Spectroscopic and Photophysical Studies of the Binding Interactions between Copper Phenanthroline Complexes and RNA

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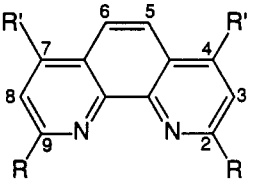
The interactions of two Cu(I) phenanthroline complexes, $Cu(bcp)_2^+$ and $Cu(dmp)_2^+$, where bcp and dmp denote 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline and 2,9-dimethyl-1,10-phenanthroline, respectively, with several different types of RNA have been investigated by means of spectroscopic, photophysical, and viscometric methods. Surface association occurs between $Cu(dmp)_2^+$ and RNA, but binding of $Cu(bcp)_2^+$ with RNA strongly perturbs the spectral properties of the metal complex in ways that depend on the nucleotide-to-copper ratio. Thus, at low ratios, an aggregated form of $Cu(bcp)_2^+$ binds to RNA. At higher ratios, the aggregates break up, but the $Cu(bcp)_2^+$ remains bound to RNA since solvent-induced quenching of the charge-transfer emission from the copper complex continues to be suppressed. Furthermore, a circular dichroism signal can be observed from $Cu(bcp)_2^+$ in the presence of high levels of poly(C)·poly(I). An intimate association with the biopolymer must occur, but viscometric measurements indicate that $Cu(bcp)_2^+$ is not intercalated into the RNA. One possibility is that the local RNA structure melts out to allow internalization of the $Cu(bcp)_2^+$ ion into the macromolecule. Another possibility is that $Cu(bcp)_2^+$ may be sandwiched between separate runs of double-helical RNA structure in solution.

Introduction

The use of metal complexes as probes of DNA structure has proven to be quite fruitful.[1-5] Information obtained from the study of small molecules binding to DNA and RNA is potentially useful in the design of new drugs which can recognize a specific site or conformation of DNA or RNA. Moreover, metal coordination complexes are flexible reagents which present the opportunity to observe the effects the central metal ion, the ligands, and the coordination geometry have on the binding event.

In our laboratory, we have begun studies of copper(I) complexes containing derivatized phenanthroline ligands in order to learn more about binding interactions involving $Cu(phen)_2^+$ (where phen denotes 1,10-phenanthroline). Sigman and co-workers have shown that $Cu(phen)_2^+$ is an efficient artificial nuclease which can be a useful probe of DNA and DNA complexes.[3,6,7] Despite intense study, the exact mode of binding remains unknown. Evidence exists which indicates that $Cu(phen)_2^+$ binds to the surface of DNA within the minor groove in a non-intercalative mode.[3,8,9] However, in studies involving mismatched strands, Williams and co-workers have obtained cleavage results which suggest that $Cu(phen)_2^+$ binds near bulges in the duplex where intercalative binding is thought to be favored.[10] Further, Veal and Rill have interpreted viscometry data to indicate that $Cu(phen)_2^+$ binds to DNA by intercalation.[11]



	R	R'
phen	H	H
dmp	Me	H
bcp	Me	Ph

Figure 1. Phenanthroline ligands and ligand abbreviations.

Studies in our laboratory[12-14] have centered around $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$, where dmp denotes 2,9-dimethyl-1,10-phenanthroline and bcp denotes 2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline, respectively, which are represented in Figure 1. These metal complexes have the same basic structure as $Cu(phen)_2^+$ but are much poorer reducing agents because of the presence of methyl substituents in the 2- and 9-positions of the ligands.[13] As a consequence, they do not combine with oxygen so readily to form hydrogen peroxide and promote oxidative cleavage of DNA. Both complexes bind to DNA as demonstrated by the hypochromism which is observed in the visible spectrum of each compound. A useful property of $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$ is that the charge-transfer (CT) luminescence is a sensitive reporter of the solution environment because the emission is subject to quenching via a type of exciplex formation involving nucleophilic attack by the solvent molecule.[15-18] This process is suppressed

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in a rigid matrix, such as a low-temperature glass,[16] or in a noncoordinating solvent, such as methylene chloride. Interestingly, we have found that the presence of DNA can inhibit the quenching of $Cu(bcp)_2^+$ in aqueous solution. On the other hand, the emission from $Cu(dmp)_2^+$ is virtually completely quenched even in the presence of DNA.[12,14] Some type of internalization, such as intercalation of $Cu(bcp)_2^+$, could explain these results either because of rigidity imposed upon the coordination sphere of the copper center or because of reduced accessibility to solvent. On the other hand, the results obtained with $Cu(dmp)_2^+$ are consistent with exterior, or surface, binding since the complex remains solvent accessible. In any case, the phenyl substituents in the 4- and 7-positions of the phenanthroline ligand clearly have a profound influence on the nature of the adduct that is formed with DNA. Major differences in DNA binding are also observed between $Cu(bap)_2^+$, where bap denotes 4,7-diphenyl-1,10-phenanthroline, and $Cu(phen)_2^+$. Indeed, Sigman and coworkers have proposed that the bap complex may bind by intercalation as opposed to surface association.[3,9]

Previous work has shown that emission can also be observed from $Cu(bcp)_2^+$ in the presence of transfer RNA (tRNA).[14] This suggests that interaction with an A-form double helix may also inhibit solvent-induced quenching. In this report, RNA-binding interactions involving $Cu(dmp)_2^+$ and $Cu(bcp)_2^+$ are examined where the dmp and the bcp complexes are considered to be analogues of $Cu(phen)_2^+$ and $Cu(bap)_2^+$, respectively. Studies involving synthetic, double-stranded RNA and yeast tRNA have been carried out by means of UV–visible, luminescence, and circular dichroism (CD) spectroscopies along with viscometry.

Experimental Section

Materials. Samples of $[Cu(dmp)_2]NO_3 \cdot 2.5H_2O$, $[Cu(bcp)_2]Cl \cdot 2.5H_2O$, and $[Cu(bcp)_2]NO_3 \cdot H_2O$ were synthesized by variations of a previously reported procedure.[19] Trisma base, Trisma·HCl, and type X-S yeast transfer RNA were purchased from Sigma Chemical Co. and used without further purification, as were the random copolymers poly(AU) and poly(CI) where A denotes the adenosine 5′-phosphate, U the uridine 5′-phosphate, C the cytidine 5′-phosphate, and I the inosine 5′-phosphate residues, respectively. The double-stranded RNA polymers poly(A)·poly(U) and poly(C)·poly(I) were purchased from Pharmacia and used without further purification. Fisher brand sodium triphosphate was purified using a literature method.[20] In order to reduce the viscosity and decrease the average chain length of the biopolymers, solutions of poly(AU), poly(CI), and tRNA were syringed through a 0.22-gauge needle and then passed through a Millipore filter (0.22-μm pore size for poly(CI), 0.45 μm for poly(AU) and tRNA). Poly(A)·poly(U) and poly(C)·poly(I) were passed through a Millipore filter with a 0.65-μm pore size. Methanol was purchased either from Fisher or Burdick & Jackson and was of spectroscopic grade. All other chemicals were reagent grade.

Methods. Spectral measurements were made at a copper concentration of 25 μM in 0.025 M pH 7.8 Tris buffer or 0.02 M pH 7.8 triphosphate buffer in combination with methanol at 25 °C.[13] Triphosphate buffer was prepared by adding diphosphoric acid ($H_4P_2O_7$) to a solution of sodium triphosphate ($Na_5P_3O_{10} \cdot 6H_2O$). Samples were typically prepared by adding an aliquot of a buffered RNA solution to a solution of the copper complex in methanol and then diluting with buffer to a final composition of 33% methanol. Concentrations of the nucleic acid solutions were determined from the reported molar absorptivity values at 260 nm: 7700 M^{-1} cm^{-1} for yeast tRNA,[21] 7140 M^{-1} cm^{-1} for poly(A)·poly(U),[22] and 5000 M^{-1} cm^{-1} for poly(C)·poly(I).[23] Molar absorptivity values at 260 nm of 9240 M^{-1} cm^{-1} for poly(AU) and 5000 M^{-1} cm^{-1} for poly(CI) were estimated from absorption data from solutions where the concentration was calculated on the basis of formula weights of the constituent nucleotides. The stoichiometry in a solution was expressed by the RNA-P/Cu ratio, which is the ratio of the RNA phosphate concentration to the copper complex concentration. For the viscometric measurements, the RNA concentration was constant at 0.1 mg/mL while the copper concentration varied in 33% MeOH/0.02 M pH 7.8 triphosphate buffer. Correction factors for the excitation spectra were calculated under the assumption that the excitation spectrum of $[Cu(bcp)_2]BF_4$ dissolved in methylene chloride matched the absorption spectrum of the complex in the same solvent. These same factors satisfactorily corrected the excitation spectrum of $Ru(bipy)_3^{2+}$, where bipy denotes 2,2′-bipyridine.

Table I. Luminescence and Absorption Data for $Cu(bcp)_2^+$ in the Presence of RNA

	RNA-P/Cu = 50			RNA-P/Cu = 100	
RNA	λ_{max}[a] (nm)	% $\Delta\epsilon$[b]	τ[c] (ns)	λ_{max} (nm)	% $\Delta\epsilon$
poly(AU)	477.5	14	89	478.5	3
poly(CI)	477.5	13	81	478.0	3
poly(A)·poly(U)	476.6	4	87		
poly(C)·poly(I)	477.1	8	80	476.1	7
tRNA	476.6	13	77	478.5	1

[a] λ_{max} represents wavelength of maximum absorbance. [b] The decrease in the apparent molar absorptivity at λ_{max} was determined from $(\epsilon_c - \epsilon_b)/\epsilon_c$, where ϵ_c represents the apparent molar absorptivity of free $Cu(bcp)_2^+$ and ϵ_b represents the apparent molar absorptivity of $Cu(bcp)_2^+$ in the presence of RNA. [c] Estimated experimental errors are ±10%.

Instrumentation. Absorption spectra were recorded with a Perkin-Elmer Lambda 4C spectrophotometer. Emission and excitation spectra were recorded with an SLM/Aminco SPF-500C spectrofluorometer with a 520-nm long-wave-pass filter between the sample and the emission monochromator. Emission spectra were recorded with 474- and 454-nm excitation for $Cu(bcp)_2^+$ and $Cu(dmp)_2^+$ solutions, respectively. Luminescence lifetimes were measured with an EG&G Princeton Applied Research Model 2100 N_2-pumped dye laser system as described previously.[24] A combination of four Corning color filters, 2-58, 2-60, 2-61, and 2-64, with cutoff wavelengths from 600 to 660 nm, or a combination of a 520-nm long-wave-pass filter, a 600-nm long-wave-pass filter, and Corning filters 2-58 and 2-64 was placed between the sample compartment and the detector. In each case, at least 5 lifetimes worth of data were collected. Decay curves were analyzed with an iterative, nonlinear least-squares algorithm. The squared residuals were weighted by $1/e_i^2$ where e_i is the experimental intensity at the ith sampling time. Plots of the weighted residuals indicated that the decay curves could be satisfactorily modeled as simple exponential decays. Circular dichroism (CD) spectra were recorded on a Jasco J-600 spectropolarimeter using a 2-cm-path cell for the visible region and either a 1-cm- or 1-mm-path cell for the UV region at 25 °C. Viscometric measurements were completed with a Cannon-Fenske No. 25 viscometer in a temperature-controlled water bath at 23 °C.

Results

UV–Visible Absorption. As was the case with DNA,[14] combining $Cu(bcp)_2^+$ with RNA induced a small red shift in the CT absorption band, which would otherwise maximize at 475.1 nm, as well as a small degree of hypochromism, i.e., a decrease in the molar absorptivity (Table I). Both effects are time-honored indications of a binding interaction,[25] especially since the addition of free bcp ligand to a solution containing $Cu(bcp)_2^+$ and DNA showed that the changes are not connected with ligand dissociation equilibria. Although the CT band of $Cu(dmp)_2^+$ did not show a significant red shift in the presence of poly(CI) or poly(AU), hypochromism was observed at the 454-nm absorbance maximum in both cases, with an absorptivity decrease of 6% and 2% for poly(CI) and poly(AU), respectively. However, there was no red shift or hypochromism in the CT band of $Cu(dmp)_2^+$ in the presence of tRNA.

Spectral titrations were performed so that the concentration of $Cu(bcp)_2^+$ was fixed at 25 μM and the concentration of each type of RNA was varied. Figure 2 presents the UV–visible spectrum of $Cu(bcp)_2^+$ in different solutions of poly(C)·poly(I).

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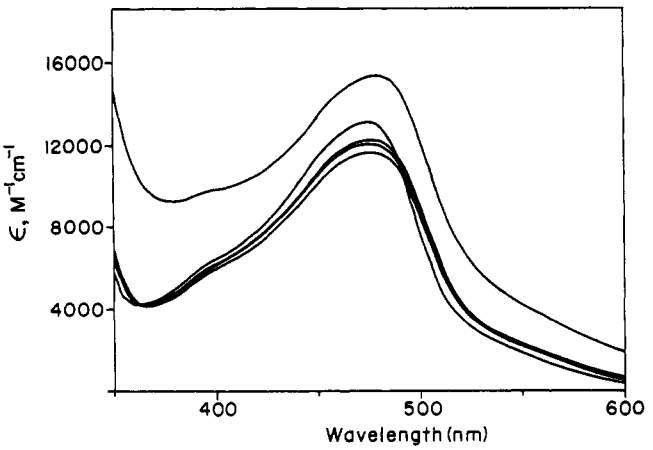

Figure 2. UV–visible spectra of $Cu(bcp)_2^+$ in the presence of different amounts of poly(C)·poly(I) in 33% methanol/0.02 M pH 7.8 triphosphate buffer. In order of decreasing absorptivity at 475 nm, the RNA-P/Cu values are 0.6, 0, 100, 50, and 27. The temperature was 25 °C.

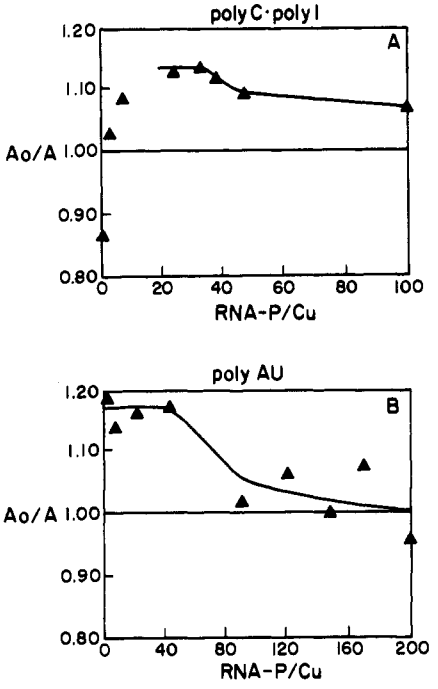

Figure 3. Hypochromicity at the CT maximum of $Cu(bcp)_2^+$. Values are plotted as a function of the RNA-P/Cu ratio for poly(C)·poly(I) (A) and poly(AU) (B), where A_0 is the absorbance of free $Cu(bcp)_2^+$ and A is the absorbance of $Cu(bcp)_2^+$ in the presence of RNA. The experimental conditions were the same as those in Figure 1. The smooth curves drawn through the experimental data points are simply visual aides. Note the scattering artifact at low ratios in (A).

The samples with RNA-P/Cu ratios greater than 0 but less than 1 were noticeably turbid, as can be appreciated from the offset baseline in the UV–visible spectrum (Figure 2). This effect probably arises from particulate formation due to the aggregation of RNA and $Cu(bcp)_2^+$. At higher RNA-P/Cu ratios, the turbidity vanished and an absorbance decrease occurred. Plots showing the hypochromicity in the CT band of $Cu(bcp)_2^+$ as a function of RNA concentration are shown in Figure 3.

The results in Figure 3A are representative of results obtained for poly(C)·poly(I) and poly(A)·poly(U), while those in Figure 3B are representative of poly(AU), poly(CI), and tRNA. The results with salmon testes DNA are similar to those in Figure 3A. In all cases, scattering effects complicated the results at RNA/Cu ratios of less than 1. Generally, the maximum hypochromism occurred at RNA/Cu ratios in the range 5–50, while at higher ratios there appeared to be a gradual diminution of the hypochromism. With poly(AU) and poly(CI), the hypochromism practically vanishes at high ratios.

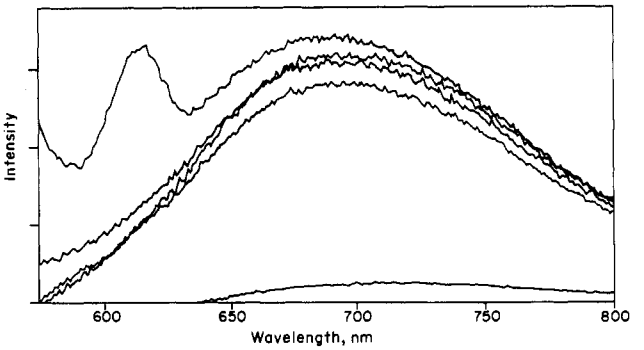

Figure 4. Uncorrected emission spectra of $Cu(bcp)_2^+$ in 33% by volume methanol/buffer solutions containing poly(C)·poly(I). In order of decreasing emission intensity at 700 nm, the RNA-P/Cu ratios are 0.6, 50, 100, 40, and 0. The excitation slit was set at 10 nm, and the emission slit was set at 20 nm.

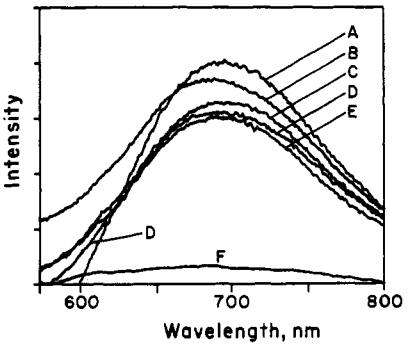

Figure 5. Uncorrected emission spectra of $Cu(bcp)_2^+$ in 33% by volume methanol/buffer solutions at RNA-P/Cu ratios of 50. RNA sources: (A) poly(AU); (B) tRNA; (C) poly(CI); (D) poly(C)·poly(I); (E) poly(A)·poly(U); (F) none. The instrumental settings were as in Figure 4.

Luminescence. The emission from $Cu(dmp)_2^+$ in 33% methanol solution was extremely weak, and the addition of RNA did not produce an enhancement. As with the dmp complex, the emission from $Cu(bcp)_2^+$ was also quite weak in 33% methanol; however, a significant increase in emission intensity was observed in the presence of RNA. In contrast to the hypochromism, the emission intensity did not fall off at high RNA/Cu ratios. Figure 4 shows the emission spectrum of $Cu(bcp)_2^+$ in the presence of different amounts of poly(C)·poly(I), while Figure 5 depicts the emission spectra which were obtained with the different types of RNA at an RNA-P/Cu ratio of 50. In each case, the corrected excitation spectrum of the sample was congruent with the visible absorption spectrum. The same emission spectrum occurs when salmon testes DNA is present, but the signal intensity is ca. 25% less than it is with poly(A)·poly(U).

An anomalous, relatively narrow signal centered around 615 nm was sometimes observed in the emission spectrum of $Cu(bcp)_2^+$ solutions containing RNA. This signal can be seen in Figure 4 in the spectrum obtained at an RNA-P/Cu ratio of 0.6. In general, this band was present for samples with RNA-P/Cu ratios between 0.2 and ca. 2. At higher RNA-P/Cu ratios the band was absent, except with poly(AU), where it became evident again at an RNA-P/Cu ratio of 200, and with poly(CI), where it reappeared at an RNA-P/Cu ratio of 75. Since placement of a notch filter (485–520-nm band-pass) between the excitation monochromator and the sample eliminated the 615-nm band, the band was attributed to the scattering of stray light by turbid samples. In the case of solutions of the random copolymers poly(AU) and poly(CI) at high RNA-P/Cu ratios, the 615-nm peak may be connected with some type of aggregation induced by self-association of the RNA. This seems likely to occur since poly(AU) and poly(CI) must

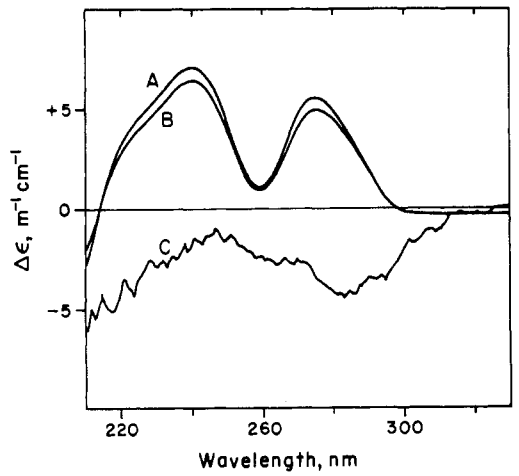

Figure 6. Circular dichroism spectra of poly(C)·poly(I) in 33% methanol/0.02 M triphosphate pH 7.8 buffer solution: (A) without $Cu(bcp)_2^+$; (B) with RNA-P/Cu = 40; (C) with RNA-P/Cu = 1.

contain long runs of bases which could support hydrogen-bonding interactions between the strands. In the case of poly(AU), short runs containing triple helices may occur, especially when the concentration of RNA is high.[26]

It can also be noted that a broad emissive shoulder from 575 to approximately 635 nm was observed in the spectra of solutions containing $Cu(bcp)_2^+$ and tRNA when the RNA-P/Cu ratios were 50 or greater (data not shown). However, this shoulder was not related to $Cu(bcp)_2^+$ as the emission was observed from blank samples containing only tRNA.

Luminescence Decay. Lifetime data for $Cu(bcp)_2^+$ obtained in the presence of RNA are presented in Table I. In general, a biphasic decay was observed, but the short-lived component was assumed to be due to scattered light since it could not be resolved from the laser pulse. The lifetime was therefore determined from the trailing edge of the decay after removing the first 50 ns worth of data.

Circular Dichroism. The CD spectrum of poly(C)·poly(I) exhibited a decrease in intensity in the presence of $Cu(bcp)_2^+$ at RNA-P/Cu = 40 (Figure 6), consistent with a change in conformation of the RNA as a result of $Cu(bcp)_2^+$ binding. Specifically, this could signal a limited melting of the helix, i.e., a more relaxed structure.[27] Alternatively, the spectral changes could be due to the bound form of $Cu(bcp)_2^+$ since the copper complex also absorbs in this region. A much more severe spectral perturbation occurred at an RNA-P/Cu ratio of 1 (Figure 6). The noise present in this spectrum is consistent with aggregation involving the copper complex and RNA.[28,29]

A positive CD signal also occurred in the vicinity of the charge-transfer absorption band of $Cu(bcp)_2^+$ in the presence of poly(C)·poly(I), as shown in Figure 7. Since poly(C)·poly(I) has no absorption bands in the visible region, this signal must be due to $Cu(bcp)_2^+$. This induced CD signal is good evidence for the formation of an adduct with poly(C)·poly(I) because the free copper complex is optically inactive. A similar spectrum is induced for $Cu(bcp)_2^+$ in the presence of salmon testes DNA.

Viscometry Results. The average chain length of the poly(C)·poly(I)polymer was determined to be 1250 base pairs. No increase in the specific viscosity was observed for poly(C)·poly(I) in the presence of $Cu(bcp)_2^+$; in fact, some decrease in the viscosity occurred.

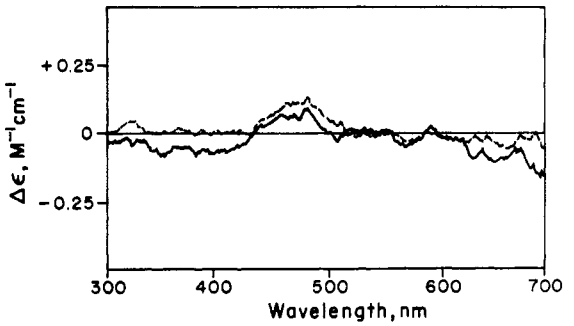

Figure 7. Induced circular dichroism spectra of $Cu(bcp)_2^+$ in the presence of poly(C)·poly(I) in 33% methanol/0.02 M triphosphate pH 7.8 buffer solution: RNA-P/Cu = 40 (dashed line); RNA-P/Cu = 2 (solid line).

Discussion

As found previously in DNA-binding studies, the presence of phenyl substituents in the 4,7-positions of the ligand has a profound influence on the binding of copper phenanthrolines to RNA in solution. An interaction occurs with $Cu(dmp)_2^+$ since hypochromism is evident in the presence of excess poly(CI) or poly(AU); however, the binding interaction has no significant influence on the CT emission intensity. More profound spectral changes occur with $Cu(bcp)_2^+$, and they differ depending on the RNA-P/Cu ratio.

Aggregation Involving $Cu(bcp)_2^+$ at Low RNA-P/Cu Ratios. Particulate formation clearly occurs when RNA-containing solutions are combined with $Cu(bcp)_2^+$ at RNA-P/Cu ratios of less than ca. 2. In some instances the samples are visibly turbid, but in all cases a pronounced offset occurs in the baseline of the visible absorption spectrum (Figure 2). The noise in the CD spectrum at low ratios is also an indication of scattering artifacts[28] as is the 615-nm signal in the emission spectrum; vide supra.

This effect is not surprising in view of previous work involving aromatic dyes interacting with polyanions.[30-32] Michaelis has found that aromatic dyes are prone to aggregation when they are bound to the surfaces of polyanions when the concentration of dye is greater than or equal to the concentration of the polyanion. However, if an excess of the polyanion is present, the dye binds to the polyanion as a monomer. Tamilarasan et al. have observed a similar effect while studying the interactions between $Cu(bcp)_2^+$ and poly(styrenesulfonate).[12] They have found that at low styrene-to-copper ratios the emission from $Cu(bcp)_2^+$ is greatly increased in aqueous methanol due to the formation of an aggregated or colloidal form of $Cu(bcp)_2^+$ which is resistant to solvent attack. However, when excess polymer is present and the complex is dispersed along the polymer, the emission is quenched. Moreover, Tamilarasan et al. have also demonstrated that at low DNA-P/Cu ratios an aggregative interaction occurs between $Cu(bcp)_2^+$ and DNA.[12]

Binding Interaction with $Cu(bcp)_2^+$ at High RNA-P/Cu Ratios. At higher RNA-P/Cu ratios, the aggregates break up because the baseline offset disappears in the visible spectrum and the 615-nm peak disappears from the emission spectrum. The CD signal which is observed at ca. 480 nm clearly establishes the fact that the complex remains bound to RNA. This is also evident from the persistence of the CT emission, which would otherwise be quenched by solvent interactions.

In the case of our DNA-binding studies involving $Cu(bcp)_2^+$, we have proposed that intercalative binding could explain the observation of emission since the rigidity inherent in the adduct

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would inhibit the ligand reorganization that is required by the exciplex quenching mechanism.[14] To test whether this occurs with RNA, we have carried out viscosity measurements because intercalation of a group between base pairs of an A-form duplex would be expected to result in elongation and an increase in the specific viscosity observed for the RNA.[33] However, instead of an increase, we have found that a small decrease in the specific viscosity of poly(C)·poly(I) occurs when the copper complex is present in solution. This strongly suggests that the binding interactions do not involve intercalation.

If we exclude intercalation, some other type of internalization or sequestration has to occur because of the inhibition of solvent-induced quenching of the emission. In the case of groove binding, strong internalization may occur if the binding of the bcp complex induces a local melting of the RNA structure something like Mn(III) porphyrins do when they bind to B-form DNA.[34] Although the free porphyrin ligand and complexes of metal ions, such as Cu(II) or Ni(II), which do not require axial ligands, are able to intercalate into DNA,[35,36] the Mn(III) complex has axial ligands and is incapable of intercalating. It binds externally and seeks out regions of DNA which are rich in adenine and thymine residues. However, Dabrowiak and co-workers have found that the presence of bulky substituents on the periphery of the porphyrin has no influence on the pattern of DNA cleavage induced by this reagent.[34,37] To explain these results, they have proposed that a disruption of the Watson-and-Crick hydrogen bonding occurs when the Mn(III) porphyrin binds and that a local melting of duplex structure occurs. If $Cu(bcp)_2^+$ becomes entangled with RNA in a similar fashion, solvent-induced quenching could be curtailed. The structure breaking involved in this type of binding could be responsible for the decrease in the specific viscosity of the RNA.

Another possibility is that $Cu(bcp)_2^+$ binds as a monomer but to more than one segment of RNA structure. For example, the phenanthroline ligands on either side of the copper center could interact with grooves in separate molecules of RNA. In this way the copper complex would induce aggregation of the RNA molecules in solution and thereby dictate the decrease in the specific viscosity due to the RNA. Interactions with two different macromolecules would inhibit the structural reorganization required at the copper center for the addition of a fifth ligand. At the same time the metal center would be heavily shielded from the solvent so that emission can occur. According to this model, the structural transition associated with the curve drawn in Figure 3A would represent the conversion from aggregated copper centers to monomeric copper complexes sandwiched between runs of RNA double helix.

Finally, it is possible that the emission persists at high RNA-P/Cu ratios because the aggregated form of $Cu(bcp)_2^+$, which is evident from the scattering artifacts observed at lower ratios, has not been fully dispersed. One type of aggregation that is observed in the solid state with $Cu(phen)_2^+$ and related systems involves the formation of chains of copper complexes linked by intermolecular stacking interactions.[38,39] RNA could conceivably stabilize such a structure; indeed, cationic porphyrins have been proposed to bind to DNA as stacked aggregates.[40] However, in the case of the porphyrins this mode of binding gives rise to a conservative CD spectrum in the region where the π-π^* absorption bands of the porphyrin occur. The fact that a simple positive CD signal is observed with $Cu(bcp)_2^+$ may be more consistent with the primary interaction being between the copper complex and RNA chromophores rather than between copper complexes clustered together on a chiral framework.

Conclusions

The spectroscopic data presented indicate that $Cu(bcp)_2^+$ binds to many different types of RNA. This is evident from the hypochromism and the induced CD intensity that occur in the CT absorption region and the CT emission that is observed. The latter observation indicates that the bound form of $Cu(bcp)_2^+$ is well protected from the solvent. Some type of particulate formation involving $Cu(bcp)_2^+$ occurs at low RNA-P/Cu ratios. These aggregates are dispersed at high RNA-P/Cu ratios, but we cannot entirely rule out the possibility that some form of aggregated $Cu(bcp)_2^+$ in contact with RNA is responsible for the emission observed at higher RNA-P/Cu ratios. However, the physical data are better explained in terms $Cu(bcp)_2^+$ monomers which are sufficiently embedded in the RNA such that solvent-induced emission quenching is inhibited. In the case of double-helical RNA, the internalization may occur along with the local melting of the A-form conformation and a decrease in the rigidity of the RNA molecule. Alternatively, $Cu(bcp)_2^+$ may find itself sandwiched between RNA molecules in solution. There is no evidence that partial intercalation into double-stranded regions of RNA occurs. Although the emission intensity and the hypochromism vary with the source, our results show that a wide variety of polynucleotides are capable of binding $Cu(bcp)_2^+$ and protecting the metal center from solvent attack. The structures include B-form DNA, A-form RNA, and synthetic polymers such as poly(AU) and poly(CI) that contain single-stranded domains. The binding interaction seems to depend more on hydrophrobic forces than it does on the recognition of specific structure within the polynucleotide host.

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