A Thermodynamic Analysis of the Influence of Simple Mono- and Divalent Cations on the Conformational Transitions of Polynucleotide Complexes†

Henry Krakauer‡

ABSTRACT: The complex dependence of the conformational transitions among the structures formed by polyadenylate and polyuridylate on the concentrations of Na^+ and Mg^{2+} is described and is satisfactorily interpreted in terms of the measured interactions of these ions with the polynucleotides. The analysis is phenomenological and not predicated on any model of the interactions, but is consistent with the notion that those with Na^+ are qualitatively different from those with Mg^{2+}, the latter but not the former resulting in a definite complex.

The identification and interpretation of the mechanisms through which a cell may induce and control the various structural rearrangements that occur in nucleic acids during the course of their utilization in the processes of replication, transcription, and translation are among the central problems of molecular biology. Because nucleic acids are polyelectrolytes and hence the stability of the various structures that they form is senstitive to the ionic environment, the manipulation of this ionic environment provides one such potential mechanism. It is, therefore, important to define the influence of the various species of electrolytes, from simplest alkali cations to the polycationic histones, on the conformational transitions of the nucleic acid complexes.

First to be obtained was the definition of the effects of monovalent cations (reviewed by Felsenfeld and Miles, 1967). Interpretations of these effects were generally given in terms of the dependence of the electrostatic free energy of the structures involved in the transitions, G_{el}, on the ionic strength. When the Debye–Hückel approximation, or linearization of the Poisson–Boltzmann equation, is employed in calculations of G_{el}, the linear charge density of the polymers must be made an adjustable parameter to permit satisfactory application to experimental results, implying the "binding" of counterions (for a recent example containing references to previous work, see Bailey, 1973). When the approximation is not made, the behavior of small ions in the close vicinity of the polymer is accounted for by theory and the assumption of counterion binding is generally not necessary. But then some other property of the reacting system such as a molecular dimension remains an adjustable parameter (see, for example, Nagasawa and Muroga, 1972). There are, however, indications of binding of counterions to polyelectrolytes (Gross and Strauss, 1966), but the manner in which its effect on the conformational transitions is to be formulated is not certain. Strauss and coworkers propose that the binding is governed by the mass action principle modified by electrostatic interactions. Manning (1969) bases his approach on the requirements, dictated by theoretical considerations, for condensation of counterions to reduce the electrostatic free energy of the polyelectrolyte below a critical value determined by the linear charge density. The remaining electrostatic interactions are computed by means of the Debye–Hückel approximation. Krakauer and Sturtevant (1968), on the basis of the "additivity rules" for activities of small ions in the presence of polyions, ascribed to "binding" all reductions in counterion activity due to counterion–polyelectrolyte interactions, whatever their nature on the molecular level, in their interpretation of the enthalpies of the conformational transitions of the poly(A) (polyadenylate)–poly(U) (polyuridylate) complexes. (Note, however, that the concept of additivity rules has been challenged by Lyons and Kotin, 1965.) Privalov *et al.* (1969) obtained, through a more general phenomenological approach, rather similar results in their analysis of the stability of DNA.

The most sensitive test of such formulations of the dependence of the thermodynamic properties of the polynucleotide complexes on monovalent cations is obtained by a comparison of observed effects on the T_m of the conformational transitions, where T_m is the temperature at which a transition is half completed, with those predicted on the basis of the formulations. The equations written down by Massoulie (1968) for this purpose for the complexes of poly(A) and poly(U) contain terms to include explicitly the contributions of the electrostatic effects and the effect of temperature on the conformation of poly(A), in addition to the intrinsic enthalpy and entropy changes of the transitions. The numerical values of all the other quantities, obtained as solutions of simultaneous equations whose independent variables are the T_m and the concentration of cations, are very sensitive to the choice of values for the ΔH and ΔS of self-stacking of poly(A), the adjustable parameters. Reasonable choices lead, however, to excellent fit of the experimental result. Nagasawa and Muroga (1972) calculated ΔG_{el} *a priori* in their comparable analysis of the thermal denaturation of DNA and were able to deduce an equation to represent the dependence of the T_m on the concentration of salt and on the guanine–cytosine content very similar to the empirical one. It is worthy of note that in both treatments, as a result of the utilization of the charged rod as a model for the polynucleotides, as well as in that of Manning (1972a) which is based on theoretically predicted counterion condensation, ΔG_{el} and the T_m are predicted to depend linearly on the logarithm of the salt or counterion concentrations.

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(Earlier treatments with similar results are reviewed by Felsenfeld and Miles (1967) and in the other references above.)

The role of divalent cations in determining the stability of the various polynucleotide structures is rather more complex. The doubly charged transition metal ions interact with both the phosphates of the backbone and with the heterocyclic bases, while the alkaline earth cations, specifically Mg^{2+}, appear to interact exclusively with the phosphates (Eichhorn and Shin, 1968; Jakabhazy and Fleming, 1966). Nevertheless, the details of the interaction of even the latter remain unclear and its quantitative experimental description in dispute. The binding of Mg^{2+} to DNA is said by Lyons and Kotin (1965) to be largely of the diffuse type, similar to that of monovalent cations, in the pure MgDNA solution but that site binding becomes prominent when $MgCl_2$ is added. Manning (1972b) maintains that the binding of mono- and that of divalent cations are both determined by the same requirement to reduce the linear charge density of the polymer below a critical value, the divalent cations binding to the full extent needed, in precedence to the monovalent, if both are present. Measurements of the binding of Mg^{2+} to nucleic acids have been performed by a variety of techniques, with contradictory results. Skerjanc and Strauss (1968) examined the distribution of Mg^{2+} at equilibrium in dialysis of DNA and determined binding constants several orders of magnitude smaller than those calculated by Clement *et al.* (1968) from potentiometric measurements and measurements in which centrifugation was used in place of a semipermeable membrane to segregate the DNA. The results of measurements of binding of Mg^{2+} to the poly(A)·poly(U) complex, obtained by the present author (Krakauer, 1971), were similar in qualitative pattern and, allowing for differences in ionic strength, quantitatively comparable to those of Clement *et al.* (1968). Characteristic of both was the anticooperative nature of the binding. In contrast, the sites on poly(A) to which Mg^{2+} binds were found by Danchin (1972) and by Cohn *et al.* (1969) to be independent and equivalent. Similar uncertainties exist in the matter of the binding of divalent cations to tRNA. Rialdi *et al.* (1972) observed two classes of independent and equivalent binding sites for Mg^{2+} on yeast tRNA, one with four and the other with 20 members, while Danchin (1972) found that the smaller class, with six members, was highly cooperative.

Beyond these lie the difficulties in interpreting the interplay of the interactions of mono- and divalent cations with the polymers. Thus, Dove and Davidson (1962) found that DNA, in the presence of one Mg^{2+} for every two nucleotides, was stabilized against thermal denaturation by increasing concentrations of Na^+ above 0.01 M and destabilized below. The one theoretical evaluation of this behavior (Manning, 1972b) accounts only for the latter effect.

It was to obtain a detailed phenomenological description of the role that simple mono- and divalent cations play in the control of the conformational transitions observed in nucleic acids, and thereby to obtain an insight into the molecular mechanisms by which they exert their effects, that a thorough investigation of the transitions of the model poly(A)·poly(U) system and of the interactions of the cations with the polynucleotides was undertaken. The experimental studies of the enthalpies of the transitions and of the parameters that govern the "binding" of Na^+ and of Mg^{2+} have been discussed elsewhere (Krakauer and Sturtevant, 1968; Krakauer, 1971; Archer *et al.*, 1972; Krakauer, 1972). Presented below will be a description of the behavior of the transitions (Stevens and Felsenfeld, 1964): (1) poly(A)·poly(U) → poly(A) + poly(U) (2→1), (2) poly(A)·poly(U) → $^1/_2$(poly(A)·2poly(U) + $^1/_2$poly(A) (2→3), (3) poly(A)·2poly(U) → poly(A)·poly(U) + poly(U) (3→2), and (4) poly(A)·2poly(U) → poly(A) + 2poly(U) (3→1), in the presence of various concentrations of Na^+ (and K^+) and Mg^{2+} and a thermodynamic analysis of this behavior, based on the results of the experimental studies listed above.

Materials and Methods

The procedures used to prepare the polynucleotides and their complexes have been given elsewhere, as have the analytical techniques used to evaluate the enthalpy changes that accompany the transitions, and the extents of binding of Na^+ and Mg^{2+} (Krakauer and Sturtevant, 1968; Krakauer, 1971; Archer *et al.*, 1972; Krakauer, 1972). The poly(A) and poly(U) were high polymers with molecular weights, verified by analytical ultracentrifugation, of about 10^5 or greater. Other chemicals were commercial reagent grade preparations and were used without further purification. Solutions of $MgCl_2$ were standardized by titration with $AgNO_3$, with K_2CrO_4 the indicator. Cacodylate buffer was prepared from solid cacodylic acid and standardized NaOH and KOH, permitting precise knowledge of its cation concentration. It was used to maintain pH in the range 6.8–7.1. All critical volumetric glassware was calibrated.

The solutions in which the conformational transitions were induced by elevation of temperature contained cacodylate buffer 0.01 M in Na^+ or K^+, polynucleotide concentrations, in terms of the constituent nucleotides, in the range 0.5–1.5 × 10^{-4} M, given as normalities N, and varying concentrations of NaCl or KCl and of $MgCl_2$.

The transitions were followed spectrophotometrically by monitoring absorbance, at 260 nm for the 2→1, 3→2, and 3→1 transitions and at 280 nm for the 2→3 and 3→1 transitions (Stevens and Felsenfeld, 1964), as a function of temperature. Temperature control was achieved by circulating water from a Precision Scientific or Neslab TE9 water bath through individually jacketed spectrophotometer cells (Helma 160QS) and its measurement by means of calibrated thermistors inserted into the cells, just above the light beam, through snugly fitting Teflon caps. The temperature was increased discontinuously to permit attainment of thermal equilibrium in the cells after each increment, manually in early experiments in which K^+ was the monovalent counterion, performed in a Beckman DU spectrophotometer, and subsequently automatically, by means of a Neslab TP2 temperature programmer, in experiments performed in a Guilford 2400 spectrophotometer.

Computations were performed on a Wang 700 programmable calculator.

Results

(A) The Transitions. The absorbance *vs.* temperature profiles of the conformational transitions in the presence or absence of Mg^{2+}, in addition to the monovalent counterion, are of conventional appearance. As was indicated above, the 3→2 and 2→1 transitions may be followed at 260 nm (Figure 1) where both result in an increase in absorbance. Note the progressive convergence of the temperatures of the two transitions as the concentration of Na^+ is increased, until coalescence. The 2→3 transition is not detectable at 260 nm, but is at 280 nm where it results in a decrease in absorbance. The subsequent 3→1 transition is accompanied by a larger increase resulting in a net gain in absorbance (Figure 2). Note

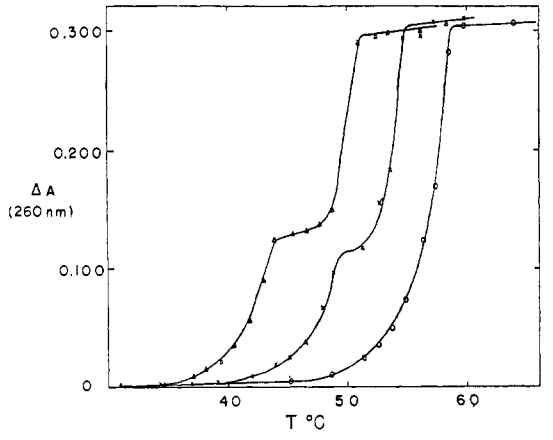

FIGURE 1: Conformational transitions of poly(A)·2poly(U): polynucleotide = 1.51×10^{-4} N, $MgCl_2$ = 1.45×10^{-4} M, Na^+ = 0.0383 M (Δ), 0.0631 M (×), 0.0175 M (○).

in this figure that the region of coalescence of the two transitions, *i.e.*, the region in which poly(A)·poly(U) is transformed into random coils in one step, occurs at intermediate concentrations of Na^+. Qualitatively similar transition profiles are obtained when Na^+ is replaced by K^+. The relative stability of two conformations is conveniently defined by the T_m of the transition between them. Figure 3 presents the variation of the T_m's of the transitions, with the exception of 3→2, with the activity of K^+ at various concentrations of Mg^{2+}. In Figure 4 is given the dependence of the T_m's of all the transitions on the concentration of Na^+. Figure 5 deals with the 2→3 and 3→1 transitions under conditions in which the molar ratio of Mg^{2+} and nucleotides is fixed at 1. It is evident from the figures that the profound effects of Mg^{2+} visible in the presence of low concentrations of monovalent cations may be masked when concentrations of the latter are sufficiently high. Concentrations of Mg^{2+} that are low, both absolutely and relatively to those of the polynucleotides, merely stabilize the three-stranded complex with respect to the two-stranded one and the latter with respect to the single-stranded forms at low concentrations of monovalent cations. In the presence of sufficient Mg^{2+} to cover a substantial fraction of the sites, the three-stranded complex is stabilized so well that the 3→2 transition is no longer observed. If the two-stranded complex is heated under such circumstances, the initial transition encountered is that to the three-stranded one (2→3) and that is followed by the disruption of the latter (3→1). It may also be noted in Figure 3 that in the presence of near physiological

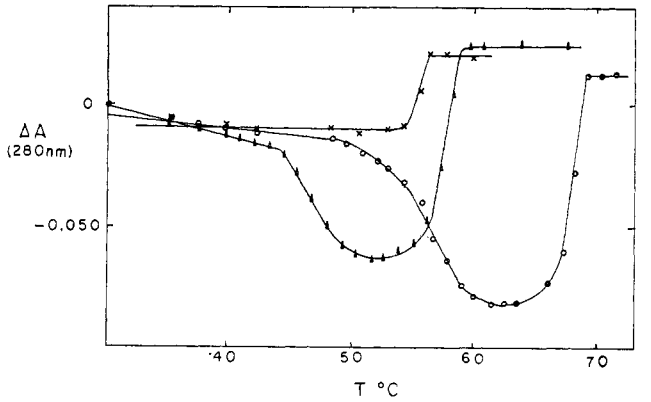

FIGURE 2: Conformational transitions of poly(A)·poly(U): polynucleotide = 1.46×10^{-4} N, $MgCl_2$ = 1.07×10^{-4} M, Na^+ = 0.0136 M (Δ), 0.0486 M (×), 0.284 M (○).

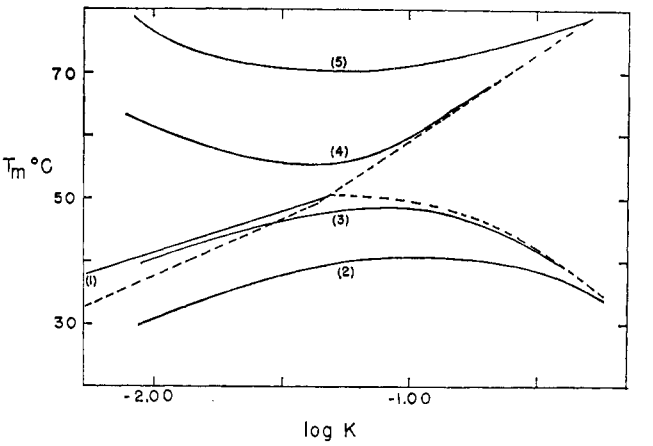

FIGURE 3: The dependence of the T_m of the conformational transitions on the activity of K^+: polynucleotide = 5.6×10^{-5} N, $MgCl_2$ = 10^{-5} M (1), 10^{-4} M (3) and (4), 10^{-3} M (2) and (5). The solid lines represent: (1) transition 2→1, (2) and (3) transition 2→3, (4) and (5) transition 3→1. The dashed lines represent the transitions in the absence of Mg^{2+} or in the presence of Mg^{2+} but at sufficient concentrations of K^+ to mask the effects of the divalent cation. The transitions defined by these lines are the same as those of the solid lines that converge on them. Poly(A) and poly(U) were present in equinormal amounts.

concentrations of Mg^{2+} and K^+, the T_m of the 2→3 transition lies also near the physiological temperature. In Figure 4 the range of Mg^{2+} concentrations in which the replacement of the 2→1 by the 2→3 and 3→1 transition takes place is explored in greater detail. Most notable are the regions of Na^+ concentration in which the 2→1 and 3→1 transitions have the same T_m's. This was verified in experiments in which two- and three-stranded complexes, prepared at room temperature from the proper stoichiometric amounts of poly(A) and poly(U), were heated and observed at both 260 and 280 nm. (The

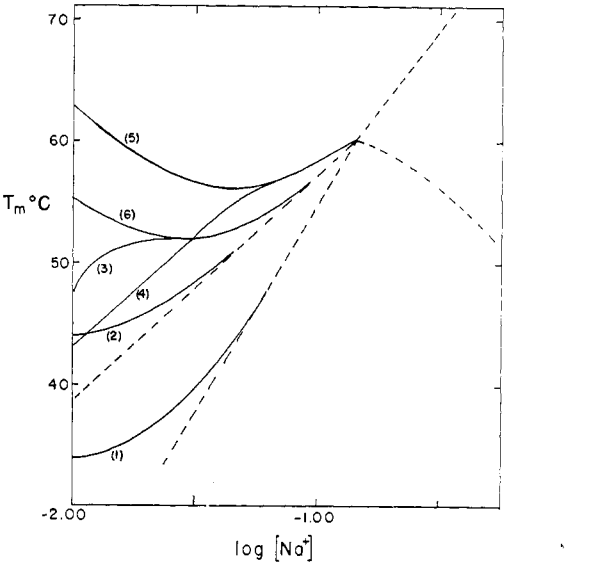

FIGURE 4: The dependence of the T_m of the conformational transitions on the concentration of Na^+: polynucleotide = 1.5×10^{-4} N, $MgCl_2$ = 1.45×10^{-5} M (1) and (2), 7.3×10^{-5} M (3) and (6), 1.45×10^{-4} M (4) and (5). The solid lines represent: (1) transition 3→2, (2) transition 2→1, (3) and (4) transition 2→3, (5) and (6) transition 3→1. After convergence of the lines for transitions 2→3 and 3→1 they are replaced by transition 2→1. The dashed lines, identified by the solid lines that join them, represent transitions in the absence of Mg^{2+} or in the presence of sufficient Na^+ to mask the effects of Mg^{2+}. The lines at the extreme right represent transitions 2→3 (lower) and 3→1 (upper).

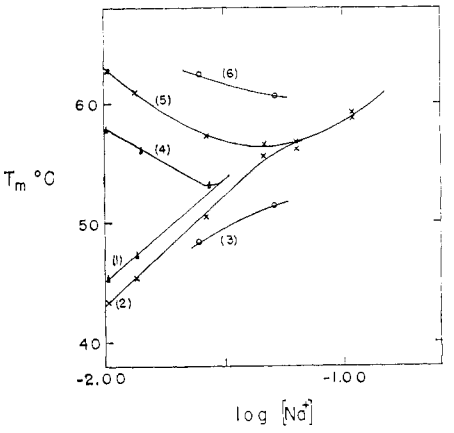

FIGURE 5: The dependence of the T_m of the transitions on the concentration of Na^+ at a fixed Mg^{2+}/polynucleotide ratio: polynucleotide = 7.3×10^{-5} N, $MgCl_2$ = 7.3×10^{-5} M (1) and (4); polynucleotide = 1.45×10^{-4} N, $MgCl_2$ = 1.45×10^{-4} M (2) and (5); polynucleotide = 3.7×10^{-4} N, $MgCl_2$ = 3.7×10^{-4} M (3) and (6); lines (1), (2), and (3) transition 2→3; lines (4), (5), and (6) transition 3→1.

experimental points defining the drawn lines were omitted from this figure and the preceding one to enhance their clarity.) It is clear from Figure 5 that the complete separation of the 2→3 and 3→1 transitions observed at high concentrations of Mg^{2+} and all concentrations of K^+ will also be encountered in the presence of Na^+. Another point made by this figure is that the transitions depend on the actual concentrations of both the polynucleotides and Mg^{2+} and not merely on their ratio or not at all and that, therefore, the interaction between those species is concentration dependent in contrast to that involving monovalent cations alone. (In the absence of Mg^{2+}, the T_m is independent of the concentration of the polynucleotides and is determined solely by that of the Na^+ or K^+.) Figure 6 displays the data of Figure 4 in a different form useful in subsequent calculations: the dependence of the T_m of the 2→3 and 3→1 transitions on the concentration of Mg^{2+} (more precisely, $MgCl_2$) at various concentrations of Na^+.

As may be seen from the theoretical evaluations discussed in the "introduction" and as will be shown below, the interpretation of the conformational transitions of the complexes of poly(A) and poly(U) requires detailed knowledge of the nature and extent of interactions of the mono- and divalent cations,

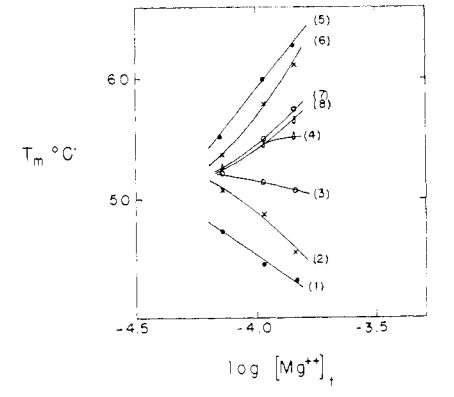

FIGURE 6: The dependence of the T_m on the total concentration of Mg^{2+} in the solution. Polynucleotide = 1.45×10^{-4} N. Na^+ = 0.0101 M (1) and (5), 0.0137 M (2) and (6), 0.0265 M (3) and (7), 0.0475 M (4) and (8). Lines (1)-(4) represent transition 2→3 and lines (5)-(8) transition 3→1. Transition 2→1 is observed along the line formed by the fusion of (4) and (8).

TABLE I: The Binding of Mg^{2+} to the Polynucleotides at 25°.

$$\ln K_\theta = \ln \frac{\theta}{1-\theta}\frac{1}{[Mg^{2+}]} = a_0 + a_1\theta^a$$

	a_0	a_1
Na^+ = 0.010 M		
Poly(A)	10.37 ± 0.14[b]	−10.34 ± 0.59
Poly(U)	7.93 ± 0.11	−3.57 ± 0.48
Poly(A)·poly(U)	11.19 ± 0.21	−10.72 ± 0.70
Poly(A)·2poly(U)	10.79 ± 0.20	−7.83 ± 0.61
Na^+ = 0.029 M		
Poly(A)	8.48 ± 0.06	−7.59 ± 0.32
Poly(U)	6.57 ± 0.03	−1.82 ± 0.22
Poly(A)·poly(U)	8.52 ± 0.18	−5.48 ± 0.98
Poly(A)·2poly(U)	8.57 ± 0.08	−3.68 ± 0.35
Na^+ = 0.060 M		
Poly(A)	6.86 ± 0.11	−4.69 ± 0.77
Poly(U)	5.66 ± 0.09	−1.38 ± 0.88
Poly(A)·poly(U)	7.15 ± 0.16	−3.70 ± 0.34
Poly(A)·2poly(U)	7.31 ± 0.07	−2.71 ± 0.26
Na^+ = 0.100 M		
Poly(A)	5.28 ± 0.12	−1.24 ± 1.00
Poly(U)	5.53 ± 0.09	−0.52 ± 0.90
Poly(A)·poly(U)	5.84 ± 0.11	−2.78 ± 0.81
Poly(A)·2poly(U)	5.70 ± 0.13	−2.39 ± 0.84

[a] θ is the fraction of sites (nucleotides) to which Mg^{2+} has been bound. [b] The uncertainties given are standard deviations [(variance)$^{1/2}$] (data taken from Krakauer (1971)).

in whose presence the transitions take place, with the polynucleotides, as well as of the enthalpy changes that accompany them.

(B) The Binding of Mg^{2+}. The extent of binding of Mg^{2+} to poly(A), poly(U), poly(A)·poly(U), and poly(A)·2poly(U) in the presence of excess Na^+ may be calculated from the quantities, taken from Krakauer (1971), in Tables I and II. The quantities that appear in these tables are K_θ, the apparent binding parameter, defined by the equation

$$\ln K_\theta = \theta/(1-\theta)[Mg^{2+}] \quad (1)$$

θ = fraction of nucleotides to which Mg^{2+} has been bound, $[Mg^{2+}]$ = concentration of free Mg^{2+}. Ln K_θ was found (Krakauer, 1971) to be a linear function of θ

$$\ln K_\theta = a_0 + a_1\theta \quad (2)$$

and to depend on the concentration of Na^+.

The quantities in these and subsequent tables were obtained by regression analysis and represent the parameters of the simplest equations that give satisfactory fit of the experimental data. (Where more "significant figures" are given than appear to be justified, it is to minimize rounding-off errors that would result from the application of the equations.)

Ln K_θ was also determined at 10 and 40°, in addition to 25°, the temperature at which the data in Tables I and II apply. The effect of changes in temperature was found to be small and therefore difficult to define precisely. However, ΔH of binding of Mg^{2+} to the polynucleotides was also measured calorimetrically over a similar range of concentrations of Na^+ (Table III (Krakauer, 1972)) permitting correction of K_θ from 25° to the temperature of the transition to be evaluated. (The accuracy of such a correction is limited by the unavailability of ΔC_p for the binding of Mg^{2+}. The error introduced

TABLE II: Analytical Expressions for the Dependence of the Binding of Mg^{2+} on the Concentration of Na^+.

	a_0[a]	a_1[a]
Poly(A)	$12.60 - 23.35[Na^+]^{1/2}$	$-14.68 + 41.74[Na^+]^{1/2}$
Poly(U)[b]	$2.101 - 1.264 \ln [Na^+]$	$2.328 + 1.255 \ln [Na^+]$
Poly(A)·poly(U)	$0.592 - 2.286 \ln [Na^+]$	$6.239 + 3.56 \ln [Na^+]$
Poly(A)·2poly(U)	$0.992 - 2.142 \ln [Na^+]$	$3.767 + 2.388 \ln [Na^+]$

[a] These are the quantities that appear in Table I. [b] These expressions are valid in the range 0.01 M < $[Na^+]$ < 0.06 M. a_0 and a_1 do not change significantly between 0.06 and 0.10 M Na^+ (data taken from Archer *et al.* (1972)).

as a result should, however, not be large because of the relatively small magnitudes of ΔH and small ranges in T over which the correction must be made.) The information in Table III is also needed to compute the contribution of changes in binding of Mg^{2+} to the enthalpies of the transitions induced in its presence.

(*C*) *The Interaction with* Na^+. The interaction of Na^+ with the polynucleotides was studied directly by potentiometry, in the presence and absence of Mg^{2+}, and indirectly by linked-function analysis (Wyman, 1964) of its effect on the binding of Mg^{2+} (Archer *et al.*, 1972). The results of both approaches were very similar in the cases of poly(U) and poly(A)·poly(U). The behavior of poly(A) is aberrant because of its tendency to self-stack, a tendency enhanced by the binding of Mg^{2+} with the result that the binding of Mg^{2+} to poly(A) is exothermic (it is endothermic in all the other polynucleotides studied) and stronger than to poly(U). Elevations in ionic strength also enhance the stacking, as is demonstrated nicely by a comparison of the heats of dilution, measured by calorimetry, of poly(A) and poly(U). The process studied was

$$\text{or } \begin{matrix}\text{poly(A)}\\\text{poly(U)}\end{matrix}\Big\}([N]_i, [Na^+]_i) \longrightarrow \text{or } \begin{matrix}\text{poly(A)}\\\text{poly(U)}\end{matrix}\Big\}([N]_f, [Na^+]_f)$$

i.e., the concentrations of both the polynucleotides, N, and of the salt, represented by $[Na^+]$, were reduced simultaneously. (Dilution of polynucleotides at fixed concentration of salt is not accompanied by measurable heat changes.) The results were

	$dH/d \log [Na^+]$ (cal/mol)
poly(A)	-263 ± 4 ($T = 25°$)
poly(U)	$+118 \pm 1$ ($T = 25°$)
poly(U)	-263 ± 3 ($T = 9°$)

TABLE III: The Differential Heats of Binding of Mg^{2+}.

$$\Delta H(\theta)^a = a + b\theta + c\theta^2$$

	a	b	c
Poly(A)			
$[Na^+]$ = 0.015 M	−2,987	10,860	−4,546
0.029 M	−1,984	6,950	−782
0.100 M	−1,600	9,214	−6,795
Poly(U)			
$[Na^+]$ = 0.015 M	3,247	−14,000	20,183
0.029 M	2,812	−12,204	18,307
0.100 M	2,349	−14,334	24,500
Poly(A)·poly(U)[b]	1,876	−5,791	9,276
Poly(A)·2poly(U)[b]	3,193	−13,958	20,255

[a] The units of $\Delta H(\theta)$ are calories per mole of Mg^{2+} bound. [b] The heat of binding of Mg^{2+} to these complexes is independent of the concentration of Na^+ (data taken from Krakauer (1972)).

$[Na^+]_i$ = 0.04 M, $[N]_i$ = 3–5 mM in nucleotides. The dilutions were up to 32-fold in several steps (T. R. Fink and H. Krakauer, manuscript in preparation).

The exothermic *dilution* of poly(U) at 25° is what is expected of conventional polyelectrolytes (Skerjanc *et al.*, 1970). As both poly(A) at room temperature (Applequist and Damle, 1966), for example, and poly(U) at lower temperature (Inners and Felsenfeld, 1970) are known to stack their bases, and as the process depends on the concentration of salt, it is reasonable to ascribe the observed endothermic dilutions to an accompanying reduction in base stacking. If we call the fraction of bases stacked x, the above data yield $dx/d \log [Na^+]$ = 0.03–0.06 for poly(A) at 25°, depending on the choice of ΔH of stacking (−6.5 to −13 kcal/mol). This effect is half as large as that calculated from a comparison of the effect of Na^+ on the heat of binding of Mg^{2+} to poly(A) and poly(U) (Krakauer, 1972). [The latter may be an overestimate because part of the difference may be due to a heat of binding of Mg^{2+} to AMP 2 kcal/mol less positive than to UMP (R. Biltonen, personal communication)]. It is difficult at present to decide whether the discrepancy is significant. It is, however, clear from both calculations that the effect of Na^+, and also of Mg^{2+}, on the structure itself of poly(A) and, more importantly on the average axial interphosphate distance, is rather small. Consequently, the direct potentiometric measurements are taken to reflect more faithfully the interactions of poly(A) with Na^+.

Both types of measurements led to the conclusion that the interaction or "binding" of Na^+ depends only on the net linear charge density of the polymers, and thus on the extent of binding of Mg^{2+} which serves to reduce it, and not, at least directly, on the concentrations of the ionic species. Table IV, therefore, presents the parameters of the quadratic equations that were found to represent the experimental data, obtained

TABLE IV: The "Binding" of Na^+.

$$\Psi = B_0 + B_1\theta + B_2\theta^2$$ [a]

	B_0	B_1	B_2
Poly(A)[b]	0.400	−0.948	0.294
Poly(U)[b]	0.375	−0.899	0.306
Poly(A)·poly(U)[b]	0.592	−1.731	1.116
Poly(A)·2poly(U)[c]	0.658	−1.948	1.264

[a] Ψ is the fraction of sites (nucleotides) to which Na^+ is "bound." [b] The coefficients of the equation for these polynucleotides are based on potentiometric measurements. The standard error of Ψ is in all three cases ±0.01. [c] These coefficients were obtained by linked-function analysis of the binding of Mg^{2+} in the presence of various concentrations of Na^+ (data taken from Archer *et al.* (1972)).

TABLE V: The Enthalpies of the Transitions in Solutions Containing Only Na Salts.

Transition	$\Delta H/T_m^2$ [a]
2→1	0.0740 ± 0.0006[d]
3→2	0.0378 ± 0.0008
3→1	0.110 ± 0.001
2→3[b]	0.0168 (T_m = 45°)
	0.0181 (T_m = 68°)
2→3[c]	0.0127 (T_m = 57.5°)

[a] The units of ΔH are calories per mole of base pairs when the initial complex is two stranded, and per mole of base triplets when it is three stranded. T_m is in °K. [b] ΔH for this transition was obtained by calculation using Hess's law. The inconstancy of $\Delta H/T_m^2$ is expected here because of the curvature of the line T_m *vs.* log [Na^+]. [c] The ΔH of this entry is a measured quantity. [d] The uncertainties are standard errors of the mean (data taken from Krakauer and Sturtevant (1968)).

in the cases of poly(A), poly(U), and poly(A)·poly(U) by potentiometric measurement, and in the case of poly(A)·2poly(U) by linked-function analysis because the low stability of this complex at the concentrations of Na^+ in the absence of Mg^{2+} at room temperature prevented the application of potentiometry to the determination of its interaction with Na^+.

(*D*) *The Enthalpy Changes that Accompany the Transitions.* The results of the calorimetric measurement of the enthalpies of transitions induced in the presence of Na^+ as the sole cation are summarized in Table V (Krakauer and Sturtevant, 1968). When applied to transitions that take place in the presence also of Mg^{2+}, correction must be made for the contribution of the enthalpy of binding of Mg^{2+} to the initial and final forms of the polynucleotides (Table III).

(*E*) *Thermodynamic Analysis of the Transitions.* A most general and useful formalism for the analysis of equilibria among, for example, macromolecular species influenced by small molecular components of the solution has been summarized by Wyman (1964). Equivalent equations may be derived through the statistical mechanical approach of Crothers (1971). That, however, specified, as an initial assumption, that the effects of the small molecules result from their being bound in the conventional manner to the macromolecules. Because of the uncertainties about the specific nature of the interaction of Na^+ and even Mg^{2+} to the polynucleotides, it was thought best to adopt the less restrictive approach of Wyman.

The conformational transitions listed in the "introduction" are all specified to be isomeric equilibria

$$A \rightleftharpoons B \qquad K_{tr} = [B]/[A] = e^{-\Delta G^\circ_{tr}/RT} \tag{3}$$

because, in the absence of Mg^{2+}, their position or, more exactly, their T_m's are not affected by the concentration of the polynucleotides over a 100-fold range (Krakauer and Sturtevant, 1968). The implication is that strand separation does not occur in the vicinity of the T_m, and this is true for complexes of long-chained polymers. Nevertheless, the average lengths of segments containing residues in either state are sufficiently great that if a state represents a mixture of species, *e.g.*, the random coils, its properties are adequately approximated by the average of those of the independent species.

The equilibria are, of course, affected by the temperature and the concentrations or activities of Na^+ and of Mg^{2+}

$$\Delta G^\circ_{tr} = f(T, \ln Na, \ln Mg) \tag{4}$$

The symbols Na and Mg refer to the activities of the ions.

$$d\left(\frac{\Delta G^\circ_{tr}}{T}\right) = \frac{1}{T}\left[\left(\frac{\partial \mu^\circ_B}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} \left(\frac{\partial \mu^\circ_A}{\partial \ln Mg}\right)_{Na,T,n_A,n_B}\right] d\ln Mg + \frac{1}{T}\left[\left(\frac{\partial \mu^\circ_B}{\partial \ln Na}\right)_{Mg,T,n_A,n_B} \left(\frac{\partial \mu^\circ_A}{\partial \ln Na}\right)_{Mg,T,n_A,n_B}\right] d\ln Na + (\Delta H)_{Na,Mg,n_A,n_B} d\left(\frac{1}{T}\right) \tag{5}$$

The symbols n_A and n_B specify the quantities of species A and B in the system. The quantities $\partial\mu^\circ_B/\partial \ln Mg$, etc., have a simple operational interpretation. For example

$$\left(\frac{\partial \mu^\circ_B}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} = \left(\frac{\partial \mu^\circ_B}{\partial n_{Mg}}\right)_{Na,T,n_A,n_B} \left(\frac{\partial n_{Mg}}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} = \left(\frac{\partial \mu_{Mg}}{\partial n_B}\right)_{Na,T,n_A,n_{Mg}} \left(\frac{\partial n_{Mg}}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} \tag{6}$$

the last because of the reciprocity relations. But, as the activity of Mg^{2+} depends on the concentration of Na and those of the polymers with which it interacts, *i.e.*, ln Mg = $f(T, Na, n_A, n_B)$

$$(d \ln Mg)_{Na,T,n_A} = \left(\frac{\partial \ln Mg}{\partial n_{Mg}}\right)_{n_A,n_B,Na,T} dn_{Mg} + \left(\frac{\partial \ln Mg}{\partial n_B}\right)_{n_A,T,Na,n_{Mg}} dn_B \tag{7}$$

so that at fixed activity of Mg^{2+}

$$0 = \left(\frac{\partial \ln Mg}{\partial n_{Mg}}\right)_{n_A,n_B,Na,T} \left(\frac{\partial n_{Mg}}{\partial n_B}\right)_{Mg,Na,n_A,T} + \left(\frac{\partial \ln Mg}{\partial n_B}\right)_{n_{Mg},Na,n_A,T} \tag{8}$$

or

$$-\left(\frac{\partial n_{Mg}}{\partial n_B}\right)_{Mg,Na,n_A,T} = \left(\frac{\partial \ln Mg}{\partial n_B}\right)_{n_{Mg},Na,n_A,T} \left(\frac{\partial \ln Mg}{\partial n_{Mg}}\right)^{-1}_{Na,T,n_A,n_B} = \frac{1}{RT}\left(\frac{\partial \mu_{Mg}}{\partial n_B}\right)_{n_{Mg},Na,n_A,T} \left(\frac{\partial n_{Mg}}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} = \frac{1}{RT}\left(\frac{\partial \mu^\circ_B}{\partial \ln Mg}\right)_{Na,T,n_A,n_B} \tag{9}$$

The last equality is from eq 6. $(\partial n_{Mg}/\partial n_B)_{Mg,Na,n_A,T}$ is simply the number of moles of Mg^{2+} that must be added or removed along with every mole of B to maintain a constant activity of Mg^{2+}, under the specified conditions. The nature of the interaction between B and Mg^{2+} which affects the activity of Mg^{2+} is not defined. In what follows "binding" will be used to denote this quantity because conventional binding is the most easily visualized type of interaction giving this result although it should not be so narrowly construed.

The following symbols will be defined for convenience.

$$\left(\frac{\partial n_{Mg}}{\partial n_B}\right)_{Mg,Na,n_A,T} \equiv \theta_B \qquad \left(\frac{\partial n_{Mg}}{\partial n_A}\right)_{Mg,Na,n_B,T} \equiv \theta_A$$

$$\left(\frac{\partial n_{Na}}{\partial n_B}\right)_{Mg,Na,n_A,T} \equiv \Psi_B \qquad \left(\frac{\partial n_{Na}}{\partial n_A}\right)_{Mg,Na,n_B,T} \equiv \Psi_A \tag{10}$$

TABLE VI: The Calculation of $\Delta\theta$ and $\int \Delta\theta \, d \ln Mg$ by Means of Equation 16.[a]

ln Mg	θ_A	θ_U	$\theta_{A \cdot U}$	$\Delta\theta$[b]	$\int \Delta\theta \, d \ln Mg$	$[Mg^{2+}]_t M$[c]
−16.0	0.004	0.001	0.012	−0.010	−0.006	
−15.5	0.006	0.002	0.020	−0.016	−0.012	
−15.0	0.010	0.002	0.030	−0.024	−0.023	
−14.5	0.016	0.003	0.041	−0.032	−0.036	
−14.0	0.024	0.004	0.056	−0.042	−0.055	
−13.5	0.034	0.008	0.073	−0.051	−0.078	
−13.0	0.048	0.013	0.094	−0.064	−0.107	
−12.9	0.052	0.014	0.099	−0.066	−0.113	1.34×10^{-5}
−12.8	0.055	0.015	0.102	−0.067	−0.120	1.42×10^{-5}
−12.7	0.060	0.017	0.108	−0.070	−0.127	1.53×10^{-5}
−12.6	0.063	0.018	0.113	−0.073	−0.1311	
−12.5	0.066	0.020	0.118	−0.015	−0.142	
−12.0	0.088	0.030	0.142	−0.083	−0.181	
−11.5	0.112	0.045	0.170	−0.092	−0.225	
−11.0	0.140	0.068	0.199	−0.095	−0.272	
−10.5	0.168	0.094	0.227	−0.096	−0.139	
−10.0	0.200	0.130	0.259	−0.094	−0.367	
−9.5	0.232	0.169	0.289			
−9.0	0.267	0.217	0.322			
−8.5	0.300	0.220	0.355			
−8.0	0.338	0.328	0.389			
−7.5	0.373	0.389	0.422			
−7.0	0.410	0.459	0.459			

[a] The experimental conditions in this example were: $T_m = 44°$, $[Na^+] = 0.010$ M, $[Mg^{2+}]_t = 1.45 \times 10^{-5}$ M, poly(A)·poly(U) = 1.45×10^{-4} N. For the purposes of application of eq 12, $[Na^+]_t = 0.019$ M. [b] $\Delta\theta = \theta_{A \cdot U} - \frac{1}{2}(\theta_A + \theta_U)$. [c] See text for the method of calculation of this quantity. It corresponds to ln $[Mg^{2+}]_t$ of Table VII.

So that

$$d(\Delta G^\circ_{tr}/T) = -\Delta\theta R \, d \ln Mg - \Delta\Psi R \, d \ln Na + (\Delta H)_{Na, Mg, n_A, n_B} d(1/T) \quad (11)$$

where $\Delta\theta = \theta_B - \theta_A$, and $\Delta\Psi = \Psi_B - \Psi_A$.

Two constraints throw eq 11 into forms useful for application to the analysis of the transitions. If it is applied at the T_m, *i.e.*, at fixed $K_{tr} = [B]/[A] (= 1)$, $d(\Delta G^\circ_{tr}/T) = 0$

$$0 = \Delta\theta \left(\frac{dT_m}{d \ln Mg}\right)^{-1} + \Delta\Psi \left(\frac{dT_m}{d \ln Na}\right)^{-1} + \frac{(\Delta H)_{Na, Mg}}{RT_m^2} \quad (12)$$

(This equation reduces to eq 13 obtained by Krakauer and Sturtevant (1968) if Mg^{2+} is not a component of the solution.) Alternatively, it may be applied at fixed temperature, *i.e.*,

$$d \ln K_{tr} = \Delta\theta \, d \ln Mg + \Delta\Psi \, d \ln Na \quad (13)$$

$$\ln (K_f/K_i) = \int_{Mg} \Delta\theta \, d \ln Mg + \int_{Na} \Delta\Psi \, d \ln Na \quad (14)$$

$d(1/T) = 0$. A convenient path along which to perform the integration is

A,B $(K_i, [Na^+]_i, [Mg^{2+}]_i = 0)$

↓

A,B $(K_i', [Na^+]_f, [Mg^{2+}]_i = 0)$

↓

A,B $(K_f, [Na^+]_f, [Mg^{2+}]_f)$

because the first step is carried out in the absence of Mg^{2+} and therefore $\Delta\Psi$ is a constant over its path: Ψ depends only on the charge density of the polynucleotides, not on their concentration or that of Na^+. In addition, we may require that $K_i = K_f$ to obtain finally

$$0 = \int_{Mg_i}^{Mg_f} \Delta\theta \, d \ln Mg + \Delta\Psi_0 \ln ((Na)_f/(Na)_i) \quad (15)$$

The K_{tr} at which the system will be maintained will be that at the T_m. The data presented in the preceding sections may be used to calculate all the parameters of eq 12 and 15.

The extent of binding of Mg^{2+}, θ, to the polynucleotides and to their complexes at specified temperatures and concentrations of Na^+ and as a function of $[Mg^{2+}]$, or in some cases the activity of Mg^{2+}, was obtained from iterative solutions of the equation

$$\ln \left(\frac{\theta}{1 - \theta [Mg^{2+}]} \frac{1}{}\right) = \ln K_\theta - \frac{\Delta H_\theta}{R} \left(\frac{1}{T} - \frac{1}{298}\right) = a_0 + a_1\theta - (a + b\theta + c\theta^2) \frac{1}{R} \left(\frac{1}{T} - \frac{1}{298}\right) \quad (16)$$

with a_0 and a_1 taken from Tables I and II and a, b, and c from Table III (T is the absolute temperature).

$\Delta\theta$ at any $[Mg^{2+}]$, at the specified T and $[Na^+]$, and for any transition is thus readily calculated.

Table VI illustrates the application of eq 16 to the 2→1 transition under the conditions specified. The last two columns contain data called for by eq 15. $[Mg^{2+}]_t$ is the sum of the concentration of free Mg^{2+}, as determined by its activity given in the first column and the activity coefficient applicable at an ionic strength of 0.01 and 25° (Krakauer, 1971), and of the concentrations of Mg^{2+} bound to the polynucleotides distributed among their various forms as though the system were at the T_m at every concentration of Mg^{2+} (in this case half in the two-stranded and half in the random coil form). Figure 7 presents data analogous to those in the fifth and six columns, but for transition 2→3. Because the pattern of this figure is highly significant (see below), its sensitivity to the experimental uncertainties was explored. The initially negative character of $\Delta\theta$, followed by the change in its sign, is not lost

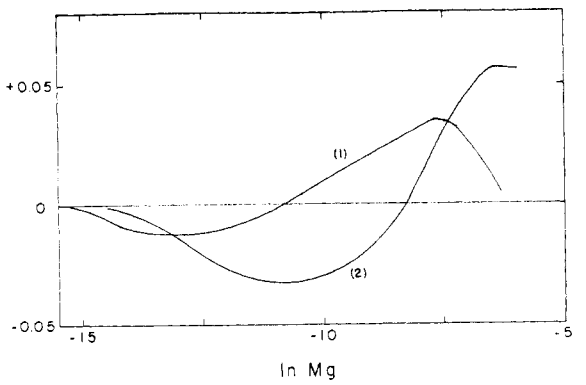

FIGURE 7: An illustration of the application of eq 15 to transition 2→3 in 0.01 M Na^+ at 25°. Line (1) gives $\Delta\theta$ and line (2) $\int_0^{Mg}\Delta\theta \cdot d \ln Mg$ (see text for details.)

until the parameters used in its calculation are altered substantially, that is until a_0 and a_1 for poly(A)·poly(U) are increased by a full standard deviation *and* those of poly(A) and poly(A)·2poly(U) are decreased by like quantities (or *vice versa*). More specifically, an alteration of a_0 and a_1, both in the same direction, by one standard deviation changes θ by about 10–12%. Because the magnitude of $\Delta\theta$ for transition 2→3 is by far the smallest, it is most substantially affected by these uncertainties and, therefore, great demands on the quantitative reliability of data calculated from it cannot be made. Greater precision of derived results may on the other hand be expected for the other transitions.

Table VII presents the application of eq 15 to the transitions encountered in the poly(A)–poly(U) system. The equation may be used to calculate $\Delta\Psi_0$, the change in binding of Na^+ that results from a conformational change *in the absence of* Mg^{2+}, if the activities of Na^+ required to give the same T_m in the absence, $(Na)_i$, and in the presence of $[Mg^{2+}]_t$, $(Na)_f$, are known.

$$\Delta\Psi_0 \text{ (eq'n)} = \frac{\int_0^{(Mg)_f} \Delta\theta \, d \ln Mg}{\ln [(Na)_f/(Na)_i]}$$

$(Mg)_f$ is the activity of Mg^{2+} at the T_m, in the presence of the polynucleotides, that corresponds to the actual total concentration of Mg^{2+}, $[Mg^{2+}]_t$, in the system. This quantity may be compared with the equivalent one calculated from measurements of Na^+ binding by potentiometric methods (at room temperature), $\Delta\Psi_0$ (EMF), or the application of eq 12, $\Delta\Psi_0$ (calorim), both in the absence of Mg^{2+}.

Alternatively, given $\Delta\Psi_0$, and here $\Delta\Psi_0$ (EMF) will be used, eq 15 may be used to calculate the concentration of Mg^{2+}, or more generally $(Mg)_f$, needed to cause a temperature to be the T_m in the presence of a specified $(Na)_f$. (This temperature is also the T_m in the presence of $(Na)_i$ and no Mg^{2+}.) This $(Mg)_f$ is simply the upper limit of the integral that results in its value exactly adding to $\Delta\Psi_0 \ln [(Na)_f/(Na)_i]$ to give a sum of zero. The next to the last column gives the logarithm of the total concentration of Mg^{2+} so calculated and the last column that determined experimentally. (The last column gives simply the logarithms of the quantities in the fourth column.)

Table VIII illustrates the application of eq 12. The first four columns identify the transitions and the conditions under which they are observed. The θ's for the various polynucleotides and complexes, and so $\Delta\theta$, may be calculated under those conditions in the manner described, and from them the various $(\Delta H)_{Na,mg}$ (Tables III and V), and the Ψ's and $\Delta\Psi$'s (Table IV). The last are identified as $\Delta\Psi$ (EMF), the last column in the table. Compared to them are the $\Delta\Psi$ (next to last column) that may be calculated by means of the equation, given all the other parameters. The quantities $dT_m/d \ln Na$ are obtained directly from graphs such as Figures 4 or 5. $dT_m/d \ln Mg$ may be obtained from Figure 6 through the transformation

$$\frac{dT_m}{d \ln Mg} = \frac{dT_m}{d \ln [Mg^{2+}]_t} \frac{d \ln [Mg^{2+}]_t}{d \ln Mg}$$

with $d \ln [Mg^{2+}]_t/d \ln Mg$ calculated from tabulations of the two quantities as in Table VI. (The transformation is required in the case of Mg^{2+} but not in that of Na^+ because the concentrations of the former are comparable to those of the polynucleotides while the latter are much greater.)

Transition 3→2 does not appear in Table VIII because $dT_m/d \ln Mg$ is not available for it. Similarly $d T_m/d \ln Na$ is not known independently for transition 2→3 at 25° in 0.01 M Na^+. The quantity in parentheses was calculated by use of $\Delta\Psi$ (EMF) and eq 12. (The concentration of Mg^{2+} of 2.63 mM, in this example, was obtained by extrapolation of line 1 of Figure 6.)

Discussion

The principal objective of these investigations was to render intelligible the complex behavior, displayed in Figures 3–6,

TABLE VII: The Consistency of the Parameters that Characterize the Transitions Tested by Means of Eq 15.

Transition	T_m (°C)	$[Na^+]_f$	$[Mg^{2+}]_t \times 10^{-4}$ M	$\Delta\Psi_0$ (eq'n)	$\Delta\Psi_0$[a] (EMF)	$\Delta\Psi_0$[b] (calorim)	Ln $[Mg^{2+}]_t$ (eq'n)	Ln $[Mg^{2+}]_t$ (exptl)
2→1	44	0.010M	0.145	−0.19			−11.05	−11.14
	52	0.027	0.73	−0.21	−0.20	−0.16	−9.63	−9.53
	57	0.063	1.45	−0.21			−8.90	−8.84
3→1	55	0.027	1.08	−0.18			−8.36	−9.13
	55	0.010	0.73	−0.19	−0.27	−0.21	−8.31	−9.53
	63	0.010	1.45	−0.19			−6.93	−8.84
3→2	34	0.010	0.145	−0.029	−0.14	−0.13	−9.66	−11.14
	36	0.020	0.145	−0.061			−10.4	−11.14
2→3	25	0.010	26.3	+0.01			−6.19	−5.94
	43	0.010	1.45	−0.01	+0.01	+0.05	−6.93	−8.84
	51	0.027	1.45	0			−7.03	−8.84

[a] Calculated from data in Table IV. [b] Calculated from data in Table V and eq 12. $\Delta\Psi$ has the units of moles of Na^+ per mole of nucleotide.

TABLE VIII: The Consistency of the Parameters that Characterize the Transitions Tested by Means of Eq 12.

Transition	T_m (°C)	[Na^+] (M)	$[Mg^{2+}]_t \times 10^{-4}$ M	dT_m/d ln Mg	$\Delta\theta$	$(\Delta H)_{Na,Mg}/RT_m^2$	$\frac{dT_m}{d \ln Na}$	$\Delta\Psi$	$\Delta\Psi^a$ (EMF)
2→1	44	0.010	0.145	+4.4	−0.067	+0.018	+2.2	−0.004	−0.07
	52	0.027	0.73	+3.2	−0.070	+0.018	0	0	−0.04
	55	0.064	0.73	+2.1	−0.031	+0.018	+7.5	−0.024	−0.13
3→1	55	0.010	0.73	+8.4	−0.127	+0.0164	−6.1	+0.008	+0.030
	55	0.027	1.08	+6.7	−0.112	+0.0166	−2.6	−0.0004	−0.009
	63	0.010	1.45	+8.8	−0.130	+0.0165	−7.1	+0.012	+0.059
2→3	25	0.010	26.3	−6.0	+0.012	+0.0031	(+10)		−0.01
	43	0.010	1.45	−4.6	+0.005	+0.0039	+8.3	−0.02	−0.02
	51	0.027	1.45	−2.0	+0.0068	+0.0042	+8.3	−0.01	−0.02

[a] Calculated from data in Table IV. Extensive quantities are given per mole of nucleotides.

of complexes of poly(A) and poly(U) in solutions containing mono- and divalent cations. The thermodynamic framework for such an undertaking is provided by eq 12 and 15 which disclose the parameters, $\Delta\theta$, $\Delta\Psi$, and ΔH, that govern this behavior and that must therefore be measured. A secondary objective was the deduction, if possible, of the nature of the interaction of the cations with the polynucleotides from the above and from the patterns of the variations of Ψ and θ. The detailed discussion of these matters, given below, may be prefaced to advantage with a brief summary.

The transitions in the absence of Mg^{2+} are simplest to interpret and illustrate in most elementary terms the operation of this principle of Le Chatelier. They are all driven in the direction written by elevation of temperature and must therefore have $\Delta H > 0$. Those for which $\Delta\Psi_0 < 0$, *i.e.*, which result in a net decrease in the effective binding (eq 10) of Na^+, must be inhibited by increases in the concentration of this ion and will, in consequence, require higher temperatures for their induction. This indeed is the pattern followed by transitions 2→1 and 3→1. Transition 2→3, for which $\Delta\Psi_0 > 0$, must follow the opposite pattern and does.

Mg^{2+} exerts its influence directly, in the manner of Na^+ just described, but also indirectly by profoundly affecting $\Delta\Psi$ and, less significantly, by its effect on ΔH. The consequences of the presence of Mg^{2+} are seen most prominently at low concentrations of Na^+, <0.1 M. There it tends to stabilize the reactants in transitions 2→1 and 3→1 with respect to the products ($\Delta\theta < 0$) and also of transition 2→3 at very low [Mg^{2+}]. At higher [Mg^{2+}], $\Delta\theta > 0$ for this last transition and it is facilitated by this ion, sufficiently so that at some point, determined in part by the concentration of Na^+, the transition becomes possible and occurs at decreasing T as [Mg^{2+}] is increased.

The striking reversal in the region of low [Na^+], compared to high [Na^+], of the variation of T_m with ln [Na^+] of transition 2→3 and 3→1 in the presence of Mg^{2+} is evidently, put a bit too simply, the result of the reversal of the sign of $\Delta\Psi$. Here transition 2→3 is accompanied by a net decrease in the effective binding of Na^+, and transition 3→1 by an increase, with the expected consequences on the required changes in the temperature at which the transitions are caused to take place as the concentration of Na^+ is altered.

To the extent that the above description is correct it supports the formulations on whose basis θ and Ψ were calculated. It is tempting to infer from them, particularly from the very different dependences of Ψ and θ on the concentrations of Na^+ and Mg^{2+}, that the nature of the interaction of these ions with the polynucleotides is different, that of Na^+ being of a diffuse electrostatic type and that of Mg^{2+} a tighter specific type.

The validity of these conclusions is dependent on whether the consistency among $\Delta\Psi$, $\Delta\theta$, and ΔH that is required by eq 12 and 15 is achieved. This is tested in Tables VII and VIII. Because, however, of the limited precision of the data used to calculate the entries in these tables, the criteria applied and, therefore, the conclusions drawn must be of a semiquantitative nature. (The enthalpies of the transitions have been estimated (Krakauer and Sturtevant, 1968) to be subject to an overall uncertainty of ±5% for the larger ones (transitions 2→1 and 3→1) and up to ±20% for the smaller (transitions 3→2 and 2→3), although the precision of the measurements is better. The standard errors to be applied to calculated values of individual Ψ_0's and Ψ's are ±0.01 (Table IV), and therefore those of the $\Delta\Psi_0$'s and $\Delta\Psi$'s may be expected to be $< \pm 0.02$. Estimates of uncertainties in the θ's and $\Delta\theta$'s have been discussed in the previous section.)

The consistency of the measured and calculated quantities for transition 2→1 shown in Table VII is satisfactory, even on a quantitative basis, and somewhat less so for transition 3→1. The reason for the low values of $\Delta\Psi_0$ (eq'n) of transition 3→2 is not clear. It may be noted, however, that the concentration of Mg^{2+} is quite low in these examples. $(Na)_i$ and $(Na)_f$ are rather close and the θ's and $\Delta\theta$ quite low ($|\Delta\theta| < 0.023$ over the range of the integration) and that, therefore, substantial uncertainty must be present in the calculation of both the integral and the second term.

Because $\Delta\theta$ for transition 2→3 is but a small difference between relatively large θ's (note the rather high concentrations of Mg^{2+}), it is best to attempt to interpret it only in a qualitative manner. This may be done most profitably by reference to Figure 7. Because $\Delta\Psi_0$ for this transition must be positive on the basis of eq 12 or 13 given by Krakauer and Sturtevant (1968) and because $(Na)_f < (Na)_i$ (*i.e.*, ln $(Na)_f/(Na)_i < 0$), eq 15 can be satisfied only if the integral is positive. Figure 7 makes it clear that this will be so only if the concentration of Mg^{2+} is sufficiently high. A decrease in $[Mg^{2+}]_t$ will result in a decrease in the value of the integral, neglecting the relatively small effect of temperature, requiring a compensating decrease in the term in $\Delta\Psi_0$. That may be achieved by narrowing the difference between $(Na)_f$ and $(Na)_i$, that is by inducing the transitions in the presence and absence of Mg^{2+} at a higher temperature (see Figure 4). Thus, we may expect the T_m to increase as the concentration of Mg^{2+} is decreased. However, at a sufficiently low concentration of Mg^{2+} at a specified concentration of Na^+, the integral will

become <0, it will no longer be possible to satisfy eq 15 and, therefore, transition 2→3 will no longer be observed. This is precisely what is revealed by Figure 5. The behavior of transition 2→3 in the presence of Mg^{2+} thus is readily intelligible simply on the basis of the differences in the binding of Mg^{2+} to reactants and products.

Table VIII also demonstrates the qualitative pattern of consistency and gives further insight into the factors that govern the transitions. Note that $\Delta\theta$ and d T_m/d ln Mg are of opposite sign, as are $\Delta\Psi$ and d T_m/d ln Na (except in one case in transition 3→1 of which too much should not be made because of the closeness of $\Delta\Psi$ to zero). (Note also that in all cases, the magnitude of $\Delta\Psi$ is determined by small differences between relatively large numbers.) The physical interpretation of these correlations was given at the beginning of this section in a simplified manner in terms of the principle of Le Chatelier. This interpretation is reinforced by the simultaneous reversal in the signs of $\Delta\Psi$ and d T_m/d ln Na when transition 2→3 and 3→1 are carried out at low [Na^+] in the presence of Mg^{2+} rather than at high [Na^+] in the absence of Mg^{2+}. The reversals in the sign of $\Delta\Psi$ are readily understood on the basis of the variation of Ψ with θ and the relative affinities of the polynucleotides for Mg^{2+}, and most clearly illustrated in transition 3→1. $\Delta\Psi_0 < 0$ in consequence of the higher charge density of the complex. It also, however, binds Mg^{2+} much more avidly than, in particular, poly(U), but also poly(A), sufficiently so that, in the presence of appropriate concentrations of Na^+ and Mg^{2+}, θ for it will be so much greater that its charge density will be reduced below that of the random coils. $\Delta\Psi > 0$ must then follow (see Figure 10 of Archer *et al.* (1972)). Any manipulation of the system that results in the reduction of the extent of binding of Mg^{2+} to all species, such as a reduction in the concentration of Mg^{2+} or an increase in that of Na^+, may be predicted to cause $\Delta\Psi$ to be less positive and dT_m/d ln Na to be less negative. Figure 4 and Table VIII bear out this prediction. (It is also to be noted that eq 12 requires in the case of transition 2→1 at $T_m = 52°$ that $\Delta\Psi = 0$ because dT_m/d ln Na = 0, irrespective of the other terms. The principle also, of course, requires this.)

It remains to determine the extent to which the above analysis clarifies the nature of the interaction of Mg^{2+} and Na^+ with the polynucleotides. The phenomenological approach employed neither requires nor provides information bearing directly on this problem. An empirical determination of the effect of the polynucleotides on the activities of Mg^{2+} and Na^+ is all that is called for by eq 9, and precisely that is supplied by the measurements described in the papers of Krakauer (1971) and Archer *et al.* (1972). If Ψ is defined in accordance with eq 9, it follows immediately, if the polymer may be approximated by a rod, that, for a fixed linear charge density Ψ will not depend on the concentration of Na^+. This is because for a rod-like polyelectrolyte (Bailey 1973; Nagasawa and Muroga, 1972) G_{el} is a linear function of the logarithm of the ionic strength or, simply, of the concentration of Na^+ in the present systems. Therefore, assuming that only G_{el} depends on the concentration of Na^+

$$\Psi = \left(\frac{\partial n_{Na}}{\partial n_p}\right) = -\frac{1}{RT}\left(\frac{\partial G_{el}}{\partial \ln Na}\right) = \text{constant} \qquad (17)$$

p = polyelectrolyte

The analysis of Manning (1972a) produces a result similar in form for $\Delta\Psi$. It is to be emphasized that no molecular basis for Ψ has been invoked at this point: Ψ merely reflects the reduction in the activity of Na^+ in the presence of the polymer, or, conversely, the dependence of G_{el} of the polymer on the concentration of Na^+.

The interaction of Mg^{2+} with the polynucleotides may also be viewed in this abstract manner, but it is likely that it is in fact site binding. The binding is anticooperative, this last being interpretable in two alternative but ultimately equivalent ways. The form of the anticooperativity suggests electrostatic interaction among the sites, or, in other words, that the binding is partly driven by reductions in $|G_{el}|$ that follow attachment of Mg^{2+} to the sites, $(\partial G_{el}/\partial\theta)$, reductions that decrease as θ increases, or as the concentration of Na^+ (or the ionic strength) is increased. Alternatively, the anticooperativity could be said to be due to a decreasing release of Na^+ that accompanies the binding of Mg^{2+}, $\partial\Psi/\partial\theta$, as θ increases, or release into solutions of Na^+ at higher chemical potential, as the concentration of Na^+ is increased. Because of eq 17, the two alternatives are equivalent. (This was not recognized by the author in a previous paper (Krakauer, 1971), although that they were indistinguishable was (p 2483).) (The contribution of self-stacking has been disregarded in the above because it is peripheral to the argument and applies only to any significant extent to poly(A).)

The matter may be pursued a bit further. G_{el} of a polyelectrolyte is in general proportional to the square of its charge. Consequently, according to eq 17, the same must be true of Ψ. If it is now specified that Mg^{2+} binds specifically to the polynucleotides but that Na^+ does not, then the charge at any extent of binding θ will be proportional to $(1 - 2\theta)$, and therefore $\Psi \propto (1 - 2\theta)^2 = (1 - 4\theta + 4\theta^2)$. Note that the experimentally determined Ψ is well represented by a quadratic function of θ (Table IV) and that, as predicted, the signs of the coefficients of θ and θ^2 are opposite. These coefficients themselves, though of similar magnitude, are, however, not equal, nor are they exactly four times the constant term. Because of the limited precision of the potentiometric measurements, they are subject individually to substantial uncertainty. For example, while the lines for Ψ *vs.* θ for poly(A)·poly(U), obtained by means of potentiometry and linked-function analysis of the binding of Mg^{2+}, are practically indistinguishable (Figure 4 of Archer *et al.* (1972)), the equations that represent them have substantially different coefficients:

$$\Psi = 0.592 - 1.731\theta + 1.116\theta^2 \text{ (potentiometry)}$$

$$= 0.588 - 2.100\theta + 1.847\theta^2 \text{ (linked-function analysis)}$$

(Table X of Archer *et al.* (1972)). Thus it again appears that overall the theoretical prediction is borne out semiquantitatively by experiment and that therefore the assumptions put forward above concerning the different interactions of Mg^{2+} and Na^+ with the polynucleotides have some basis.

Equations that relate Ψ to θ have also been devised by Manning (personal communication) within the framework of his theory of counterion condensation.

$$\Psi = {}^1/_4\xi(1 - 2\theta)^2, \qquad \xi < (1 - 2\theta)^{-1}$$

$$= 1 - {}^3/_4\xi^{-1} - 2\theta, \qquad \xi > (1 - 2\theta)^{-1}$$

ξ is a parameter proportional to the linear charge density. $\xi = 1$ for poly(A) and poly(U) and 4.2 for poly(A)·poly(U) on the basis of their dimensions but must be 1.3 and 2.0, respectively, to be consistent with measured Ψ_0's. Because of the minimal counterion condensation on the single-stranded polymers, the theoretical predictions of this and the approach above are very similar for them. The observed variation of Ψ with θ shows, however, less curvature than expected. Both

formulations also produce equally good fits of the data for poly(A)·poly(U) if ξ is taken as 2 in Manning's.

It must be pointed out that the results on which the above analysis is based are at variance with those of a number of other studies, as was noted in the "introduction" in the case of Mg^{2+} binding and as may be most easily seen in Figure 4 of Auer and Alexandrowicz (1969) in the matter of the interactions with Na^+. The discrepancies can neither be reconciled nor accounted for. The uncertainties raised in consequence, as well as those due to the limited precision of the measurements themselves, interfere with the satisfactory testing of theoretical models of these interactions, particularly of the very interesting one of Manning (1969). The alternative test, that is of the ability of that model to account for the behavior of the transitions in the mixed electrolyte system, will not be attempted here because it is in preparation by its author (G. S. Manning, personal communication).

Finally, it should be noted that the elucidation of possible mechanisms of control of the conformations of macromolecular complexes by manipulation of the ionic environment is by no means of importance only in nucleic acids. A mechanism of this very sort has recently been proposed in connection with nerve excitability (Neumann *et al.*, 1973).

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