation. On the other hand, the slope for the Ag-RNA was monophasic with the apparent binding constant of $K = 1.5 \times 10^5\ M^{-1}$, due to the Ag-guanine binding (Fig. 7 *B*). The Ag-binding affinity for the RNA is approximately two times higher than that of DNA. This is probably attributed to the fact that RNA has mainly single-stranded structure, and the guanine N-1 proton can readily be transferred to the O6 atom to cause keto and enol tautomerism (Muller et al., 2000). As a result, Ag may be easily transferred to the C6OH, substituting the proton from the enol group, and therefore, the additional stability of the Ag-RNA over Ag-DNA adducts can be related to the cation chelation via guanine N7 and O6 in the Ag-RNA complexes.

SUMMARY

Using FTIR spectroscopy and capillary electrophoresis for the structural characterizations of the Ag-DNA and Ag-RNA complexes in aqueous solution, the following points are important to note: 1) first, in the type I silver-DNA complexes, Ag binds DNA at guanine N7 atom at low cation concentration ($r = 1/80$), whereas at higher metal ion content ($r = 1/10$), Ag-adenine interaction occurs via N7 site; 2) in the type II Ag-DNA complexes ($r = 1/2$), cation binding to both G-C and A-T base pairs are observed; 3) in the Ag-RNA complexes, silver ion binds to guanine N7 and O6; 4) Ag-phosphate interaction is not observed; 5) there are two distinct binding sites in the type I silver-DNA complexes with $K_1 = 8.3 \times 10^4\ M^{-1}$ and $K_2 = 1.5 \times 10^4\ M^{-1}$, due to the guanine and adenine bases, respectively; 6) one binding with $K = 1.5 \times 10^5\ M^{-1}$ is estimated for the Ag-RNA complexes; and 7) no conformational transition occurs for polynucleotides upon Ag complexation with DNA remaining in B-family structure and RNA in A-conformation.

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