infer that orotidine is in an unstrained syn conformation with a C(2′)-endo ribose.

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Interaction of Metal Ions with Polynucleotides and Related Compounds. XXI. Metal Ions as Agents for the Stacking of Nucleotides. A Specific Interaction of Zinc(II) and Adenosine Monophosphate

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Abstract: Zinc(II) ions react with 5′-AMP at 1×10^{-3} *M* concentration in the presence of a polycation to produce a highly rotatory complex with conservative circular dichroism bands. This complex is characterized in the uv by hypochromicity and shoulders at 280 and 290 nm, and it exhibits a titration curve in which the phosphate p*K* is lowered and the p*K* for zinc hydrolysis is raised. Conservative CD bands are also produced by Zn(II) and 3′-AMP. These observations indicate that the zinc promotes parallel stacking of 3′- and 5′-AMP. The effect is remarkably specific. Other AMP isomers (2′,2′,3′ cyclic, and 3′,5′ cyclic) and other nucleotides (5′-GMP, 3′-GMP, 5′-CMP, 5′-UMP, and 5′-IMP) do not produce a highly rotatory complex. Metal ions other than Zn(II), *e.g.*, Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Hg(II), Ag(I), Fe(III), Al(III), and Ce(III), also do not produce such a complex. The failure to produce the effects that have been noted does not indicate a lack of complex formation, but only the inability of the metal to induce parallel stacking of the nucleotides. A metal that does not induce parallel stacking at low concentration may do so at high concentration, *e.g.*, Cu(II), but some metals apparently do not induce such stacking at any concentration. Highly rotatory complexes that do not display the conservative CD effect are formed between Pb(II) and 5′-AMP and Zn(II) and 3′- and 5′-dAMP. The absence of the 2′-OH group produces a much greater change in the CD effect of the 3′-AMP complex than of the 5′ complex, suggesting that the 2′-OH group plays a more significant role in the former than in the latter.

The participation of metal ions in the biochemical reactions of nucleotides and nucleic acids has provided a great deal of interest in the determination of the structures of the metal–nucleotide complexes.[1,2] Recently a nuclear magnetic resonance study has suggested that the reaction of Cu(II) with 3′-AMP and 5′-AMP results in a complex containing two atoms of Cu(II) and two molecules of AMP, with the AMP molecules located in such a way as to permit π interaction between the purines.[3] If metal ions are able to induce such stacking of nucleotide bases, this stacking should result in the drastic alterations of their ORD and CD spectra. The present study reveals that metal ions can indeed produce large increases in the rotatory strength of nucleotides which are consistent with an interaction between the bases. Perhaps the most striking aspect of the production of these Cotton effects is that, far from constituting a general phenomenon, they are produced only in certain very restricted cases. Copper ions unexpectedly do not produce the effects. Only zinc(II) and lead(II), of all common metal ions, and only AMP (either 3′ or 5′), of all the common nucleotides, will produce Cotton effects, and only the optical characteristics of zinc, and not lead, are indicative of parallel stacking. Such specificity in the reaction of metal and ligand is quite remarkable.

This paper describes the studies that demonstrate this specificity and provides some clues for its occurrence.

The study of the optical properties of metal–nucleotide complexes is complicated by the fact that many of these complexes precipitate at neutral pH. This problem has been overcome by carrying out the optical studies in the presence of poly-L-lysine and certain other polycations. It is then possible to probe the effect of metal ions on the uv, ORD, and CD spectra of various nucleotides and thus to determine whether metal ions can induce the stacking of nucleotide bases.

Experimental Section

The nucleosides and nucleotides were obtained from Sigma except for 3′-GMP which was obtained from P. L. Biochemicals. The basic polypeptides were obtained from Pilot Chemicals, polyethyleneimine from Pfaltz and Bauer, and DEAE-dextran from Pharmacia. All other chemicals were reagent grade.

The concentrations of nucleotides were determined from their uv spectra. The concentrations of basic polypeptides were deter-

(1) G. L. Eichhorn, N. Berger, J. Butzow, P. Clark, J. Rifkind, Y. Shin, and E. Tarien, *Advan. Chem. Ser.*, No. **100**, 135 (1971).
(2) U. Weser, *Struct. Bonding (Berlin)*, **5**, 41 (1968).
(3) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).

mined by the method of Lowry which was checked by nitrogen analysis. The concentrations of metal ions were determined with a Zeiss atomic absorption spectrometer.

Titrations were performed using a Radiometer TTA3 titration assembly with an ABU1 autoburet. Titrations were performed at 25° under nitrogen using nitrate salts. All titrations were made at 5 min/pH unit and at a slower rate of 20–200 min/pH unit. The faster and slower results were always qualitatively similar and often identical.

Uv spectra were measured using a Cary 14 spectrophotometer, and ORD and CD spectra were obtained with a Cary 60 spectropolarimeter with the 6001 circular dichroism attachment. Cylindrical cells of 1 mm path length at an ambient temperature of 24 ± 1° were used unless specified otherwise.

In the preparation of samples for uv, CD, and ORD spectroscopy the acetate salts were frequently used because of the greater solubility of zinc acetate. However, the same results are obtained with the chloride or nitrate salts, and when the acetate was not available other salts were used.

The solutions were usually prepared by adding the nucleotide to the polycation, then adding the metal, and finally adjusting the pH with a Radiometer Model 25 pH meter to the desired pH. Variations in the protocol were investigated, and particularly with 3′-AMP some of the details of the spectra are sensitive to the manner in which the solutions are prepared.

The spectra of 5′-AMP in the presence of poly-L-lysine and Zn(II) are insensitive to the order in which the solutions are added, but some of the qualitative features of the spectra do depend on pH, particularly in the region of pH 6. The position of the peaks and the relative intensity of the peaks are very sensitive to the relative concentrations of polypeptide, nucleotide and metal. For this reason all reported spectra are at pH 7.0 with a 2:1:1 polypeptide:nucleotide:metal concentration.

In all cases the effect of time on the observed spectra was investigated. Particularly in those cases in which no significant effect on the CD spectra was observed, the solutions were reexamined after standing for at least 1 day. When the elapse of time produced a significant enhancement in CD, as was the case for the solutions of Zn(II) 2′-AMP in Figure 9, and the solutions used in Figure 11, the later spectrum is reported.

Results and Discussion

The Reaction of Zn(II) with 5′-AMP. The addition of a twofold excess of poly-L-lysine to 5′-AMP produces a 9% decrease in the extinction coefficient at 259 nm at neutral pH[4] and no significant effect on the ORD or CD spectrum of 5′-AMP. The further addition of zinc(II) has a dramatic effect on all three types of spectra (Figure 1). The uv spectrum exhibits a hypochromicity of 37% at 259 nm relative to that of the already hypochromic 5′-AMP after the addition of poly-L-lysine, or a total hypochromicity of 42%. In addition, two shoulders appear, at 280 and 290 nm. The ORD and CD spectra indicate a very large increase in rotatory strength to a value that is somewhat greater than that obtained for the ordered conformations of polyadenylic acid. The CD spectrum consists of two sets of conservative CD bands and at least one nonconservative band indicated by the high-wavelength minimum at 282 nm. The near-uv conservative bands consist of a minimum at ~277 nm (corresponding to a shoulder) and a maximum at 251 nm, while the far-uv conservative bands consist of a minimum at 222 nm and a maximum at 212 nm. The presence of the conservative CD bands constitutes a clear indication that the Zn(II) holds the nucleotide bases in a parallel stacked configuration which splits the $\pi-\pi^*$ bands into two bands whose rotatory strengths are approximately equal but opposite in sign.[5,6] Such a conclusion is

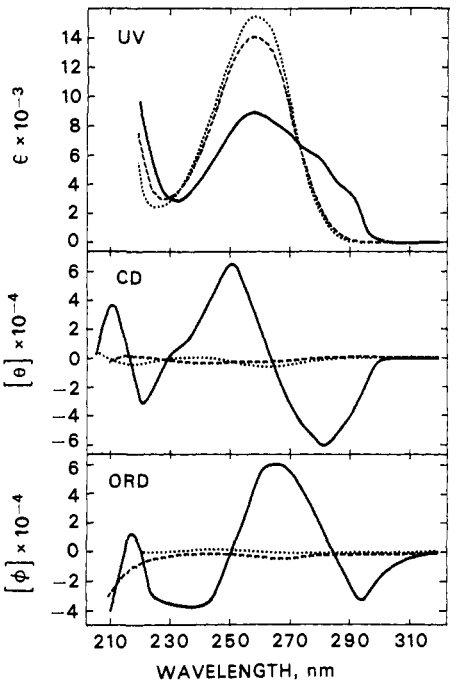


Figure 1. Uv, CD, and ORD spectra of a solution of 1×10^{-3} M 5′-AMP at pH 7: (···) without further addition; (- - -) in the presence of 2×10^{-3} M poly-L-lysine; (——) in the presence of 2×10^{-3} M poly-L-lysine and 1×10^{-3} M zinc(II).

strengthened by the fact that the inversion of all of the bands of Figure 1 produces a spectrum that is strikingly similar to the CD spectrum of poly A at neutral pH,[7] or of an adenosine dinucleotide,[7] in both of which the bases are considered to be in a parallel stacked helical configuration. The inversion of the bands in the Zn(II) 5′-AMP spectrum can readily be explained by a change in the relative orientation of the bases and their transition moments which determine the signs and magnitudes of the CD bands.[6] Such an inversion has been observed in a dinucleotide in which the D-ribose is replaced by L-ribose,[8] as well as in a dinucleotide in which the 5′ positions are separated by three or four phosphates.[9]

The poly-L-lysine is not required for the production of these optical effects; other polypeptides, *e.g.*, poly-D-lysine, poly-L-ornithine, and poly-L-arginine, may be substituted. Indeed, other unrelated polycations, *e.g.*, polyethylenimine, a globular polyamine containing primary, secondary, and tertiary amines, and DEAE-dextran, a polysaccharide with positive diethylamine ethyl ether substituents, can be used instead of polylysine. The range of structural differences that can be tolerated in the polycation suggests that it brings the zinc(II)–AMP complex into solution by a process that leaves the complex intact,[10] though some interaction with the polypeptide probably takes place.

The addition of zinc(II) to 1×10^{-3} M 5′-AMP in the absence of polycation immediately precipitates most of the nucleotide at neutral pH. The uv spectrum of the 5′-AMP remaining in solution when zinc(II) is

(4) K. G. Wagner and R. Arav, *Biochemistry*, 7, 1771 (1968).
(5) I. Tinoco, Jr., *J. Amer. Chem. Soc.*, **86**, 297 (1964).
(6) C. A. Bush and I. Tinoco, Jr., *J. Mol. Biol.*, **23**, 601 (1967).
(7) C. A. Bush and H. A. Scheraga, *Biopolymers*, 7, 395 (1969).
(8) I. Tazawa, S. Tazawa, L. M. Stempel, and P. O. P. Ts'o, *Biochemistry*, **9**, 3499 (1970).
(9) J. F. Scott and P. C. Zamecnik, *Proc. Nat. Acad. Sci. U. S.*, **64**, 1308 (1969).
(10) A very large excess (*e.g.*, 40-fold) of polycation, however, will disrupt the complex.

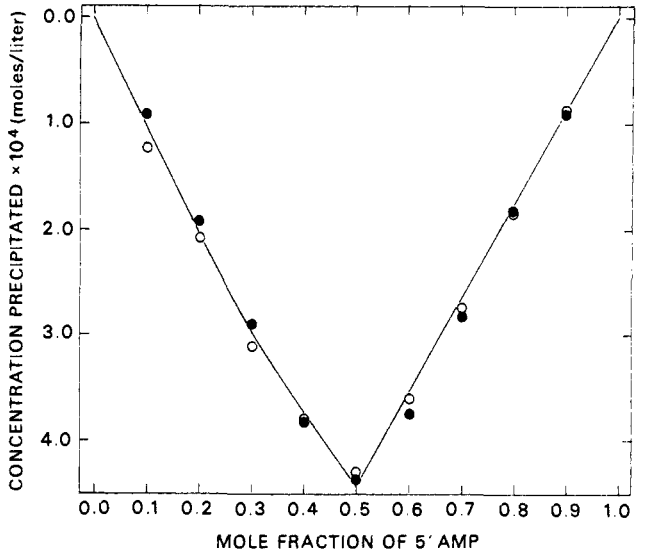


Figure 2. Continuous variation curve to determine stoichiometry of the Zn(II)–5′-AMP complex. Stock solutions of 5′-AMP and Zn(II) acetate, both 1×10^{-3} *M*, were mixed so as to produce solutions with the same total concentration of Zn(II) and 5′-AMP, but varying ratios, as indicated in the abscissa: analytical measurements performed on supernatant; precipitate concentration determined by difference; (○) concentration of 5′-AMP, from absorbance remaining after precipitation; (●) concentration of Zn(II), by atomic absorption spectrophotometry.

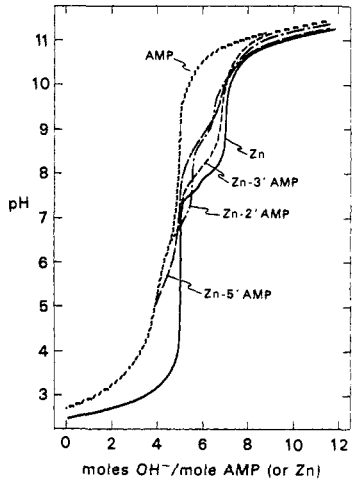


Figure 3. Titration curves of the zinc(II) complexes of the isomers of AMP compared with the titration curves of the components. Solutions 1×10^{-3} *M* in nucleotide and/or $Zn(NO_3)_2$. Enough HNO_3 was added to each solution to make it 5×10^{-3} *M* in HNO_3 before titration with 5×10^{-2} *M* NaOH under nitrogen at 25°: (—) $Zn(NO_3)_2$; (- - -) AMP; (— · —) 2′-AMP and $Zn(NO_3)_2$; (— — —) 3′-AMP and $Zn(NO_3)_2$; (— —) 5′-AMP and $Zn(NO_3)_2$.

added to the nucleotide is virtually identical with that of 5′-AMP in the absence of zinc, and it appears therefore that the zinc(II)–AMP complex is insoluble. The continuous variation curve (Figure 2) measuring the absorbance and zinc concentration of solutions of zinc(II) and 5′-AMP, from which precipitate has been removed, exhibits a minimum at a 1:1 ratio of the constituents, indicating an equimolar ratio of zinc and 5′-AMP in the complex.

The zinc(II)–5′-AMP complex dissolves at high and low pH. It is possible to study the formation of an insoluble complex by a titration that is carried out slowly

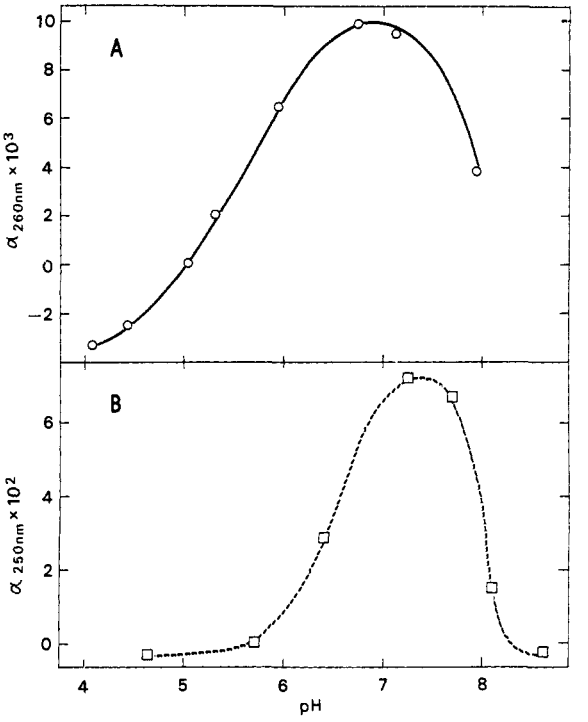


Figure 4. Spectropolarimetric titration of solutions containing (A) 5×10^{-4} *M* 5′-AMP and zinc(II) acetate and 1×10^{-3} *M* poly-L-lysine, and (B) 1×10^{-3} *M* 3′-AMP and zinc(II) acetate and 2×10^{-3} *M* poly-L-lysine. The solutions were adjusted with concentrated NaOH.

enough to attain equilibrium[11] at all points of the titration curve. In Figure 3 the titration curve of a 1:1 mixture of Zn(II) and 5′-AMP is compared with titration curves of the components. Two features of the titration of the complex may be noted. First, complex formation lowers the p*K* of the secondary phosphate. Second, the hydrolysis of zinc(II) occurs at higher pH in the presence of the nucleotide.[12]

These results indicate that the complex formed involves the phosphate of the nucleotide and that the complexed zinc is protected from the action of hydroxide ion. The requirement of doubly negative phosphate prevents formation of the complex at low pH and the hydrolysis above pH 8 destabilizes the complex at high pH. Such a stability range is demonstrated by the spectropolarimetric titration in the presence of poly-L-lysine shown in Figure 4A. The pH titration and the spectropolarimetric titration lead to the same conclusion. The fact that the stability range of the complex as determined by pH titration in the absence of poly-L-lysine and by spectropolarimetric titration in the presence of polylysine is so similar again indicates that a similar complex is formed in the presence or absence of poly-L-lysine.

When the concentrations of all components of a 2:1:1 mixture of poly-L-lysine, 5′-AMP, and zinc(II) are gradually decreased, the rotatory strength of the solution decreases in such a way that at a 1×10^{-4} *M* concentration of zinc no more complex formation is

(11) G. L. Eichhorn and P. Clark, *J. Amer. Chem. Soc.*, **85**, 4020 (1963).

(12) A titration was also carried out in the presence of poly-L-lysine; the p*K* of the secondary phosphate of 5′-AMP is lowered as in Figure 3. However, the effect on the Zn(II) hydrolysis is masked by titration in that region of the polypeptide ε-amino group, whose titration could itself be altered by the presence of the Zn(II)–AMP complex.

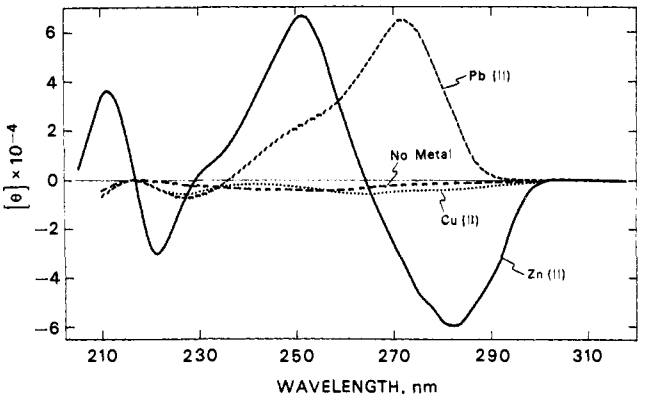


Figure 5. Comparison of CD spectra at pH 7 of solutions of 1×10^{-3} *M* 5′-AMP and 2×10^{-3} *M* poly-L-lysine: (– – –) in the absence of divalent metal ions; (——) in the presence of 1×10^{-3} *M* zinc acetate; (- - -) in the presence of 1×10^{-3} *M* lead nitrate; (· · ·) in the presence of 1×10^{-3} *M* copper(II) chloride. Other metal ions that, like Cu(II), produced no appreciable enhancement are Ca(II), Mg(II), Mn(II), Co(II), Ni(II), Cd(II), Hg(II), Ag(I), Fe(III), Cd(III), and Al(III).

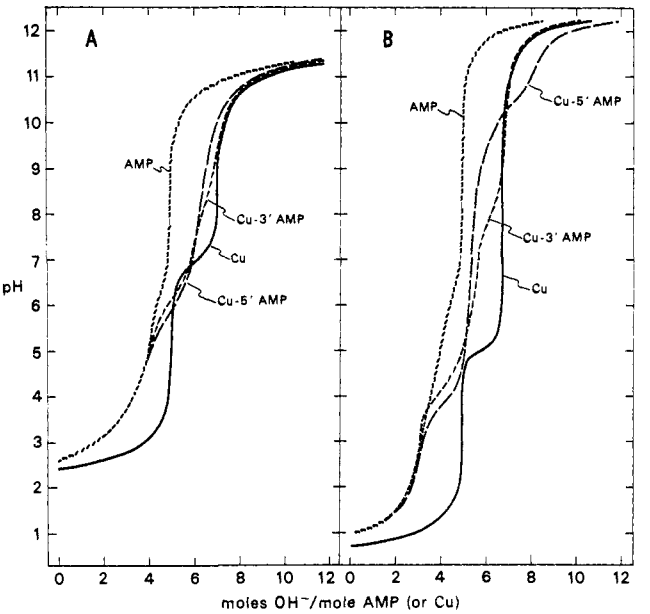


Figure 6. Titration curves of the copper(II) complexes of the isomers of AMP, compared with the titration curves of the components: (A) solutions 1×10^{-3} *M* in nucleotide and (or) $Cu(NO_3)_2$; enough HNO_3 was added to each solution to make it 5×10^{-3} *M* in HNO_3 before titration with 5×10^{-2} *M* NaOH under nitrogen at 25°; (B) solutions 5×10^{-2} *M* in nucleotide and (or) $Cu(NO_3)_2$; enough HNO_3 was added to each solution to make it 2.5×10^{-1} *M* in HNO_3 before titration with 2.57 *M* NaOH under nitrogen at 25°; (——) $Cu(NO_3)_2$; (- - -) AMP; (– – –) 3′-AMP and $Cu(NO_3)_2$; (— —) 5′-AMP and $Cu(NO_3)_2$.

indicated. This concentration is only slightly higher than the concentration of unprecipitated Zn(II) at a 1:1 ratio of 5′-AMP and Zn(II) in the absence of poly-L-lysine (Figure 2). These results indicate that the insoluble complex in the absence of polycation and the polycation-solubilized complex are both disrupted at similar concentrations.

The rotatory strength of the complex is not affected by heating to 75° (2×10^{-3} *M* poly-L-lysine, 1×10^{-3} *M* $Zn(NO_3)_2$, and 1×10^{-3} *M* 5′-AMP at pH 8.0), and is diminished by only 25% at 98°.

Are Conservative CD Bands Found When Zn Is Replaced by Other Metal Ions? Figure 5 shows the effect on the CD spectrum of replacing Zn(II) by other metal ions. In the presence of Cu(II), Ca(II), Mg(II), Mn(II), Co(II), Ni(II), Cd(II), Hg(II), Ag(I), Fe(III), Ce(III), and Al(III) the effect on the circular dichroism of the nucleotide is insignificant. Pb(II) is the only other metal ion besides Zn(II) which was found to produce a large effect on the CD spectrum of the nucleotide. Some of these metals have minor effects on the uv spectrum. However, no metal other than Zn(II) produces the shoulders at 280 and 290 nm, and no other metal produces a conservative CD effect. The near-uv absorption peak of 5′-AMP is shifted in the presence of Pb(II) to 262 nm with a small decrease in extinction coefficient. The titration curve of a 1:1 mixture of 5′-AMP and Pb(II) reveals that lead has an even greater effect on the phosphate p*K* (~5) and the metal hydrolysis (>9) than Zn(II). On the other hand, lead produces no significant effects on the uv and CD spectra of 3′-AMP and deoxy-5′-AMP, in contrast to the behavior with Zn(II). Thus, lead exhibits an even greater specificity than zinc in its reaction with the ribose–phosphate portion of the nucleotide.

These results imply that the effect of lead on 5′-AMP may be produced by a different type of complex than the effect of zinc. A rotatory strength much greater than that of 5′-AMP has been observed with some adenosine derivatives in which the ribose is rigidly attached to the base.[13,14] If the lead causes a similar rigidity in the ribose–base attachment, the large rotatory strength observed with Pb(II) can be explained. Alternatively, the CD spectrum can be interpreted as resulting from a complex similar to that found for zinc, with the difference that lead produces a nonparallel interaction between bases. Such an interaction would result in nonconservative CD bands.[15,16] A comparison of the titration results indicates a similarity between the zinc and lead complexes.

The failure of Cu(II) to produce any effect on the CD spectrum is surprising in view of the nmr evidence indicating a stacked structure for the Cu(II)–AMP complex.[3]

The apparent discrepancy between these nmr results, which were obtained at a high concentration of AMP, and the uv and CD results, obtained at a much lower concentration, has perhaps been resolved by titration of Cu(II)–5′-AMP mixtures at two different concentrations. Figure 6A contains titration curves at 1×10^{-3} *M* concentration. At this concentration the p*K* of the phosphate is lowered in a manner analogous to that observed with zinc. However, one OH^- reacts with Cu(II) in the presence of 5′-AMP at a lower pH than in its absence; the second OH^- requires higher pH. As Figure 3 has shown, both OH^- ions in Zn(II) 5′-AMP are titrated at a higher pH, and this phenomenon has been attributed to a structure in which the zinc ions are relatively protected from the action of OH^- ions. Apparently copper ions react with *one* OH^- ion under

(13) D. W. Miles, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, 57, 1138 (1967).

(14) D. W. Miles, M. T. Robins, R. K. Robins, and H. Eyring, *Proc. Nat. Acad. Sci. U. S.*, 62, 22 (1969).

(15) J. T. Yang and T. Samejima, *Biochem. Biophys. Res. Commun.*, 33 739 (1968).

(16) W. C. Johnson, Jr., and I. Tinoco, Jr., *Biopolymers*, 7, 727 (1969).

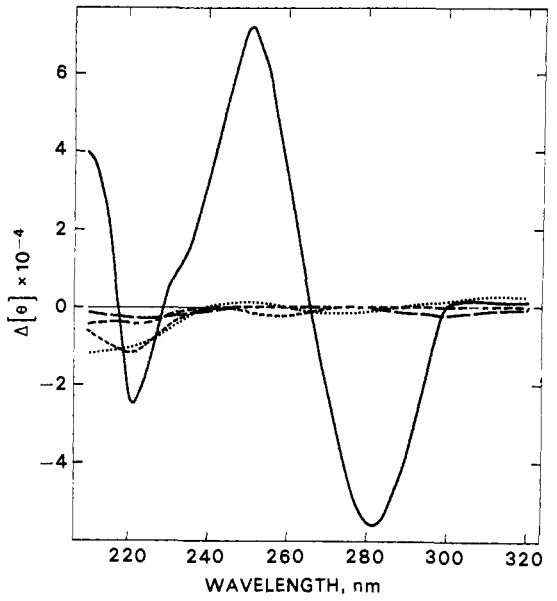
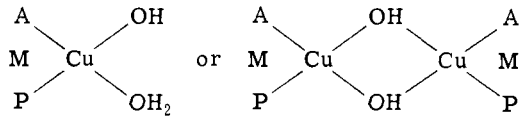


Figure 7. Difference CD spectra of the zinc(II) complexes of nucleotides. The spectrum of a solution containing 1×10^{-3} *M* nucleotide and 2×10^{-3} *M* poly-L-lysine is subtracted from the spectrum of a solution containing 1×10^{-3} *M* Zn(II) and nucleotide and 2×10^{-3} *M* poly-L-lysine. Nucleotides are: (——) 5′-AMP; (···) 5′-GMP; (- - -) 5′-IMP; (— —) 5′-UMP; (- - —) 5′-CMP.

these conditions. Such titration behavior indicates the stabilization of a complex containing one OH^- per metal ion, as in the following structures.[17] In these



structures the adenine bases are presumably not stacked. It must be remembered, however, that the nmr experiments with Cu(II) were conducted at much higher concentrations. When a titration is carried out at a concentration of 5×10^{-2} *M* 5′-AMP (approaching the nmr concentrations) the Cu(II) ions do indeed react with *both* OH^- ions at a much higher pH (Figure 6B). Thus, Cu(II) ions at high concentration behave very much like zinc(II) ions at lower concentration. The possibility exists therefore that the zinc complex studied here resembles the copper complex previously studied by nmr, but that the latter requires higher concentration to form. This requirement of higher concentration to achieve the stacked conformation with copper could be due to the greater stability of an unstacked structure, such as one of those shown above, in the case of copper.

When a solution of Ni(II) 5′-AMP is titrated even at the high concentration of 5×10^{-2} *M*, the titration curve resembles Figure 6A rather than 6B; 1 equiv of base is titrated at the pH for the hydrolysis of Ni(II) in the absence of AMP, whereas the second equivalent is titrated at higher pH. The retarded one-step hydrolysis attributed to the presence of a stacked nucleotide complex thus does not occur with Ni(II) even at high concentration.

(17) S. Chaberek and A. E. Martell, "Organic Sequestering Agents," Wiley, New York, N. Y., 1959, p 77.

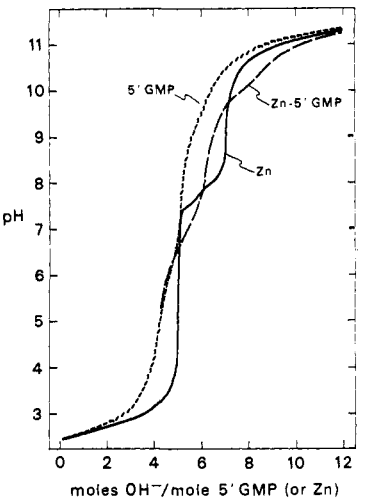


Figure 8. Titration curves of (——) $Zn(NO_3)_2$, (- - -) 5′-GMP, and (— —) $Zn(NO_3)_2$ and 5′-GMP. The concentrations of solutions are as in Figure 3.

A comparison of the concentration dependence of the titration curves of solutions containing 5′-AMP and zinc, copper, and nickel thus indicates an increasingly high concentration required for the formation of what is presumed to be the stacked complex. For zinc, but not copper or nickel, a 1×10^{-3} *M* concentration is adequate; for copper, but not for nickel, stacking appears to occur at 5×10^{-2} *M* concentration. Perhaps the stacked complex cannot be produced with nickel at any feasible concentration.

Is the Conservative CD Effect Produced When Adenine Base Is Substituted by Other Bases? When zinc(II) is added to a mixture of poly-L-lysine and 5′-GMP, 5′-UMP, 5′-CMP, and 5′-IMP, no large increases in rotatory strength indicating an ordered structure of the nucleotides are observed (Figure 7). Slight changes occur in the uv spectrum, but nothing as dramatic as the effect with 5′-AMP can be observed. The addition of poly-L-lysine to 5′-GMP produces a small effect on the ORD and CD of the nucleotide[18,19] in the absence of zinc(II), but the addition of zinc does not significantly enhance this effect; hence it cannot result from metal complex formation. The titration curve (Figure 8) of 1×10^{-3} *M* Zn(II)–5′-GMP also indicates that a stacked complex is not formed. The zinc in the presence of 5′-GMP does not exhibit the totally retarded hydrolysis at higher pH characteristic of Zn(II)–5′-AMP, but rather reacts with one OH^- at lower pH, and with a second OH^- at higher pH, than in the absence of the GMP. In this respect the zinc hydrolysis in the presence of 5′-GMP mimics the copper hydrolysis in the presence of 5′-AMP at low concentration (Figure 6A), and can also be explained on the basis of a mononuclear complex or an unstacked olated binuclear complex. Zn(II) also does not affect the p*K* of the phosphate group of 5′-GMP (Figure 8). It is evident that the conservative CD effects generated by zinc(II) in the absorbance region of 5′-AMP in the presence of polypeptide require the proper nucleotide base component, and therefore presumably involve binding to base as well as to phosphate.

(18) J. M. Rifkind and G. L. Eichhorn, *Biochemistry*, **9**, 1753 (1970).
(19) K. Wulff, H. Wolf, and K. G. Wagner, *Biochem. Biophys. Res. Commun.*, **39**, 870 (1970).

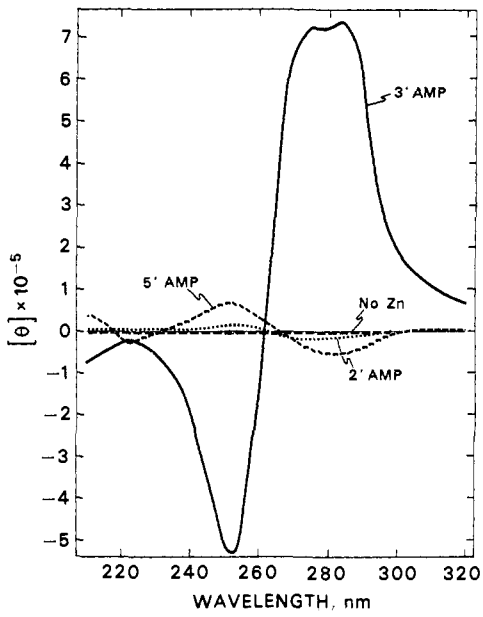


Figure 9. Comparison of CD spectra of the zinc(II) complexes of AMP isomers: (- - -) 1×10^{-3} *M* AMP; (· · ·) 1×10^{-3} *M* 2′-AMP, 1×10^{-3} *M* Zn(II); (——) 1×10^{-3} *M* 3′-AMP, 1×10^{-3} *M* Zn(II); (- · -) 1×10^{-3} *M* 5′-AMP, 1×10^{-3} *M* Zn(II). All solutions contain 2×10^{-3} *M* poly-L-lysine and are at pH 7.

How Does the Position of the Phosphate Affect the Conservative CD Complex? The titration results of Figure 3 clearly implicate the phosphate group in the complex which elicits the conservative CD bands. Such a complex is not produced by adenosine; this fact furnishes additional evidence for the participation of the phosphate in the formation of the complex.

It is of considerable interest to examine the effect of variations in the position of the phosphate on the adenine nucleotide. No significant spectral changes are observed when zinc(II) in the presence of poly-L-lysine is allowed to react with 2′,3′-cyclic AMP or 3′,5′-cyclic AMP, in which the phosphate has only a uninegative charge and is rigidly fixed, preventing any interaction with a metal on an adjacent stacked nucleotide. With 5′-ADP, a small change in the uv spectrum, unaccompanied by any noticeable effect in the ORD, is observed. None of these substances form the highly rotatory complex.

When the phosphate is located in the 3′ position a complex is obtained whose rotatory strength in the near-uv CD bands is almost an order of magnitude greater than in the complex with the phosphate in the 5′ position (Figure 9). The solutions containing 3′-AMP are always somewhat turbid even in the presence of the polypeptide, but the CD spectrum obtained with this isomer, though not completely accurate, cannot be an artifact of the turbidity, since the major effect of turbidity should be to produce a decrease in the apparent rotatory strength.[20,21] This spectrum appears to contain the same near-uv conservative bands as the 5′-AMP spectrum, with the bands inverted. The complex obtained with the 3′ isomer thus appears to induce parallel base stacking analogous to that observed with the 5′ isomer. A nonconservative band

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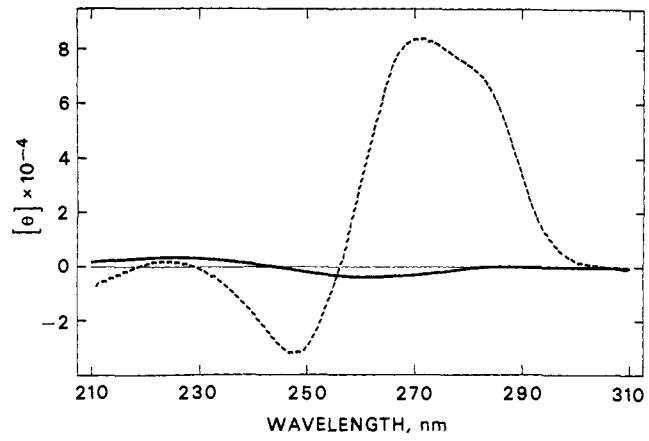

Figure 10. The enhancement of the CD spectrum of 3′-AMP by Zn(II) in the absence of polycation at pH 7: (——) 1×10^{-3} *M* 3′-AMP; (- - - -) 1×10^{-3} *M* Zn(II) and 1×10^{-3} *M* 3′-AMP.

analogous to that indicated in the 5′-AMP spectrum (Figure 1) is observed at 283 nm.

The inversion of the CD bands can be readily explained by a structure in which the metal coordinates between the phosphate of one nucleotide and the base of an adjacent nucleotide. The movement of the phosphate from the 5′ to the 3′ position rotates its position relative to the bases, and therefore the transition moments of the two bases in the complex will be rotated relative to each other. Since the sign and magnitude of the CD bands due to parallel stacking depend on the angle between the transition moments of the two bases,[5] the sign and magnitude of the CD bands are expected to change. The 280- and 290-nm shoulders are not resolved in the uv spectrum of the 3′-AMP complex because of the turbidity.

The titration curve of Zn(II)–3′-AMP does not indicate a decrease in the p*K* of the phosphate group (Figure 3) even though the spectropolarimetric titration indicates that the complex is not formed while the phosphate is protonated (Figure 4B). The effect on the zinc hydrolysis is similar to that noted with 5′-AMP; the OH^- ions react only at higher pH (Figure 3). The retardation of the OH^- reaction is not pushed to nearly as high pH in the 3′-AMP complex, and this fact, together with the failure of the phosphate p*K* to decrease, is an indication that the 3′-AMP complex is less stable than the 5′ complex. This conclusion is consistent with a decrease in the thermal stability of the 3′-AMP complex. The rotatory strength of the latter diminishes above 60°, while no decrease is observed with 5′-AMP even at 75°.

Unlike the Zn(II)–5′-AMP complex, the 3′ complex does not immediately precipitate in the absence of polypeptide, and it is therefore possible to obtain a CD spectrum of the latter in the absence of polypeptide. This spectrum (Figure 10) is quite similar to the spectrum in the presence of polypeptide, although it exhibits considerably lower rotatory strength. The similarity of the spectra supports the hypothesis that the rotatory dispersion effect is due primarily to the interaction of zinc and AMP, and that the main role of the polypeptide is to enhance the solubility. The fact that the spectrum in the absence of the polypeptide (Figure 10) is somewhat less conservative and slightly blue shifted does, however, suggest that the interaction with

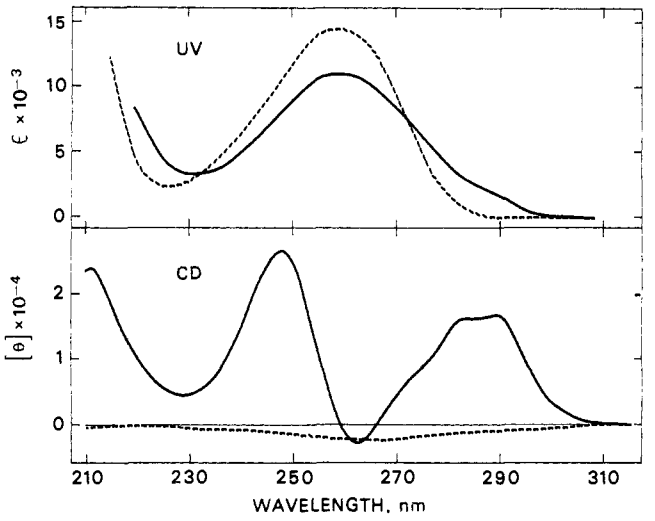

Figure 11. Uv and CD spectra of a solution of 1×10^{-3} *M* 5′-dAMP at pH 7: (----) without further addition; (——) in the presence of 2×10^{-3} *M* poly-L-lysine and 1×10^{-3} *M* zinc(II).

the polypeptide can perturb the structure of the complex.

The structural relationship between the 5′ and 3′ complexes is further demonstrated by a study of the reactions of a large number of metal ions with 3′-AMP, and comparing the results with those already noted for the 5′ isomers. Mg(II), Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Hg(II), Ag(I), Fe(III), Cr(III), Al(III), and Pb(II) were investigated. The CD spectrum of none of the metals except Zn(II) produces a CD spectrum of substantial magnitude. Not even lead produces the effect with the 3′ isomer. The titration curve of Cu(II)–3′-AMP (in Figure 6A) resembles that of Cu(II)–5′-AMP and not that of the Zn(II)–AMP isomers. The base specificity of the 3′ isomer was checked by studying the effect on the ORD spectrum of adding Zn(II) and poly-L-lysine to 3′-GMP. As in the reaction of Zn(II) and 5′-GMP, no significant increase in rotatory strength is observed.

The similarity of the 3′- and 5′-AMP complexes becomes particularly evident, however, when their properties are compared with those of the 2′-AMP complex. Figure 3 demonstrates that the titration of Zn(II)–2′-AMP proceeds very differently from the titration of the complexes of the other isomers. Zinc hydrolysis, rather than occurring at elevated pH, actually begins at a lower pH than in the absence of nucleotide, and produces a complex containing only one OH^- ion per two zinc(II). The uv spectrum of 2′-AMP is not at all affected by Zn(II). The effect of Zn(II) on the CD spectrum of 2′-AMP (in the presence of poly-L-lysine) is much smaller than that on 5′-AMP, and the minimum is at 270 nm, corresponding to a shift of ~10 nm relative to 5′ and 3′-AMP (Figure 9). 2′-AMP differs from 3′- and 5′-AMP in that the phosphate group can be made to approach closely to N-3, thus making possible the formation of a chelate involving the phosphate and N-3. Such a chelate has been postulated for Cu(II)–2′-AMP on the basis of nmr evidence[3] and may be postulated for the zinc complex to explain its differences from the zinc complexes of the other isomers. The stoichiometry of the reaction with OH^- ions indicates that one OH^- is shared between two zinc(II) atoms. It is not obvious why this should be, but it can be explained by the formation of a binuclear complex in which an OH^- ion constitutes a common corner of two zinc tetrahedra.

The Role of the 2′-Hydroxyl Group. When zinc is added to 5′-dAMP in the presence of poly-L-lysine the CD and uv spectra of Figure 11 are obtained. It can be seen from this figure that the removal of the 2′-OH group from the 5′ isomer produces a highly rotatory complex, but one which does not have conservative bands, indicating that while the bases do interact they are no longer oriented parallel to each other. The uv spectrum shows a large hypochromicity[22,23] and a shoulder at 290 nm. The titration curve of zinc(II)–5′-dAMP is superimposable on the titration curve of 5′-AMP. These phenomena support the conclusion that similar but not identical complexes are produced in the presence or absence of the 2′-OH group. The zinc complex of 3′-dAMP has a CD spectrum which is less intense than that of the 5′-dAMP complex even though the 3′-ribo complex has a rotatory strength that is so much larger than that of the 5′-ribo complex (Figure 9). Thus, the 2′-OH group seems to play a more important role in the 3′-AMP complex than in the 5′-AMP complex.

The Specificity of the Reaction. The experiments described above demonstrate that a complex which induces parallel base stacking is produced by the interaction of 1×10^{-3} *M* 5′-AMP or 3′-AMP with Zn(II), generally in the presence of a polycation. Parallel base stacking is not observed under such conditions when numerous other metals are substituted, or when other nucleotides are employed or when the AMP phosphate is 2′, or 2′,3′ cyclic, or 3′,5′ cyclic, or when the 2′-OH group is removed.

The specificity of this effect, restricted as it is to two isomers of one nucleotide and one metal ion,[24] is highly unusual, especially when it is considered that many of the metal ions that do not produce a complex with conservative CD bands do nevertheless form AMP complexes that have been detected by other means.[1-3,25] Furthermore, other nucleotides[26,27] (and nucleosides[28]) form complexes with zinc. The specific requirement of Zn(II) and certain isomers of AMP for the conservative CD effect observed at low concentration thus does not imply that other metal ions and other nucleotides do not form complexes. Complex formation of Pb(II) with 5′-AMP (Figure 5) and Zn(II) with the deoxy isomers of AMP (Figure 11) is already apparent from the CD spectra. The titration curves of 1×10^{-3} *M* Zn(II) with 5′-GMP (Figure 8) and 1×10^{-3} *M* Cu(II) with 5′-AMP and 3′-AMP (Figure 6A) indicate that a complex involving one OH^- per metal ion can be produced in the absence of any significant ORD or CD effects. The specificity can be explained in terms of an equilibrium between an unstacked complex and a

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(24) If a large increase in rotatory strength instead of the conservative CD spectrum and the parallel stacking deduced therefrom is used as the criterion for specificity, it will be restricted to two metal ions.
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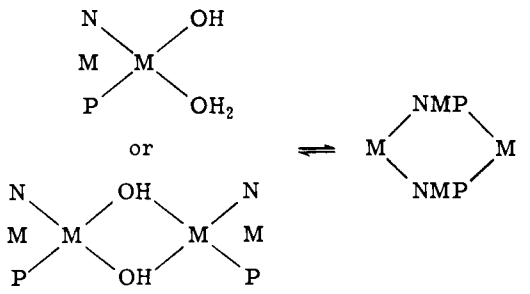
stacked complex as follows



where M represents a metal and NMP a nucleotide. The dominant complex formed by any metal or any nucleotide under any set of conditions is determined by the relative stability of the two types of complexes. The formation of a stacked complex is no indication of a greater tendency to form a metal complex but only that the stacked complex in this case is the more stable complex.

The fact that different reactions occur between AMP and Cu(II) at two different concentrations (Figure 6) suggests that the equilibrium between the various types of complexes can be shifted by a change in concentration.[29] Copper ions, which generally form stronger complexes than zinc ions, would be expected to complex AMP at least as readily as zinc, but the complex produced probably does not contain stacked bases at low concentration, so that higher concentrations are required to produce the stacking. Some metal ions and some nucleotides perhaps never produce a stacked conformation at any concentration.

The requirement of the adenine base for the formation of the stacked complex at a concentration of 1×10^{-3} *M* may be related to the fact that adenine is the only purine studied which does not have an oxygen at position 6. It has been postulated that metal binding to guanosine and inosine[30] involves chelation through N-7 and O-6. Such chelation, if it occurs, could account for the greater stability of the nonstacked complexes.

A previous study of Zn(II) nucleoside diphosphate complexes can be interpreted as indicating a specific reaction with ADP.[31] It was found that ADP broadens the nmr spectrum of ^{35}Cl from $ZnCl_2$ to a greater extent than any of the other diphosphates. Furthermore, a difference spectrum is observed between Zn(II)–ADP and ADP which, though much less intense than those observed here with AMP, is much more intense than with Mn(II) or Mg(II). These results suggest the possibility that a similar specific Zn(II) complex is formed with ADP, but that the additional phosphate greatly diminishes the spectral effects observed. This possibility is consistent with the fact that the replacement of ADP by ATP, involving yet an additional phosphate, further decreases the uv difference spectrum with zinc by 80%.[31]

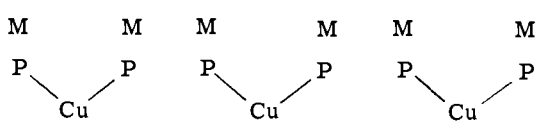
Differences in the reactivities of 2′ nucleotides and 3′ and 5′ nucleotides have been previously observed. In the binding of GMP to basic polypeptides there is a cooperative interaction between nucleotides which is greater for the 5′ and 3′ isomer than the 2′ isomer.[32,33] The stacking of AMP at high concentration is greater for 5′- and 3′-AMP than 2′-AMP and also qualitatively different.[34] As already noted, Cu(II) reacts differently with the 2′ isomer than with the 3′ and 5′ isomers.[3]

Since AMP and Zn(II) are both important cellular constituents, the stacking tendency induced in the former by the latter could have important biological implications.

On the Structure of the Zn–AMP Complex. The fact that the conservative CD bands in the absorbance region of the adenine base of the complex are strikingly similar to those observed with the dinucleotide and the single-stranded polymer leads to the conclusion that the bases are held in a position favorable for parallel stacking. The lack of such an effect with adenosine and the lowering of the phosphate p*K* of 5′-AMP by zinc(II) implicate the phosphate group as a binding site, and the base specificity suggests that a site on the base is also bound. These observations suggest a structure for the complex in which nucleotides are linked together by a phosphate–metal–base linkage instead of the phosphodiester linkage characteristic of oligo- and polynucleotides. Corey–Pauling–Koltun (CPK) models indicate that such a linkage is compatible with parallel stacking of the bases.

The phosphate–metal–base linkage can also perhaps account for the prominent shoulders at 280 and 290 nm in the uv spectrum of the 5′-AMP complex (Figure 1) as well as the nonconservative CD bands in this region of the spectra for both the 5′ and 3′-AMP complexes (Figures 1 and 9). The very weak absorption bands occasionally resolved on the long-wavelength side of the major $\pi-\pi^*$ transition of adenine have usually been attributed to $n-\pi^*$ transitions,[7,13,14,35] even though other assignments seem to be required in certain cases.[14,36] $n-\pi^*$ transitions oriented perpendicular to the plane of the base display a hyperchromic effect due to stacking[22,35] and nonconservative CD bands.[7] The particular prominence of these bands in the Zn(II)–AMP stacked complexes, relative to stacked dinucleotides and polynucleotides, could be attributed to an enhanced perturbation perpendicular to the plane of the base, produced by the interaction of the metal and per-

(29) The titration of Cu(II)–AMP at low concentration (Figure 6A) does not of course differentiate between a mononuclear complex and a binuclear olated complex. The shift in the equilibrium to the right by an increase in concentration is most easily explained on the basis of a mononuclear structure for the substance on the left. The olated binuclear complex and the stacked complex represent the same degree of association so that the equilibrium between them should not be concentration dependent. If the stacked complex is visualized as a continuous array of parallel-stacked nucleotides, with metal ions holding together pairs, as shown



then the concentration dependence of an equilibrium between olated and stacked structures can nevertheless be explained.

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(36) M. Gellert, *ibid.*, **83**, 4664 (1961).

haps the ribose–phosphate grouping of one nucleotide with the base of the other nucleotide.

The exact position of the binding site on the adenine base is as yet unknown. Nmr studies on the copper complexes of 3′- and 5′-AMP have indicated that copper binds to N-7 on the base,[3] but since the zinc complexes are formed under somewhat different conditions from those for the copper complexes, the binding sites may also be different. It can be demonstrated, through the use of CPK atomic models, that it is possible to obtain a parallel stacked structure maintaining the anti configuration of the base by linking the 5′ or 3′ phosphate to N_3, N_1, or N_7. CPK model studies also indicate that two types of stacked structures are possible. The two opposite sides of the base can be in contact, as in poly(A), or the same side of each base can be in contact, as has been proposed for the Cu–AMP complex,[3] for stacked nucleotides at high concentration,[34] and for 5′,5′-diadenosine.[9,37]

The continuous variation study shows that the complex contains the same number of zinc(II) atoms and AMP molecules. This 1:1 stoichiometry can be satisfied by a 2:2 complex of the type postulated for the Cu(II) reaction in which the face of one nucleotide is in contact with the same face of the other.[3] The CD bands which result from π interaction in the stacked bases of the dinucleotides linked through the 5′ phosphate are red shifted relative to the bands of the 3′–5′ dimer.[9,37] The conservative CD bands of the Zn–AMP complex are also shifted to the red, in line with the 2:2 binuclear structure.

The observed stoichiometry is also consistent with a face to back stacking that would be characteristic of a long polymeric structure in which each phosphate is linked by zinc(II) to the next base of the chain. Such a possibility is consistent with the precipitation of the nucleotide by Zn(II).

The role of the 2′-OH is particularly interesting because of the biological importance of the absence or presence of this group in a nucleotide. CPK models indicate that for the 3′ isomer it is possible for a Zn(II) ion which is coordinated to the phosphate and the base to bind also to the 2′-OH. Such additional coordination would increase the rigidity of the orientation of the bases relative to each other and thus help to contribute to the extremely high rotatory strength of the 3′ ribonucleotide complex. The removal of the 2′-OH would then weaken the structure and produce a drastic decrease in the rotatory strength.

On the other hand, with the 5′ isomer the 2′-OH cannot be directly involved in the complex unless N_3 is the base binding site. Furthermore, the titration behavior and uv spectra indicate that the 5′-dAMP complex is very similar to the 5′-AMP complex. The added possibility thus exists that the effect of the 2′-OH group is an indirect one on the configuration of the nucleotide, affecting the relative orientation of the bases in the complex. Such a possibility is consistent with the difference in the furanose ring conformation found for deoxyadenosine and riboadenosine.[38] A relative decrease in the rotatory strength is also found when the deoxy dimer and polymer are compared with the ribo dimer and polymer, and poly(dA), unlike poly(rA), does not exhibit conservative CD bands.[7]

The mechanism by which polypeptides or other polycations cause solubilization of the zinc nucleotide complex is not certain. Positive charges on the polypeptide may bind electrostatically to residual negative charges on the phosphates, or electron donor groups (such as amino groups) may bind to the zinc ions. The fact that the partly soluble Zn(II)–3′-AMP produces a similar conservative CD effect in the presence or absence of polypeptide indicates that the effect itself does not depend on the polypeptide.

Acknowledgment. The excellent technical assistance of Mrs. Joan Green, Mrs. William Heim, and Mr. Edward Tarien is gratefully acknowledged.

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Communications to the Editor

Oxidation of Cobalt(I) Carbonyl Complexes and Cobalt(I)-Catalyzed Oxidation of Carbon Monoxide

Sir:

In the context of the intense current interest in reactions of coordinated ligands and in the roles of such reactions in homogeneous catalysis, we wish to describe some novel reactions involving the oxidation of cobalt(I) carbonyl complexes and the catalysis by such complexes of the oxidation of carbon monoxide. Our results are also of some interest in connection with recent controversial accounts concerning the mechanisms of reduction of vitamin B_{12a} and related cobalt(III) complexes by CO.[1-4]

Our studies relate to the oxidation of the ion $[Co^{I}(CN)_2(PEt_3)_2(CO)]^-$ (**1**), whose preparation we have recently described,[5] by $Fe(CN)_6^{3-}$. In alkaline aqueous solutions containing excess $Fe(CN)_6^{3-}$, the overall oxidation of **1** proceeds quantitatively in accord with eq 1 to yield $[(NC)_5Fe^{II}CNCo^{III}(CN)_2(PEt_3)_2(H_2O)]^{3-}$ [**2**, λ_{max} 410 nm (ϵ_{max} 1.54×10^3)] which we have isolated in pure form as the potassium salt and fully characterized.[5] **2** is the analog of the ion, $[(NC)_5Fe^{II}CNCo^{III}$-

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