ulative, there is at least some physical evidence for each of its features. These features are generally susceptible to test by further spectroscopic studies. Experiments and conformational calculations are now in progress to test these various features.

Acknowledgments

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References

Aubert, J. M., Biserte, G., & Loucheux-Lefebvre, M. H. (1976) *Arch. Biochem. Biophys. 175*, 410.
Beeley, J. G. (1977) *Biochem. Biophys. Res. Commun. 76*, 1051.
Bolton, C. H., Hough, L., & Khan, M. Y. (1966) *Biochem. J. 101*, 184.
Bush, C. A., & Duben, A. J. (1978) *J. Am. Chem. Soc. 100*, 4987.
Chen, W. W., & Lennarz, W. J. (1978) *J. Biol. Chem. 253*, 5775.
Chou, P., & Fasman, G. D. (1977) *J. Mol. Biol. 115*, 135.
Coduti, P. L., Gordon, E. C., & Bush, C. A. (1977) *Anal. Biochem. 78*, 9.
Delbaere, L. T. J. (1974) *Biochem. J. 143*, 197.
Dorland, L., Schut, B. L., Vliegenthart, J. F. G., Strecker, G., Fournet, B., Spik, G., & Montreuil, J. (1977) *Eur. J. Biochem. 73*, 93.
Duben, A. J., & Bush, C. A. (1979) *Anal. Chem.* (in press).
Feeney, J. (1976) *J. Magn. Reson. 21*, 473.
Hirano, S. (1972) *Agric. Biol. Chem. 36*, 1071.
Horton, D. (1964) *J. Org. Chem. 29*, 1776.
Kopple, K. D., & Go, A. (1977) *J. Am. Chem. Soc. 99*, 7698.
Li, E., Tabas, I., & Kornfeld, S. (1978) *J. Biol. Chem. 253*, 7762.
Lis, H., Lotan, R., & Sharon, N. (1974) *Methods Enzymol. 34*, 341.
Ramachandran, G. N., Chandrasekaran, R., & Kopple, K. D. (1971) *Biopolymers 10*, 2113.

Stabilization of Nucleic Acid Secondary Structure by Cationic Metal Complexes†

Richard L. Karpel,‡ Arthur H. Bertelsen, and Jacques R. Fresco*

ABSTRACT: Complexes of inert (slowly exchanging) multipositive transition metal cations and neutral ligands very effectively stabilize both RNA and DNA double helices against thermal denaturation. This indicates that these cations prefer binding sites on double helices over those on single strands. The preferential affinity of the tripositive complexes for the double helices poly(I·C) and poly(U·U) is so great that it results at low [cation]/[polynucleotide phosphate] ratios in biphasic melting of these helices. The interaction between the complex cations and helical nucleic acids has a strong electrostatic component since T_m enhancement is diminished with increasing sodium ion concentration. In addition, the trivalent complexes, $Co(NH_3)_6^{3+}$, $Co(en)_3^{3+}$, and $Pt(en_2)(en\text{-}H)^{3+}$, show greater enhancement of T_m than the divalent ones, $Ir(NH_3)_5Cl^{2+}$ and $Pt(NH_3)_4^{2+}$. These tripositive complexes are similarly effective in stabilizing poly(I·C), suggesting that the helical binding sites for these cations are less discriminating than the sites to which they bind in stabilizing the tertiary structure of $tRNA_3^{Leu}$ [Karpel, R. L., Miller, N. S., Lesk, A. M., & Fresco, J. R. (1975) *J. Mol. Biol. 97*, 519-532]. On the other hand, $Ir(NH_3)_5Cl^{2+}$ was more effective in stabilizing poly(I·C) than was $Pt(NH_3)_4^{2+}$. The two groups of complexes had a lesser effect on the thermal stability of DNA helices. A quantitative treatment of the dependence of T_m on complex cation concentration is used to obtain association constants and the helical binding site size.

Nucleic acids, by virtue of their polyanionic nature, require the presence of cations to stabilize double-helical secondary structure. Cations can reduce the electrostatic energy of these molecules through two different mechanisms, by Debye-Huckel shielding of the phosphates and by site binding to them [cf. Felsenfeld & Miles (1967)]. The requirement for cations is well illustrated by the variation of the thermal denaturation of DNA with the concentration of shielding monovalent alkali metal ions such as Na^+ and K^+, which shows a linear dependence of the melting temperature, T_m (the temperature at the midpoint of the cooperative helix-coil transition), on log cation concentration (Schildkraut & Lifson, 1965). Obviously, diminution of the electrostatic repulsion of interstrand phosphates by cations increases the stability of the helix. Indeed, divalent alkaline earth metal ions such as Mg^{2+} or Ca^{2+}, which site bind (only) to the phosphate moieties, increase the T_m of nucleic acid helices even more dramatically (Dove & Davidson, 1962).

All metal ions do not, however, affect nucleic acids in this manner. Several transition metal ions with an affinity for the phosphates exhibit complex dependencies of T_m on cation concentration because of the additional tendency to coordinate to purines and, to a lesser extent, to pyrimidines (Izatt et al., 1971; Eichorn, 1973). Some of these cations, e.g., Cu^{2+}, even unwind nucleic acid helices because they cannot be accommodated in the helix when they bind tightly to the bases (Eichhorn & Shin, 1968; Eichhorn & Tarien, 1967), while others, such as Zn^{2+} and La^{3+}, also catalyze the scission of

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the phosphodiester bonds of polyribonucleotides (Butzow & Eichorn, 1965, 1971).

The interaction of multivalent metal complex cations with nucleic acids has until now received little attention. Previously there have been reports of the binding of several Co(III) complexes to native and denatured calf thymus DNA (Ascoli et al., 1972, 1973) and of various multivalent complexes to tRNA (Karpel et al., 1975). As the present and these earlier studies show, complex cations are unusually effective in stabilizing the secondary and tertiary structures of nucleic acids. The particular complexes employed in the present work consist of charged (≥2+) central metal ions fully coordinated to the nonexchanging neutral ligands, ammonia or ethylenediamine. Substitution of the inner coordination sphere of the complex is virtually precluded, and interaction with nucleic acids is presumably restricted to "outer-sphere" bonding to phosphates, so that there is little likelihood of hydrolysis of the polynucleotide chain.

The stabilization of several synthetic and natural helical polynucleotides, poly(I·C), poly(U·U), poly[d(A-T)], calf thymus DNA, and *Clostridium perfringens* DNA, by the octahedral cations $Pt(en)_2(en\text{-}H)^{3+}$, $Co(en)_3^{3+}$, $Co(NH_3)_6^{3+}$, and $Ir(NH_3)_5Cl^{2+}$ (see footnote 1) and the square-planar cation $Pt(NH_3)_4^{2+}$ was investigated. The results of this work indicate that several of the complex cations bind unusually tightly and selectively to helical forms of nucleic acids when the concentration of alkali metal cations is very low. These complex metal ions should therefore prove useful in probing nucleic acid and nucleoprotein structure by a variety of techniques.

Materials and Methods

Complex Cations. Tris(ethylenediamine)cobalt(III) chloride $[Co(en)_3Cl_3]$ was prepared according to Work (1946), pentaminechloroiridium(III) chloride $[[Ir(NH_3)_5Cl]Cl_2]$ was prepared according to Schmidt (1970), and tris(ethylenediamine)platinum(IV) chloride $[Pt(en)_3Cl_4]$ was prepared according to Giedt & Nyman (1966) and then exhaustively recrystallized from HCl–H_2O. Hexaminecobalt(III) chloride $[Co(NH_3)_6Cl_3]$ was the gift of Professor T. G. Spiro, and tetramineplatinum(II) chloride $[Pt(NH_3)_4Cl_2]$ was the gift of Matthey-Bishop, Inc. All starting materials in the syntheses were of reagent or comparable grade, as were all other chemicals used.

Polynucleotides. Poly(I) and poly(C) were prepared by the action of polynucleotide phosphorylase from *Mycobacterium lysodeikticus* on their respective nucleoside disphosphates (Grunberg-Manago et al., 1956). The polymers were purified and then dialyzed exhaustively vs. 0.01 M EDTA to remove divalent cations and then vs. H_2O. The several preparations of each polynucleotide used exhibited no significant differences in melting profile. Poly[d(A-T)] was from P-L Biochemicals, poly(U) was from Miles Laboratories, and calf thymus DNA was from Worthington Biochemical Corp. *C. perfringens* DNA was a gift from Dr. J. Marmur. All these nucleic acids were of a size range where T_m is essentially independent of helix length.

Samples for Absorbance–Temperature Profiles. Stock solutions of cations, nucleic acids, and salts were prepared in the standard minimal buffer (0.004 M Na^+ and 0.005 M cacodylate, pH 6.9) and were stored at 4 °C. Nucleic acid concentrations were determined spectrophotometrically, and complex cation concentrations were checked, where feasible, by conductance measurements.

Test solutions prepared from such stocks were $(4–5) \times 10^{-5}$ M (P) in nucleic acid (as indicated), 1×10^{-5} or 1×10^{-4} M in Na_2EDTA (to sequester any heavy metal impurities), $10^{-7}–10^{-5}$ M in complex cation (as desired), 0.005 M in cacodylate, pH 6.9, and 0.004 M or greater in Na^+ (as indicated).

Absorbance–Temperature Profiles. Teflon stoppered quartz cuvettes containing 0.5 mL of the test solution were placed in a Gilford Model 2000 recording spectrophotometer designed to raise the temperature from 0 to 98 °C at a constant rate of 21 °C/h or slower. To assure that equilibrium melting profiles were obtained, we checked them for reproducibility at two different rates of temperature increase. Cuvette temperature was monitored to a certainty that decreased from ±0.2 °C below 30 °C to a maximum of ±2 °C at 95 °C. Evaporation of samples during the course of such experiments was negligible. Absorbance was measured at the wavelength of maximum hyperchromic change for each nucleic acid by using a solvent blank that contained no complex cation. Control measurements of absorbance of the complex cation as temperature was varied over the full range revealed changes that were negligible relative to the absorbance changes of the nucleic acid samples, except at high complex cation concentrations and short wavelengths. Moreover, these absorbance changes were monotonic and did not affect the T_m values determined from the inflection points of the transitions. The latter were determined by differentiating the measured absorbance–temperature profiles (which were not corrected for thermal expansion). The reproducibility of the T_m values was ±1 °C or better, except where profiles were extremely broad.

Results

The complex cations significantly increase the T_m of both synthetic and natural nucleic acid helices. Helices stabilized by the dipositive ions $Pt(NH_3)_4^{2+}$ and $Ir(NH_3)_5Cl^{2+}$ display monophasic melting profiles and a positive dependence of T_m on log cation concentration. The tripositive complexes $Co(en)_3^{3+}$, $Co(NH_3)_6^{3+}$, and $Pt(en)_2(en\text{-}H)^{3+}$ (see footnote 2), which ought to bind more tightly, produce a more dramatic enhancement of T_m and under certain conditions result in biphasic melting of helices of homogeneous sequence. In aperiodic DNA, melting profiles are broad, and discrete biphasic melting is not observed. These results suggest especially strong affinities of the trivalent cations for double-helical regions relative to the component single strands. In keeping with the significant electrostatic contribution to the site binding of these cations to nucleic acids, high Na^+ levels markedly reduce their enhancement of T_m.

RNA Helices. (1) Poly(I·C). This two-stranded helix displays a monophasic absorbance–temperature profile with $T_m = 32$ °C in the standard buffer ($[Na^+] = 0.004$ M). The addition of $Ir(NH_3)_5Cl^{2+}$ or $Pt(NH_3)_4^{2+}$ increases T_m monotonically with log [complex cation] (Figure 1a). Above 5×10^{-7} M ([cation]/[phosphate] ≡ R of 0.0125), $Ir(NH_3)_5Cl^{2+}$ is more effective in raising T_m than is $Pt(NH_3)_4^{2+}$. This is

[1] Although the chloride ligand coordinated to the Ir(III) ion reduces the charge of this complex, like the amines, it is nonexchanging (Schmidt, 1970).

[2] The salt, $Pt(en)_3Cl_4$, exists in aqueous solution in several protolytic species, with pK_a values of 5.5, 9.7, and 10.7 (Grinberg, 1962). At neutral pH, therefore, the dominant free species is $Pt(en)_2(en\text{-}H)^{3+}$, where en-H is $H_2NCH_2CH_2NH^-$. Our studies on the interaction of $Pt(en)_3Cl_4$ with yeast $tRNA_3^{Leu}$ in 0.15 M K^+ and 0.01 M cacodylate, pH 7.4, indicate that $Pt(en)_2(en\text{-}H)^{3+}$ is the major binding species (Karpel et al., 1975), and there is no reason to suspect that it is also not the dominant species interacting with polynucleotide helices.

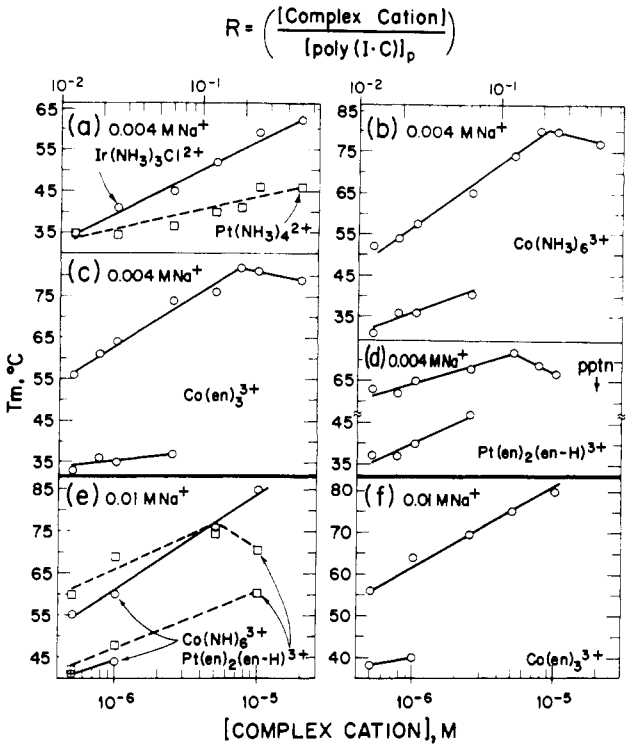

FIGURE 1: T_m variation of poly(I·C), 4.0×10^{-5} M (P), with complex cation concentration. In the absence of added complex cation, T_m = 32 °C in 0.004 M Na^+ and 38 °C in 0.01 M Na^+. Note that there are two plots in (b–f) because melting in these cases is biphasic (see text and Figure 2). (a) $Ir(NH_3)_5Cl^{2+}$, dT_m/d log [cation] = 17 °C; $Pt(NH_3)_4^{2+}$, dT_m/d log [cation] = 8 °C. (b) $Co(NH_3)_6^{3+}$, dT_m/d log [cation] = 24 °C (upper plot). (c) $Co(en)_3^{3+}$, dT_m/d log [cation] = 21 °C (upper plot). (d) $Pt(en)_2(en\text{-}H)^{3+}$, dT_m/d log [cation] = 10 °C (upper plot). (e) $Co(NH_3)_6^{3+}$, dT_m/d log [cation] = 23 °C (upper plot); $Pt(en)_2(en\text{-}H)^{3+}$, dT_m/d log [cation] = 13 °C (upper plot). (Melting in 5.0×10^{-6} M complex cation was monophasic but was reproducibly biphasic at 1.0×10^{-5} M.) (f) $Co(en)_3^{3+}$, dT_m/d log [cation] = 20 °C (upper plot). (The melting profiles in 2.5×10^{-6} and 5.0×10^{-6} M cation were extremely broad.)

reflected in the much higher dependence of T_m on the concentration of the Ir cation, 18 °C (dependences are expressed throughout as dT_m/d log [complex]), than on that of the Pt cation, 8 °C. However, in 0.15 M Na^+ (data not shown), where T_m in the absence of complex cation is 61 °C, neither dipositive ion produces any change in the melting profile, even at 1×10^{-5} M (R = 0.25). This inhibition of complex cation enhancement of T_m by high levels of Na^+ suggests that the interaction between the complexes and the polyanionic poly(I·C) has a major electrostatic component. While it is possible that Na^+ competes with the multivalent cations for specific binding sites on the polynucleotide, it seems more likely that site binding of the complex cations is weakened by the Debye–Huckel shielding of 0.15 M Na^+.

The effect of the tripositive complexes, $Co(NH_3)_6^{3+}$, $Co(en)_3^{3+}$ and $Pt(en)_2(en\text{-}H)^{3+}$, on the thermal denaturation of poly(I·C) differs from that of the divalent cations in two important aspects. First, in 0.004 M Na^+ they markedly raise T_m, even at low R values (upper plots of Figure 1b–d). For example, at a tripositive cation concentration of 5×10^{-6} M (R = 0.125; charge ratio = $3R$), T_m is raised 40 °C or more. At the same R value (charge ratio = $2R$), T_m is increased 20 °C by $Ir(NH_3)_5Cl^{2+}$ and only 8 °C by $Pt(NH_3)_4^{2+}$.

The second and more striking aspect of the interaction of the trivalent complexes with poly(I·C) in 0.004 M Na^+ is that biphasic melting occurs. This is clearly evident in Figure 2 in both the observed and differentiated profiles.

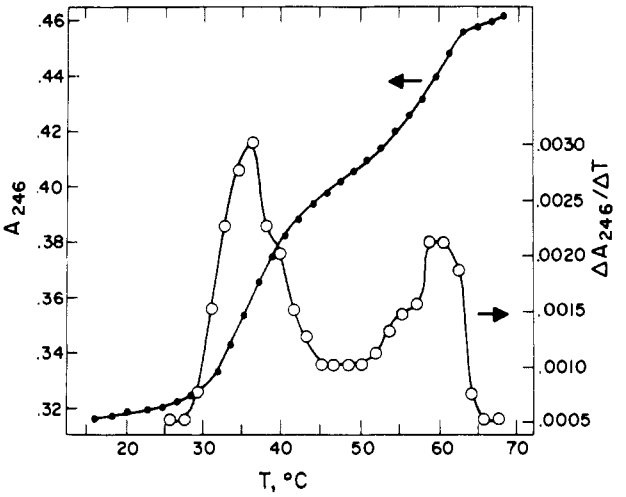

FIGURE 2: Biphasic melting of poly(I·C), 4.0×10^{-5} M (P), in the presence of 7.5×10^{-7} M $Pt(en)_2(en\text{-}H)^{3+}$ and 0.004 M Na^+. Both absorbance and derivative profiles are shown. Similar profiles were obtained with the same concentration of $Co(en)_3^{3+}$ and $Co(NH_3)_6^{3+}$.

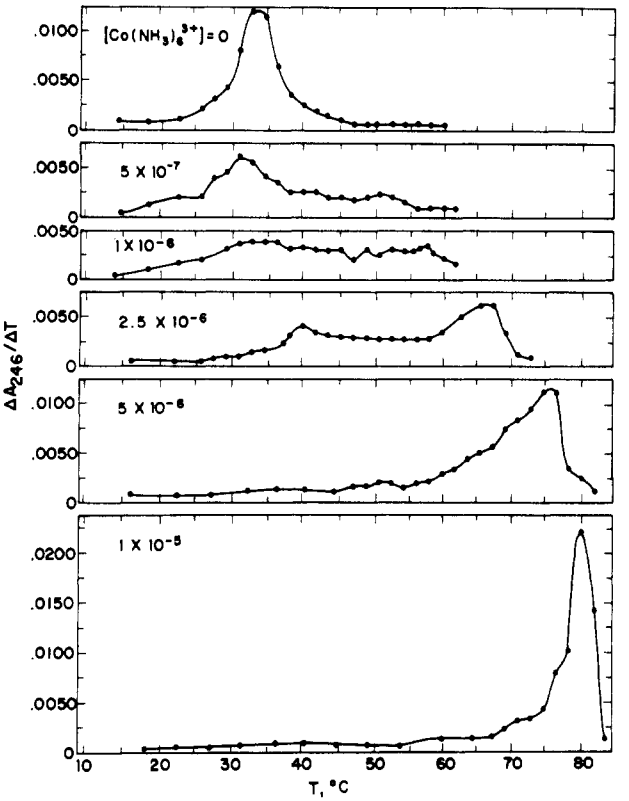

FIGURE 3: Derivative melting profiles showing biphasic melting of poly(I·C), 4.0×10^{-5} M (P) and 0.004 M Na^+, with varying $Co(NH_3)_6^{3+}$ concentration. Similar behavior was obtained with the other two trivalent complex cations.

Figure 3 shows a series of differentiated melting profiles of a constant concentration of poly(I·C) containing increasing levels of $Co(NH_3)_6^{3+}$. The observation of biphasic melting at low R values for the homogeneous poly(I·C) helix can be easily explained qualitatively. As melting proceeds, cations migrate from those double-stranded regions just denatured to residual double-helical segments. This effect is a likely consequence of the stronger affinity of the complex cations for double- vs. single-stranded regions (see below and Discussion). The *effective* R values for these remaining segments are thereby raised, adding to their stability, so that a portion of the helix melts at a higher temperature. Indeed, Figure 3

shows that, as R is raised, that portion increases, eventually encompassing 100% of the helix, hence, the reappearance of monophasic melting at high complex cation concentration. An alternative explanation for the biphasic melting, that it is due to the formation of some structural variant of the poly(I·C) helix at intermediate temperatures, appears unlikely since the difference spectra resulting from melting through the first and second transitions were not significantly different.

The preferential affinity of the complexes for double-stranded regions was further examined by observing the effect of added poly(C) (single stranded) on T_m of poly(I·C) in the presence of 5×10^{-6} M $Co(en)_3^{3+}$ ($R = 0.125$) and 0.004 M Na^+. Under these conditions, in which the complex cation is not saturating, the melting of poly(I·C) is monophasic, and the addition of poly(C) was such that the ratio of single-stranded to double-stranded residues *at* T_m was 3 or 5, T_m was depressed by only 5.5 or 11 °C, respectively. Extrapolating these results, the effect of complex cation binding to the single strands present at T_m in the absence of added poly(C) would be only about 2 °C. When compared with the 44 °C enhancement of T_m by the complex cation under these conditions, this very small T_m depression demonstrates minimal complex cation affinity for single-stranded regions, relative to double-stranded regions.

dT_m/d log [complex] for the higher transition observed with either trivalent cobalt complex [22 °C for $Co(en)_3^{3+}$; 23 °C for $Co(NH_3)_6^{3+}$], not surprisingly, is greater than that for the single transition observed in the presence of the divalent cations [18 °C for $Ir(NH_3)_5Cl^{2+}$; 8 °C for $Pt(NH_3)_4^{2+}$]. On the other hand, this parameter is only 11 °C for trivalent $Pt(en)_2(en\text{-}H)^{3+}$. However, since precipitation occurred on raising the temperature of a solution of poly(I·C) containing 2.0×10^{-5} M $Pt(en)_2(en\text{-}H)^{3+}$, aggregation or even some precipitation probably occurred at lower concentrations of this complex cation, thereby lowering the effective R. This could account for the falloff in T_m observed above 5.0×10^{-6} M as well as the curiously small dT_m/d log [complex] for this ion. Although no precipitation was observed with the trivalent cobalt complexes, there was also some falloff in T_m at high R values with these ions. A low level of hydrolysis at elevated temperature could also have contributed to this falloff in T_m, since generally only 80–90% of the original hyperchromic change was attained after remelting samples that had been reequilibrated overnight at 4 °C. On the other hand, this decrease in hyperchromicity could be due to a low level of amine ligand exchange from the complexes at high temperature; this would free some (central) metal ions, which could bind to bases of the melted single strands and so prevent full renaturation of the helix.

Another possible cause of the falloff in T_m at high R might be the existence of helix-destabilizing van der Waals interactions between polarizable solvent anions and stacked bases of the helix (Daune, 1974). Such interactions could only occur when the negative charge of the phosphates, which in the absence of the complex cations excludes anions from the helix, is sufficiently neutralized. Whatever the explanation of this inverse dependence of T_m at high complex cation concentration, the critical features of the absorbance–temperature profile were not affected significantly since, upon remelting such samples, the T_m values were reproduced. (A change in R of 10–20%, which could be brought about by a low level of polynucleotide hydrolysis, would not produce a deviation in T_m of more than 1–2 °C; viz., Figure 1.)

Even without these complications, if the equilibrium binding constant between complex and helix is sufficiently large, and the complex cations can closely pack in groove sections containing three phosphates, a discontinuity in the plot of T_m for poly(I·C) vs. [trivalent complex] would be expected at $R = 0.33$, the equivalents point for cation titration of polynucleotide in the absence of a strongly competing ionic medium. In Mg^{2+}–DNA mixtures, in the virtual absence of competing ions, this end point (discontinuity) occurs at $R = 0.5$, and above this ratio, the increase in T_m is much more gradual (Dove & Davidson, 1962). However, for the trivalent complexes in 0.004 M Na^+, the titration end point is reached *below* $R = 0.33$ (Figure 1b–d). This suggests that poly(I·C) is apparently saturated by site-bound cations at less than complete ionic neutralization, even though the 0.004 M Na^+ may slightly reduce the binding affinities of polynucleotide for complex cations (see Appendix). Consistent with this interpretation is the observation that single-phase melting occurs at high temperature also before complete neutralization with these complex cations.

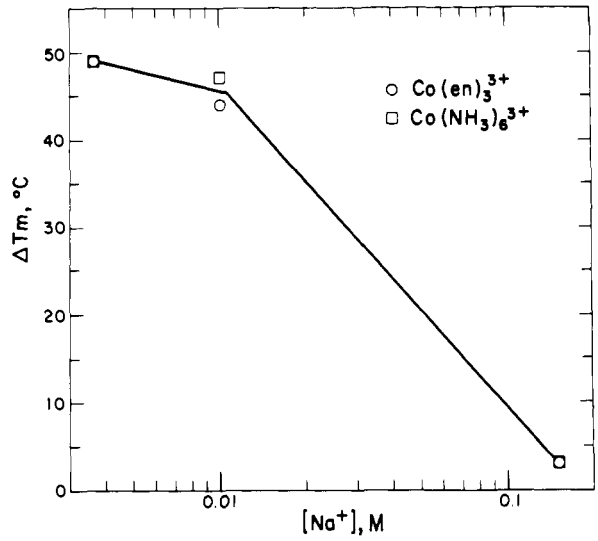

FIGURE 4: Variation of ΔT_m of poly(I·C), 4.0×10^{-5} M (P), with increasing $[Na^+]$ in the presence of 1.0×10^{-5} M $Co(en)_3^{3+}$ or $Co(NH_3)_6^{3+}$. ($\Delta T_m \equiv$ the difference between T_m in the presence and absence of complex cation.)

An increase in $[Na^+]$ of the solvent from 0.004 to 0.010 M does not alter the general melting behavior. This must be because the helical structure is still largely stabilized by the complex cations, whose Coulombic affinity for polynucleotide sites is not effectively reduced by the 2.5-fold increase in $[Na^+]$. Thus, without complex cations, T_m is an expected 6 °C higher. In the presence of low levels of the tripositive complexes, biphasic melting occurs as before. At given R values, T_m of the first transition occurs 5–10 °C above that obtained in 0.004 M Na^+ (Figure 1e,f), but T_m for the upper transition is hardly responsive to the higher $[Na^+]$. This is as expected because the low melting helices are unsaturated with complex cations and therefore sensitive to Debye–Huckel shielding, whereas the high-temperature helices are saturated and therefore insensitive to 0.01 M Na^+.

At much higher $[Na^+]$, 0.15 M, the effects of the tripositive complexes become minimal; biphasic melting does not occur and T_m enhancement of the intrinsic value of 61 °C is marginal, showing there is very little complex cation site bound to helix. For example, at 1.0×10^{-5} M, either cobalt complex raises T_m only 3 °C and $Pt(en)_2(en\text{-}H)^{3+}$ raises T_m only 4 °C. At this R value, these complexes cause a ΔT_m of ≥40 °C in 0.004 M Na^+. This difference shows that the interaction of these complexes with the polynucleotide is largely electrostatic. A plot of ΔT_m vs. log $[Na^+]$ at 1.0×10^{-5} M complex cations (conditions leading to a single transition; Figure 4) shows that

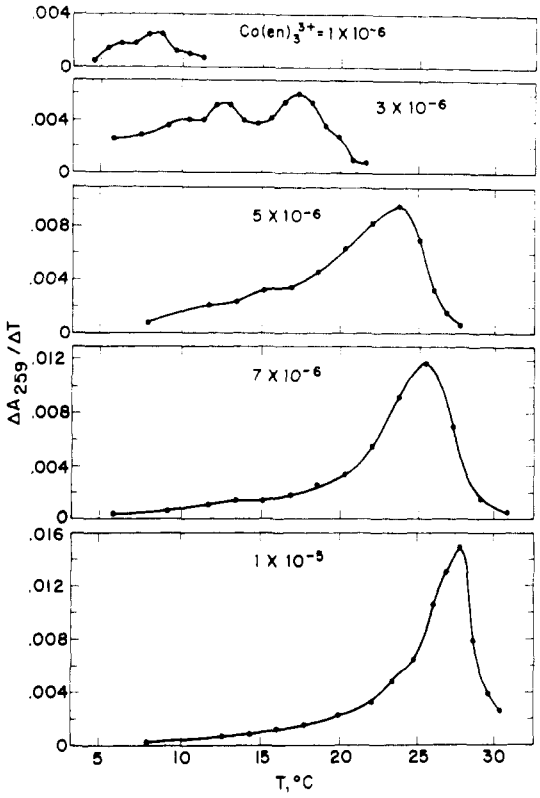

FIGURE 5: Derivative melting profiles showing biphasic melting of poly(U·U), 4.1 × 10^{-5} M (P) and 0.004 M Na^+, with varying Co-$(en)_3^{3+}$ concentration.

Na^+ does not effectively compete with the complex cation below 0.01 M, which is still 3 orders of magnitude greater than the complex ion concentration.

(*2*) *Poly(U·U).* In the presence of low levels of multivalent cations or molar concentrations of alkali metal ions, poly(U) forms a helix that is stable only at low temperature and is probably two-stranded and antiparallel (Zimmerman, 1976; Lipsett, 1960). The effect of $Co(en)_3^{3+}$ on the thermal stability of poly(U·U) was examined in order to determine whether the biphasic melting behavior observed with poly(I·C) might have wider relevance. It was also of interest to explore the feasibility of using cationic complexes in structural studies of helices, such as poly(U·U), that are otherwise unstable at room temperature.

In 0.004 M Na^+, maximal formation of poly(U·U) occurs only when the concentration of $Co(en)_3^{3+}$ reaches 5.0 × 10^{-6} M (R = 0.11) (Figure 5). Above this R value, T_m approaches a limiting value of 28 °C and the transition sharpens, but the amount of polynucleotide in helix, as judged from the hyperchromic change, while constant, is 10–15% less than that observed with the same amount of polymer stabilized by 1.2 × 10^{-5} M spermine (T_m = 29 °C). The temperature-dependent difference spectra of poly(U·U) stabilized by Co-$(en)_3^{3+}$ and spermine are nevertheless the same, suggesting that the structures are not different. The smaller hyperchromic change for the $Co(en)_3^{3+}$-stabilized helix was observed for all poly(U) samples studied, regardless of how the helix was allowed to form. Moreover, when melted solutions of poly(U) and $Co(en)_3^{3+}$ were cooled, the original A_{259} at 0 °C was attained, and on remelting, T_m values were reproduced. The interaction of this complex cation with poly(U) is thus fully reversible, with no evidence of hydrolysis.

At an intermediate concentration of $Co(en)_3^{3+}$, 3.0 × 10^{-6} M (R = 0.069), where helix formation is less than maximal, melting of poly(U·U) is clearly biphasic (Figure 5). With increasing R, the peak at lower temperature degenerates to a shoulder and eventually disappears (Figure 5). At constant $[Co(en)_3^{3+}]$ (2.0 × 10^{-5} M; R = 0.46), T_m drops dramatically with increasing $[Na^+]$ above 0.004 M (Figure 6), so that, at 0.25 M Na^+, T_m = 7 °C, only 1 °C above that obtained in the absence of $Co(en)_3^{3+}$. Again, a strong electrostatic component of complex cation–polynucleotide helix interaction is indicated.

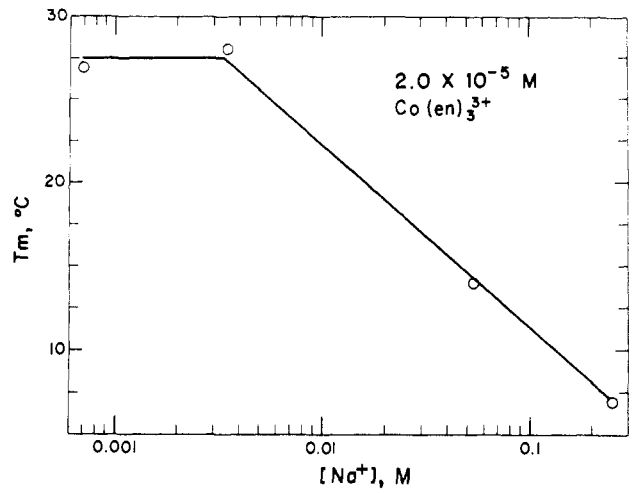

FIGURE 6: Variation of T_m of poly(U·U), 4.1 × 10^{-5} M (P), with increasing $[Na^+]$ in the presence of 2.0 × 10^{-5} M $Co(en)_3^{3+}$.

Though similar to $Co(en)_3^{3+}$ in its ability to stabilize poly(I·C), $Co(NH_3)_6^{3+}$ in 0.004 M Na^+ is less effective with poly(U·U). In 1.0 × 10^{-5} M $Co(NH_3)_6^{3+}$ (R = 0.23), poly-(U·U) displays biphasic melting with T_m = 19 and 23 °C, while with the same level of $Pt(en)_2(en\text{-}H)^{3+}$ only a smaller abrupt absorbance change at ~23 °C was observed. These results show that, as with poly(I·C), the trivalent cationic complexes stabilize poly(U·U); the somewhat different pattern of effectiveness might reflect the different topologies of the two helices. The effectiveness of the metal complexes is not markedly different from that of biogenic polyamines.

DNA Helices. (*1*) *Poly[d(A-T)].* In 0.004 M Na^+, the enhancement of T_m by $Co(en)_3^{3+}$ is somewhat less with this helix than with poly(I·C) (Figure 7). At low R (≤0.05) the dependence of T_m on complex cation concentration is considerably less (8 °C) than that seen with poly(I·C), but at R ≥ 0.05, it is the same (22 °C) for both (cf. Figure 1). The differential melting curve at R = 0.048 is considerably broader than the profile obtained in the absence of the complex (Figure 8). Two maxima are reproducibly observed under these conditions, a major peak at 40 °C (the T_m value plotted in Figure 7) and a smaller one at 42 °C.[3] At higher R values, the breadth of the melting profile narrows and the apparent heterogeneity disappears. It was not possible in these experiments to follow the dependence of T_m above 2 × 10^{-5} M (R = 0.4) because of precipitation. Melting of poly[d(A-T)] was completely reversible in the presence of complex cation.

(*2*) *Natural DNAs.* As a consequence of their sequence heterogeneity, both *C. perfringens* and calf thymus DNA should exhibit considerably broader melting transitions than

[3] In the absence of added complex, differentiation of the absorbance–temperature profiles of poly[d(A-T)] samples in 0.004 M Na^+ often exhibited a small shoulder to the right of the T_m peak(s), corresponding to about 10–15% of the hyperchromic change. This shoulder was also seen in some of the $Co(en)_3^{3+}$–poly[d(A-T)] samples of low R value. The appearance and magnitude of this shoulder did not in any way affect the observed T_m value(s), and it is obviously an intrinsic minor property of the poly[d(A-T)] sample rather than of its interaction with complex cation.

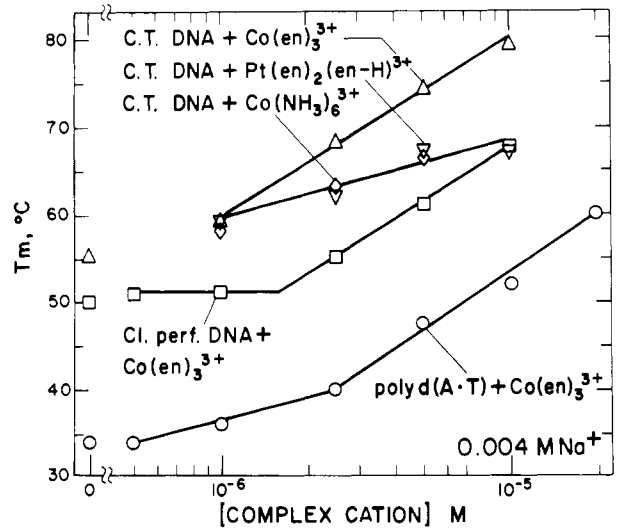

FIGURE 7: T_m variation of DNA helices with complex cation concentration, poly[d(A-T)], 5.2×10^{-5} M (P), and $Co(en)_3^{3+}$; dT_m/d log [cation^{3+}] = 22 °C. At 2.5×10^{-6} M $Co(en)_3^{3+}$, where melting of poly[d(A-T)] is biphasic (see Figure 8), a weighted-average T_m value of 40 °C was determined. *C. perfringens* DNA: 4.0×10^{-5} M (P) and $Co(en)_3^{3+}$; dT_m/d log [cation] = 21 °C. Calf thymus DNA: 4.0×10^{-5} M (P) and $Pt(en)_2(en\text{-}H)^{3+}$; dT_m/d log [cation] = 9 °C. Calf thymus DNA: 4.0×10^{-5} M (P) and $Co(NH_3)_6^{3+}$; dT_m/d log [cation] = 12 °C. Calf thymus DNA: 4.0×10^{-5} M (P) and $Co(en)_3^{3+}$; dT_m/d log [cation] = 20 °C. In 0.004 M Na^+, T_m of calf thymus DNA is 57 °C in the presence of 1.0×10^{-6} M $Ir(NH_3)_5Cl^{2+}$ and 56 °C in the presence of 1.0×10^{-6} M $Pt(NH_3)_4^{2+}$.

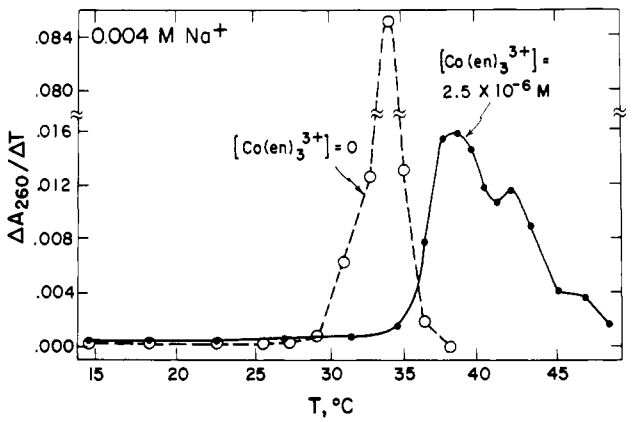

FIGURE 8: Derivative melting profiles of poly[d(A-T)], 5.2×10^{-5} M (P), in the presence or absence of $Co(en)_3^{3+}$.

poly[d(A-T)]. It is not surprising therefore that they did not display discrete biphasic melting in the presence of low levels of complex cations. Still, *C. perfringens* DNA did exhibit the broadest melting transition at an R value (0.0625) that produced a bimodal profile for poly[d(A-T)]. In other respects, their melting behavior in the presence of $Co(en)_3^{3+}$ is similar to that of poly[d(A-T)]; T_m dependence on [$Co(en)_3^{3+}$] is the same (Figure 7).

While $Co(NH_3)_6^{3+}$ and $Pt(en)_2(en\text{-}H)^{3+}$ enhanced the stability of calf thymus DNA (Figure 7), dT_m/d log [complex] was considerably smaller (~9 °C) than with $Co(en)_3^{3+}$. In this respect, the results are like those of poly(U·U). The divalent cations, $Pt(NH_3)_4^{2+}$ and $Ir(NH_3)_5Cl^{2+}$, are less effective in raising the T_m of calf thymus DNA. In 1.0×10^{-6} M cation, $R = 0.025$, $\Delta T_m = 1$ °C for $Pt(NH_3)_4^{2+}$ and 2 °C for $Ir(NH_3)_5Cl^{2+}$. These results with DNA helices suggest that, to a first approximation, the effects of the cationic metal complexes on the stability of double-stranded polynucleotides are not significantly affected by base composition.

Discussion

The ability of multipositive metal complexes to effect marked increases in the thermal stability of nucleic acids is surely the result of the very strong selective attraction of these cations to the phosphate groups of the polymers in their double-helical states. All indications from this study are that these cations interact much more strongly with the double-helical than the single-stranded forms because of the higher charge density of the helices and the favorable geometric distribution of the charges within the grooves of the helices, where site binding occurs. The helix–coil equilibrium is shifted accordingly, and the transition occurs at a higher temperature.

It is likely that the most important aspect of the cation–helix interaction is Coulombic attraction. The ethylenediamine or ammonia ligands in the complexes are tightly bound, so that covalent binding of the central metal ion to purine or pyrimidine N donors or ribose O donors cannot occur. Moreover, as shown above, high [Na^+] (0.15 M) virtually eliminates the enhancement of T_m by the complexes, and the cations with the highest charge increase T_m the most. Nevertheless, hydrogen bonding by the en or NH_3 ligands to phosphates or to water molecules, themselves hydrogen bonded to phosphates, could also contribute to the cation–helix interaction (see below).

Biphasic Melting. In the presence of the trivalent complexes, the most notable feature of the thermal melting of the model polynucleotide helices (which are homogeneous with respect to composition and sequence) is the occurrence of biphasic melting when $R < 0.125$. This behavior is consistent with the following model.

At less than limiting [cation]/[phosphate], the complexes are site bound more or less regularly along the helix, so as to distribute the electrostatic energy of the helix uniformly. As the temperature is raised, cations which had been located at newly melted regions of the polymer will migrate to the residual double-stranded areas since, as has been demonstrated above, the cationic complexes have a much greater affinity for double-helical regions than for single-stranded ones. At some elevated temperature, enough denaturation and cation release will occur, so that the binding sites on the remaining double-stranded segments become saturated with cations. With a higher cation density than at the start, these residual segments are now more resistant to thermal denaturation. Consequently, further melting only occurs at significantly higher temperature, producing biphasic melting. Such behavior for very tightly binding molecules was suggested in a statistical thermodynamic treatment of melting transitions (Crothers, 1971; McGhee, 1976) and was observed in poly[d(A-T)] melting experiments conducted in the presence of the helix-binding drug netropsin (McGhee, 1976).[4]

If the trivalent cations were able to bind strongly to adjacent sites on the helix, each consisting of three phosphates, our model would predict a symmetric biphasic derivative melting profile at $R = 0.167$; i.e., the cation-saturated helix would form after 50% of the polymer had melted. However, profiles with such symmetry are observed at much smaller R values, ≤0.025

[4] Recent studies of the effect of a hexavalent complex cationic dye on the melting of nucleic acid helices demonstrate well separated biphasic melting transitions (Karpel, unpublished experiments). The circular dichroism induced in the dye chromophore on binding to the nucleic acid helix is virtually unaltered upon undergoing the first thermal transition, but it is totally lost coincident with the second transition. This observation indicates that the nonoptically active dye remains bound to the asymmetric nucleic acid helix through the first transition but dissociates from it during the second.

(Figure 3). Apparently, under these conditions, cation–cation electrostatic repulsion prevents closest packing along the helix, so that "adjacent" binding sites are farther apart (see Appendix).

Note that the trivalent but not the divalent complex cations induce multiphasic melting. Perhaps the degree of saturation of helical regions with the divalent complexes is less than with the trivalent ones because the electrostatic affinities are not sufficiently high in this case to favor a major migration of cations from newly denatured to remaining helical regions. Alternatively, divalent cation saturated residual helical regions might not be significantly more resistant to thermal denaturation than near-saturated regions. In either case, the result would be a broadened melting profile, as is observed with the dipositive complexes and also with Mg^{2+} (Dove & Davidson, 1962; Record, 1975).

Differential Affinities of Complex Cations for RNA and DNA Helices. Biphasic melting in the presence of trivalent complex cations is not so well-defined with poly[d(A-T)], although in the presence of $Co(en)_3^{3+}$, $R = 0.048$, it displays a derivative melting profile with two closely spaced maxima (Figure 8). At extremely low R values, the T_m enhancement of the DNA helices by $Co(en)_3^{3+}$ is not as great as that seen at $R > 0.02$ (Figure 7). This is not the case for poly(I·C) where $dT_m/d \log$ [complex cation] is linear up to $R \sim 0.2$. The geometry of the grooves into which the complex cations must bind is different for RNA and DNA helices [cf. Arnott (1976, 1977)] and could also depend on base composition and sequence. The differences in the thermal stabilization of RNA and DNA helices by complex cations must therefore be a reflection of subtle as well as gross differences in helix structure.

In this study, the experiments have been confined to two different types of polyribonucleotide helices and to one model polydeoxyribonucleotide and two DNAs of quite different base composition and sequence. It is difficult from this limited range of polymers to ascribe the greater stabilization of the two model RNA-type helices to very general structural differences. Conceivably, the 2′-OH in the RNA backbone helps optimize complex cation binding through hydrogen bonding; but this is not obvious from the comparison of complex cation fit or hydrogen bonding in the grooves of RNA vs. DNA helices. The general observation from examination of Corey–Pauling–Koltun space-filling models of DNA 10- and RNA 11-fold helices and consideration of the detailed data on the grooves of these structures given by Arnott (1976, 1977) was that any of the trivalent complex cations studied could form an hydrogen-bonded network linking two phosphates of one strand and one phosphate of the opposite strand in alternating sequence in each groove. For example, such networks could be readily constructed in several different ways across either groove of DNA by formation of hydrogen bonds between amine hydrogens and phosphate oxygens through water molecule intermediaries. In the wide groove of DNA each phosphate could be hydrogen-bonded to a water molecule, which in turn could be hydrogen-bonded to the complex, while in the narrow groove only two of the phosphates had to be hydrogen-bonded through water to the complex in order to span the phosphates of opposite strands. Such hydrogen bonding could significantly contribute to the stability of cation–helix interactions. The situation was rather similar for RNA helixes.

The basic differences observed in the differential affinities of particular complex cations to DNA or RNA are not unreasonable when it is recognized that the major groove in DNA is twice as wide and slightly deeper than the narrow groove, and also twice as wide but half as deep as the major groove of RNA, but the same width and twice the depth of the minor groove of RNA. Given the multiplicity of satisfying binding arrangements in either type of helix and the variety of groove parameters, no clear-cut steric factors are apparent to us that might explain the differential T_m enhancement of RNA and DNA helices.

Consideration of Alternative Models of Complex Cation Binding. On the basis of minor perturbations of the CD spectrum of native calf thymus DNA by $Co(en)_3^{3+}$ and other cationic polyamine–cobalt(III) complexes, Ascoli et al. (1972) suggested that DNA undergoes some conformational change upon complex cation binding. Such a change must be subtle, since the major features of the CD spectrum are unaltered. The importance of any structural perturbation, if it is real, could be that RNA helices may be more readily receptive to complex cation binding, whereas DNA helices may have to undergo a more serious reorientation of their helical arrangement to make for a more stable interaction with the cations, with a resultant smaller decrease in free energy. In that event, an alternative explanation for the biphasic melting of the RNA polymers at low R could be that there are domains of altered helical structure saturated with complex cation, while other parts of helix remain in their original conformation with few or no bound complex cations.

Contrary to the results reported here, Ascoli et al. (1973) have also reported similar affinity between $Co(en)_3^{3+}$ and denatured and native calf thymus DNA, even though previously they had shown (Ascoli et al., 1972) some T_m enhancement of native DNA by this complex. Their conditions were different from those of the present study: they used $R \geq 0.5$ and $[DNA]_P = 2 \times 10^{-4}$ M, whereas in the present work $R < 0.5$ and nucleic acid concentrations were generally 5 times lower. At very high [complex]/[nucleic acid phosphate], the differential affinity for double- vs. single-stranded polymer segments will be masked. In this connection a quantitative approach to this differential affinity is given in the Appendix.

Conclusion

The demonstration of high affinity of cationic metal complexes for double helices suggests a variety of uses for them. They could have application to electron microscopy, where heavy metal complexes (e.g., those for Pt, Ir, Os, and Au) might prove useful as positive nucleic acid stains, with the possibility of employing conditions for staining single- and/or double-stranded domains. "Open" nucleic acid regions of nucleoprotein particles not in intimate contact with protein might also be visualized in this manner. The complex cations might also provide a satisfactory means of preparing stable oriented nucleic acid fibers and crystals for X-ray diffraction studies from solutions free of monovalent cations which generally interfere in such work. Indeed, these complexes have been shown also to be very effective in stabilizing the native tertiary structure of tRNA (Karpel et al., 1975), and they have also provided heavy atom isomorphous crystalline derivatives useful in the X-ray structure analysis of tRNA (Wright et al., 1979). It is interesting to note that the affinity of these complex cations for select sites in the tRNA tertiary structure is substantially greater than that for the double helices, so that the binding is not inhibited even in the presence of 0.15 M monovalent cation.

Appendix

Under Results and Discussion, we noted that the melting behavior qualitatively fits a model which assumes marked

preference of the complex cations for binding sites on double helices over those on single strands. The following approach, which resembles that of Herrick and Alberts for unwinding protein–nucleic acid interaction (Herrick, 1973; Herrick & Alberts, 1976), was taken to estimate binding constants from the dependence of T_m of the double helices on complex cation concentration. Assuming that the complex cation interacts only with double-helical sites (and this is essentially the case), the binding interactions can be described as

$$\begin{array}{ccc} C + H & \overset{K_c}{\rightleftharpoons} & CH \\ & K_1 \downarrow\uparrow & \\ & S & \end{array}$$

where C = free cation, H = unoccupied double-helical binding sites = (double-helical nucleic acid)$_P/r$ (r = minimum number of residues per binding site), S = (single-strand nucleic acid)$_P/r$, and CH = double-helical binding sites occupied by complex cations, so that $K_c = [CH]/[C][H]$, and $K_1 = [S]/[H]$.

At T_m,

$$\begin{aligned}[S] &= [H] + [CH] \\ &= [H](1 + K_c[C])\end{aligned}$$

Dividing by [H]

$$K_1 = 1 + K_c[C] \quad (1)$$

K_1 is readily calculated from the melting data, since at T_m^0, i.e., T_m in the absence of complex cation,

$$\Delta G^\circ = -RT_m^0 \ln K_1 = \Delta H^\circ = T_m^0 \Delta S = 0$$

Therefore,

$$\Delta H^\circ = T_m^0 \Delta S^\circ$$

where ΔG°, ΔH°, and ΔS° are the standard free energy, enthalpy, and entropy per binding site for the H $\rightleftarrows$ S transition in the absence of complex cation.

At T_m^c, i.e., T_m in the presence of added cation,

$$\begin{aligned}\Delta G^\circ = -RT_m^c \ln K_1 &= \Delta H^\circ - T_m^c \Delta S^\circ \\ &= T_m^0 \Delta S^\circ - T_m^c \Delta S^\circ\end{aligned}$$

Rearranging,

$$K_1 = \exp\left[\frac{\Delta S^\circ}{R}\left(1 - \frac{T_m^0}{T_m^c}\right)\right] \quad (2)$$

ΔS° for double-helix denaturation has generally been found to be $\sim$25 cal deg^{-1} mol^{-1} per base pair of 12.5 cal deg^{-1} mol^{-1} per residue (Blake, 1973; Bloomfield et al., 1974). Therefore, $\Delta S^\circ = 12.5r$.

Knowing K_1, $[C]_{total}$, and $[H]_{total}$, K_c can be determined as

$$\begin{aligned}[C]_{total} &= [C] + [CH] \\ &= [C](1 + K_c[H])\end{aligned}$$

Solving for [C] and substituting into eq 1

$$K_1 = 1 + [C]_{total}\frac{K_c}{1 + K_c[H]} \quad (3)$$

At T_m, $[S] = [H]_{total}/2$, so

$$[H] = \frac{[S]}{K_1} = \frac{[H]_{total}}{2K_1} \quad (4)$$

Substituting eq 4 into eq 3 and solving for K_c,

$$K_c = \frac{K_1^2 - K_1}{[H]_{total}/2 + K_1([C]_{total} - [H]_{total}/2)} \quad (5)$$

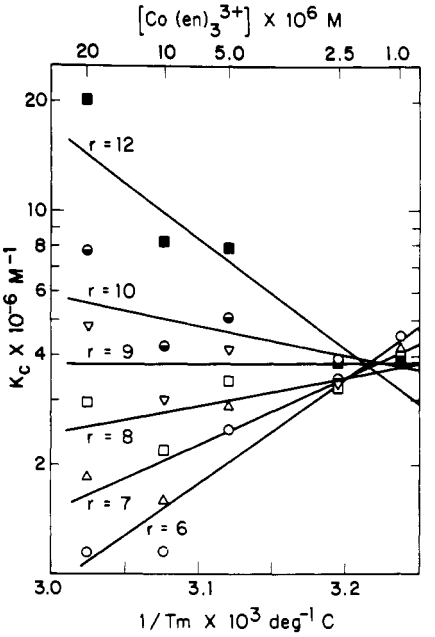

FIGURE 9: Dependence of K_c, the association constant for the helix cation interaction, on r, the minimum number of poly[d(A-T)] residues per cation binding site, and on T_m (or [Co(en)$_3^{3+}$]). The values calculated for K_c at 1.0 X 10^{-5} M Co(en)$_3^{3+}$ show better fit to the linear plots if the T_m value is taken to be 2 °C higher than that measured.

In this treatment it is evident that the calculation of K_1 and K_c is critically dependent on the choice of r, the number of nucleotide residues per cation binding site, which is equivalent to the minimum number of residues per bound cation. If the size and nature of the site for a particular complex cation are invariant over the range of R, then K_c will also be invariant. The least complicated case to apply these considerations to is that of the poly[d(A-T)]–Co(en)$_3^{3+}$ interaction, since the melting profiles, with one exception at an intermediate R value, are monophasic. For poly[d(A-T)], when r is set at 3, K_c varies greatly with [Co(en)$_3^{3+}$], and at 2.5 X 10^{-6} M complex cation, $K_c \leq 0$. Since this is physically meaningless, r must be a higher number. Semilogarithmic plots of K_c vs. $1/T_m$ for $6 \leq r \leq 12$ are shown in Figure 9. For values of $r = 6$ or 7, K_c decreases with increasing [Co(en)$_3^{3+}$] and T_m, and $\Delta H^\circ_c \sim -6$ and ~ -4 kcal mol^{-1}, respectively. However, when $r = 12$, K_c increases with increasing [complex cation] and T_m, and $\Delta H^\circ_c \sim 6$ kcal mol^{-1}. Only at $r = 8$–10 does K_c appear to have little dependence on both complex cation level and T_m (within the experimental scatter of the data); thus, $\Delta H^\circ_c \sim 0$. While the enthalpy of the binding of Co(en)$_3^{3+}$ to poly[d(A-T)] cannot be directly determined from the results of the present study, a predominantly electrostatic interaction of complex cations with nucleic acids should have little intrinsic temperature dependence. In fact, ΔH° of ion-pair formation of the tripositive complexes with simple inorganic ligands is generally ±2 kcal mol^{-1} or less (Sillen & Martell, 1964, 1971). Hence, values of $r = 8$–10 are consistent with other binding properties of Co(en)$_3^{3+}$. Our analysis suggests therefore that each Co(en)$_3^{3+}$ can be no closer than eight to ten nucleotide residues (four to five base pairs) to its adjacent complex on the poly[d(A-T)] helix. While the model building showed the possibility of close packing of one complex cation for every three residues at cation saturation, the electrostatic repulsion between adjacent trivalent cations might necessitate cation separation along the helix to give the calculated values of 8–10. The latter number corresponds to two complex cations per turn of helix, instead of the ca. seven sterically allowable. The most

favorable distribution of the two complex cations would be to have them located regularly in each half turn of helix, so they are displaced 180° from their two nearest neighbors along the helix screw, i.e., alternately distributed on opposite sides of the helix.

Having obtained an estimate of the frequency of binding of complex cations under the most favorable conditions, we can attempt to explain the absence of a saturation point in the dependence of T_m on [complex cation] (Figure 7). If K_c is sufficiently large, $[CH] \simeq [C]_{total}$ when $[C]_{total} \leq [H]_{total}/r$, i.e., at $R \leq 1/r$. The dependence of T_m on $[Co(en)_3^{3+}]$ would be expected to be significantly greater at $R \leq 1/r$ than at $R > 1/r$, with a discontinuity at $R = 1/r$, corresponding to the point of saturation of the helix by cations. However, this is clearly not the case for poly[d(A-T)] (Figure 7), showing that [CH] is always less than $[C]_{total}$.

Using the T_m data for the poly[d(A-T)]–$Co(en)_3^{3+}$ interaction, and the calculated values of K_1 and K_c at $r = 9$, we calculated the concentrations at T_m of free cation, [C], and bound cation, [CH], using eq 5. At 1.0×10^{-6} M cation these values show that 89% of the total complex cations are still bound to helix, and 31% of the residual helical sites are occupied by these cations. Despite this indication of strong complex cation affinity for helix, saturation at $R = 1/r = 0.111$ or greater is not seen. Apparently, the binding constant is not sufficiently high, which agrees with the absence of a stoichiometric end point at $R = 1/r$ in the plot of T_m vs. $[Co(en)_3^{3+}]$.

At low R values, the biphasic melting of poly(I·C) makes it very difficult to estimate [H] + [CH] at T_m with sufficient accuracy to apply the above treatment. At high R values, where melting is monophasic, K_c for poly(I·C) is about 2 orders of magnitude greater than the value for poly[d(A-T)], $\sim 10^8$ M^{-1}, assuming $r \sim 8$–10. Since T_m^0 is required for these calculations, no estimate could be obtained for poly(U·U).

The model presented under Discussion predicts that, within the range of R values yielding biphasic melting profiles, the proportion of helix melting at the higher transition increases, as it does. However, the model does not predict an increase in T_m with increasing R, as is observed. Conceivably, during the melting process, the "remaining helical segments" may not be completely saturated by cations. Thus, as R increases, the degree of cation saturation of these remaining segments increases (by mass action), and the higher T_m increases.

If the size of the poly(I·C) helix binding site is comparable to that of the poly[d(A-T)] helix, then the helix would be half-saturated at $R \geq 1/(2r)$, or $\gtrsim 1/20$ (0.05) ($R = 1/(2r)$ only if K_c is sufficiently large). Biphasic melting profiles of approximately equal peak area are seen at $R \sim 1/40$ (0.025), about half the predicted value. Conceivably, r may be greater than 10 for poly(I·C) or the higher transition represents melting of a less than fully saturated helix. Interestingly, the poly[d(A-T)] helix exhibits a biphasic derivative melting profile with comparable peak sizes at $R = 0.048$, in accordance with the prediction of the analysis. This approach is therefore quantitatively consistent with the poly[d(A-T)] melting data and provides a qualitative rationale for the poly(I·C) data.

References

Arnott, S. (1976) in *Organization and Expression of Chromosomes* (Berlin: Dahlem Konferenzen, 1976) (Allfrey, V. G., Bautz, E. K. F., McCarthy, B. J., Schimke, R. T., & Tissieres, A., Eds.) pp 209–222.

Arnott, S. (1977) *Proc. Cleveland Symp. Macromol., 1st, 1976*, 87–104.

Ascoli, F., Branca, M., Mancini, C., & Pispisa, B. (1972) *J. Chem. Soc., Faraday Trans. 1 68*, 1213–1226.

Ascoli, F., Branca, M., Mancini, C., & Pispisa, B. (1973) *Biopolymers 12*, 2431–2434.

Blake, R. D. (1973) *Biophys. Chem. 1*, 24–34.

Bloomfield, V. A., Crothers, D. M., & Tinoco, I., Jr. (1974) *Physical Chemistry of Nucleic Acids*, p 315, Harper and Row, New York.

Butzow, J. J., & Eichhorn, G. L. (1965) *Biopolymers 3*, 95–107.

Butzow, J. J., & Eichhorn, G. L. (1971) *Biochemistry 10*, 2019–2027.

Crothers, D. M. (1971) *Biopolymers 10*, 2147–2160.

Daune, M. (1974) in *Metal Ions in Biological Systems* (Siegel, H., Ed.) Vol. 3, p 24, Marcel Dekker, New York.

Dove, W. F., & Davidson, N. (1962) *J. Mol. Biol. 5*, 467–478.

Eichhorn, G. L. (1973) in *Inorganic Biochemistry* (Eichhorn, G. L., Ed.) Vol. 2, pp 1210–1243, Elsevier, New York.

Eichhorn, G. L., & Tarien, E. (1967) *Biopolymers, 5*, 273–281.

Eichhorn, G. L., & Shin, Y. A. (1968) *J. Am. Chem. Soc. 90*, 7323–7328.

Felsenfeld, G., & Miles, H. T. (1967) *Annu. Rev. Biochem. 36*, 407–448.

Giedt, D. C., & Nyman, C. J. (1966) *Inorg. Synth. 8*, 239–241.

Grinberg, A. A. (1962) in *An Introduction to the Chemistry of Complex Compounds*, p 243, Addison-Wesley, Reading, MA.

Grunberg-Manago, M., Ortiz, P. J., & Ochoa, S. (1956) *Biochim. Biophys. Acta 20*, 269–285.

Herrick, G. A. (1973) Ph.D. Thesis, Princeton University, Princeton, NJ.

Herrick, G., & Alberts, B. (1976) *J. Biol. Chem. 251*, 2133–2141.

Izatt, R. M., Christensen, J. J., & Rytting, J. H. (1971) *Chem. Rev. 71*, 439–481.

Karpel, R. L., Miller, N. S., Lesk, A. M., & Fresco, J. R. (1975) *J. Mol. Biol. 97*, 519–532.

Lipsett, M. N. (1960) *Proc. Natl. Acad. Sci. U.S.A. 46*, 445–446.

McGhee, J. D. (1976) *Biopolymers 15*, 1345–1375.

Record, M. T., Jr. (1975) *Biopolymers 14*, 2137–2158.

Schildkraut, C., & Lifson, S. (1965) *Biopolymers 3*, 195–208.

Schmidt, H. H. (1970) *Inorg. Synth. 12*, 243–247.

Sillen, L. G., & Martell, A. E., Eds. (1964) *Stability Constants of Metal-Ion Complexes*, The Chemical Society, London.

Sillen, L. G., & Martell, A. E., Eds. (1971) *Stability Constants of Metal-Ion Complexes*, Suppl. No. 1, The Chemical Society, London.

Topal, M. D., & Fresco, J. R. (1974) *Biophys. Chem. 2*, 193–195.

Work, J. B. (1946) *Inorg. Synth. 2*, 221–222.

Wright, H. T., Manor, P. C., Beurling, K., Karpel, R. L., & Fresco, J. R. (1979) in *Transfer RNA: Structure, Properties and Recognition* (Abelson, J., Schimmel, P., & Soll, D., Eds.) pp 145–160, Cold Spring Harbor Laboratory, New York.

Zimmerman, S. B. (1976) *J. Mol. Biol. 101*, 563–565.