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Proximity of Nucleic Base and Phosphate Groups in Metal Ion Complexes of Adenine Nucleotides

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Perturbation of the adenine ring ultraviolet absorption spectrum is used to detect its inner sphere coordination by metal ions. Perturbations in adenine nucleotides are compared with those derived from complexes of aqueous metal ions and protonated tripolyphosphate complexed metal ions, $HOP_3O_9M^{2-}$. $HOP_3O_9Ni^{2-}$ forms an 11 times stronger complex with adenosine than aqueous Ni^{2+}, and also shows greater selectivity for complexation at N7 over N1. It is estimated that for Ni(II) intramolecular macrochelate formation between the phosphate residues and an inner sphere coordinated adenine base occurs in ATP^{4-}, ADP^{3-}, and AMP^{2-} with mole percentages 30%, 65%, and 80%, respectively. Thus increasing the number and hence denticity of phosphate residues reduces the ability of the nucleic base portion of a nucleotide to enter into inner sphere coordination in a monomeric complex. For other complexes with ATP^{4-}, mol percentages of macrochelate are about 10% for Mn^{2+} and zero for Mg^{2+}. It is suggested that small percentages of an intramolecular outer sphere adenine complexed species may occur with Ni(II) and ATP^{4-} and ADP^{3-}

Introduction

More than twenty years ago Szent-Gyorgyi suggested that adenosine-5′-triphosphate (ATP) may be folded so that close juxtaposition of the adenine base and triphosphate group permits energy to be transferred between them [1]. Folding is necessary for this energy transfer because in ATP a ribose sugar ring, which does not pass energy, separates the adenine base and triphosphate group. He also suggested that under some conditions metal ions such as Mg^{2+} may facilitate the folding by coordinating simultaneously to both phosphate oxygens and adenine nitrogens and thus bridge these functional groups of ATP in what has since been termed a macrochelate.

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There is good evidence that in neutral aqueous solutions nucleotides are predominantly folded in the same *anti* conformation that has also been found in many crystal structures [2]. Though this conformation brings the phosphate groups and the 5-membered ring of the purines into the same vicinity, the available evidence suggests that they are not close enough for energy transfer to take place [2, 3]. There is, however, a considerable body of information indicating that macrochelate formation does occur in the presence of some transition metal ions [2]. Most metal ions bind predominantly at the phosphate groups of nucleotides and the question becomes: "To what extent is the molecule folded over so that the base also interacts with a phosphate bound metal ion?" Many studies have been performed at nucleotide to metal ion molar ratios greater than 1:1 or at high concentrations of nucleotides where stacking takes place; both of these conditions lead to intermolecular interactions and compromise interpretation in terms of the extent of folding of monomeric 1:1 complexes.

Our approach to determine the degree of interaction of the adenine base with phosphate bound metal ions in adenine nucleotides is to measure the perturbation of the adenine ultraviolet absorption spectrum and compare that magnitude with a standard derived from model complexes. An early study employed aqueous metal ion perturbations of the nucleoside adenosine as the control [4]. In this paper we report the results of an investigation of metal ion complexes of monoprotonated tripolyphosphate, $HOP_3O_9^{4-}$, on the complexation equilibria and spectral perturbation of adenosine. By utilizing this sensitive technique for study of interactions between a metal ion and two parts of the ATP molecule, we are able to relate the results to the degree of macrochelate formation in monomeric ATP at low concentrations. Since it is anticipated that only adenine rings bound in the inner coordination sphere of metal ions will produce difference spectra, the percentages of macrochelate ring formation refer to inner sphere coordinated adenine macrochelates.

In neutral solutions ATP^{4-} bears four negative charges, all on the substituted tripolyphosphate chain. The simplest model for the tripolyphosphate chain in ATP is protonated tripolyphosphate of the identical charge, $HOP_3O_9^{4-}$. This species strongly binds Ni^{2+} and Co^{2+} with stability constants of $10^{5.01}$ and $10^{5.17}$ respectively [5]. The highest concentrations of the species $HOP_3O_9M^{2-}$ occur near pH 5.5 and we have therefore conducted our investigations of tripolyphosphate complexes near this pH. Because a great deal of useful complementary information exists on the interaction of nucleotides with Ni^{2+}, we have used it as a prototype for other transition metal ions. The stability constants and spectral perturbation produced by complexation of adenosine with $HOP_3O_9Ni^{2-}$ is compared to those with aqueous Ni^{2+}. These results are then applied to the interaction of Ni^{2+} with the 5′-nucleotides, adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP). These investigations provide a background for the mode of interaction of other metal ions including Mg^{2+}, the metal ion most often associated with ATP in living organisms.

Experimental

Nucleotides were the best products available from Sigma Chemical Corp. Metal ion reagents were perchlorates and sometimes chlorides but never nitrates, which absorb in the 280 nm region. Spectrophotometric measurements were conducted on a Cary model 14R spectrophotometer of notably low stray light characteristics. Two kinds of 1 cm absorption cell configurations were employed with similar results. There was always a cell containing a solution of both ligand and transition metal ion in the sample compartment and a cell containing a solution of ligand at the same concentration in the reference compartment. In this two-cell configuration any absorption due to metal ion (and its tripolyphosphate complex) was subtracted. Alternatively, a cell containing a solution of metal ion (and tripolyphosphate) was added to the reference compartment, a cell containing water to the sample compartment, and the differential absorptivity read directly. With adenosine and derivatives metal ions induce a red shift and the difference spectra are similar to those already pictured [4] with a maximum near 275 nm; all our results are reported for 280 nm where there is a lesser absorption due to phosphate–metal ion complexation. Ionic strength was maintained with $NaClO_4$ at 3 *M* with aqueous metal ions, at 0.2 *M* with tripolyphosphate complexes, and at 0.2 *M* with nucleotides. All results were obtained at room temperature, near 23 °C.

Results

For complex formation between a metal ion, M, and a ligand, L, we define the stability constant, K, in terms of molar concentrations.

$$M + L \rightleftharpoons ML \qquad K = [ML]/[M][L]$$

Conservation of mass requires that for the total molar concentrations of metal ion, C_M, and ligand, C_L, we have

$$C_M = [M] + [ML] \qquad C_L = [L] + [ML].$$

The difference in absorptivities between a solution of metal ion and ligand in the sample compartment and the same total concentration of ligand in the reference compartment is given by

$$\Delta A = \epsilon_L[L] + \epsilon_{ML}[ML] - \epsilon_L C_L = [ML](\Delta\epsilon),$$

where $\Delta\epsilon$ is the difference in molar absorptivities of complex, ϵ_{ML}, and ligand, ϵ_L. In solutions containing a large excess of metal ion over ligand, C_M reduces to [M] and substitution into and rearrangement of the stability constant equation yields

$$C_M C_L/(\Delta A) = C_M/(\Delta\epsilon) + 1/K(\Delta\epsilon). \qquad (1)$$

Thus a plot of the left hand side of the equation *versus* C_M should yield a straight line of slope $1/(\Delta\epsilon)$, ordinate intercept $1/K(\Delta\epsilon)$ and negative abscissa intercept of $-1/K$. Thus the two unknowns, the stability constant, K, and the differential molar absorptivity, $\Delta\epsilon$, produced by complexation of metal ion at a mol of ligand, are separable quantities. Features of this analysis are formally similar to that of enzyme–substrate complexes, but the final equation is cast into the form of eq. 1 rather than into that of a double reciprocal plot, as the former is markedly superior for treatment of results by least squares [6].

The association of adenosine with Ni^{2+} was investigated at pH 5 by observing the spectral perturbations of the adenine ring at 280 nm produced by addition of 0.1 to 0.5 *M* transition metal ion. The results of a least squares treatment with standard deviations are tabulated in Table I. The stability constant for Ni^{2+} and adenosine agrees with two values in the literature [7]. Perturbation of the

TABLE I. Nickel Stability Constants and Differential Molar Absorptivities at 280 nm.

	K, M^{-1}	$\Delta\epsilon$, mM^{-1}
Adenosine	2.1 ± 0.2	0.92 ± 0.07
+ Tripolyphosphate	23 ± 2	1.22 ± 0.08
ATP^{4-}		0.33
ADP^{3-}		0.71
AMP^{2-}	304 ± 28	0.78 ± 0.02

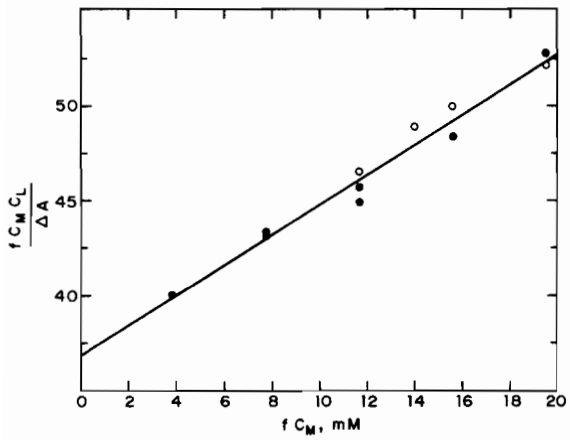

Fig. 1. Plot of product of millimolar concentrations of Ni-tripolyphosphate and adenosine divided by adenosine absorptivity difference at 280 nm *versus* millimolar concentration of $HOP_3O_9Ni^{2-}$ at pH 5.5. Solid circles refer to 0.40 to 0.48 m*M* and open circles to 0.24 m*M* adenosine concentrations. Shown is least squares line through 11 points.

adenosine absorption was also produced by the aqueous complex of Co^{2+} and more weakly by both Mn^{2+} and Mg^{2+}.

Of greater interest for our purposes is the stability constant and differential molar absorptivity produced by association of adenosine to a divalent transition metal ion strongly complexed to monoprotonated tripolyphosphate, $HOP_3O_9M^{2-}$. The logarithms of the stability constants for the Ni^{2+} and Co^{2+} tripolyphosphate complexes are 5.0 and 5.2, respectively [5], so that under our conditions they are nearly fully formed. Nevertheless, we have corrected for M^{2+} not complexed by $HOP_3O_9^{4-}$ by multiplying C_M by the fraction of M^{2+} complexed as $HOP_3O_9M^{2-}$, in all cases $f \geqslant 0.95$. All our results were obtained in equimolar solutions of M^{2+} and $HOP_3O_9^{4-}$ at pH 5.5, the optimum for formation of $HOP_3O_9M^{2-}$ for both Ni^{2+} and Co^{2+} [5].

Figure 1 shows a plot, the left-hand side of eq. 1 *versus* the molar concentration of $HOP_3O_3Ni^{2-}$. In order to check the validity of the analysis adenosine concentrations were varied by a factor of two and Fig. 1 shows good agreement over the span of concentrations. Results of the least squares treatment are given in Table I. For the corresponding Co^{2+} complex we obtained $K = 23 \pm 1\ M^{-1}$ and $\Delta\epsilon = 0.82 \pm 0.04\ mM^{-1}$. Weak perturbations of the adenosine absorption were also produced by the tripolyphosphate complexes of both Mn^{2+} and Mg^{2+}. Attempts to conduct similar experiments with Ni^{2+} and diphosphate were thwarted by precipitation at the relatively high concentrations required.

In order to assess the selectivity of metal ions for complexation at N1 or N7 of adenosine, we have conducted some ^1H nuclear magnetic resonance experiments with paramagnetic ions. It had been found that addition near pH 6 of tripolyphosphate to a solution of aqueous Ni^{2+} increases the broadening of adenosine H8 compared to H2 [8]. We have confirmed these results for Ni^{2+} and also find that addition of isocitrate at pH 6 has an effect similar to tripolyphosphate. In addition an increased adenosine H8 to H2 chemical shift occurs when either tripolyphosphate or isocitrate is added to a Co^{2+} solution of adenosine at pH 6. Thus compared to the aqueous metal ions, complexation of either Ni^{2+} or Co^{2+} with negatively charged tripolyphosphate or isocitrate ligands markedly increases the selectivity for coordination at N7 over N1 in adenosine.

Though aqueous Ni^{2+} in excess was able to perturb the ultraviolet absorption spectrum of the nucleoside cytidine (with a 6-membered nucleic base ring similar to that of adenosine) no perturbation was observed in 0.3 m*M* equimolar solutions of Ni^{2+} and cytidine-5′-triphosphate. This result is consistent with the conclusions from other studies that macrochelate formation occurs if at all only rarely in cytosine nucleotides [2].

Equimolar solutions of metal ions and ATP^{4-} or ADP^{3-} were investigated from pH 6.2 to 7.3 in the broad plateau region of predominance of 1:1 complexes [9]. Excess ligand or metal ions are apt to compromise the difference spectra interpretation. Solutions were investigated at 0.5 m*M* concentration where stacking interactions are insignificant [10]. The differential molar absorptivities at 280 nm recorded in Table I were calculated by dividing the observed absorptivity difference by the product of the concentration and the fraction of complexed nucleotide ($f > 0.84$) as calculated from published stability constants [11, 12]. On the same basis at the ATP^{4-} results reported in Table I, for Co^{2+}, $\Delta\epsilon = 0.29$ and for Mn^{2+}, $\Delta\epsilon \cong 0.10\ mM^{-1}$. A similar equimolar solution of ATP^{4-} and Mg^{2+} did not yield a difference spectrum in the 280 nm region. Cu^{2+} was not investigated due to its catalysis of ATP hydrolysis [9]. The result reported for AMP^{2-} and Ni^{2+} in Table I was obtained as those mentioned above for adenosine with excess Ni^{2+} at pH 7.3 and the stability constant is in superb agreement with those determined by a variety of methods [7, 13].

Discussion

As shown in Table I, the adenosine stability constant for Ni^{2+} in the net negatively charged $HOP_3O_9Ni^{2-}$ complex is 11 times greater than that for aqueous Ni^{2+} even though a statistical contribution operates in the opposite direction (no electrostatic contributions occur when one of the ligands is uncharged) [14]. The augmentation of mixed complexes of phosphates and aromatic amines has been observed in other systems [15], but not so drama-

tically as in this study. The enhanced stability of mixed complexes of tripolyphosphate and metal ions suggests that macrochelation in nucleoside phosphates should be more favorable than might otherwise have been anticipated.

At least a partial explanation for the favoring mixed complexed formation is the apparent greater tendency of phosphate compared to aqueous complexes of transition metal ions to coordinate at N7 rather than at N1 of adenosine. We have determined that the stability constant of Cu^{2+} with adenosine exceeds that with cytidine, for which K = 25 [16], despite the 4-fold greater basicity of the latter. This comparison supports significant aqueous Cu^{2+} binding at N7 of adenosine. The conclusion in the Results section that addition of tripolyphosphate selectively favors coordination at N7 over N1 in adenosine is important for it is coordination at N7 that takes place upon macrochelate formation in nucleotides [2]. For the interaction of phosphate bound metal ions with the adenine ring of ATP^{4-} and ADP^{3-} in a macrochelate, a more appropriate model complex for the interaction with adenosine is the coordination of $HOP_3O_9M^{2-}$ rather than aqueous M^{2+}.

The percentage of ATP^{4-} complexes in a macrochelate may be calculated from the ratio of $\Delta\epsilon$ at 280 nm for the nucleotide to that derived from the spectrophotometric analysis of $HOP_3O_9M^{2-}$ with adenosine. On this basis for ATP^{4-}, 27% of the Ni^{2+} and 35% of the Co^{2+} equimolar complexes occur as a macrochelate in which a single metal ion bridges the tripolyphosphate group and adenine ring in an inner sphere coordination. For Mn^{2+} the percentage is significantly less, of the order of 10%, but the weakness of the tripolyphosphate complex with adenosine makes difficult a more quantitative analysis. A difference absorption was not observed in the 280 nm region for Mg^{2+} and ATP^{4-} but significantly aqueous Mg^{2+} and $HOP_3O_9Mg^{2-}$ did induce difference spectra in adenosine. Thus Mg^{2+} possesses the capability of perturbing the adenosine absorption spectrum and the fact it does not do so in ATP^{4-} indicates that the amount of macrochelate is near 0%. This conclusion for Mg^{2+} is in agreement with results obtained by other methods [2].

In order to find the fraction α of adenine base inner sphere coordinated to Ni^{2+} in the series of nucleotides AMP^{2-}, ADP^{3-}, and ATP^{4-}, it is necessary to establish the appropriate base line 280 nm $\Delta\epsilon$ values for each case. As indicated in Table I, aqueous Ni^{2+} binds less strongly than $HOP_3O_9Ni^{2-}$ to adenosine and also yields a difference spectrum $\Delta\epsilon$ only 75% as great. Thus as the combined formal net charge on the nickel ion and the inner coordination sphere varies from 2+ for aqueous Ni^{2+} to 2− for $HOP_3O_9Ni^{2-}$, $\Delta\epsilon$ increases from 0.92 to 1.22 mM^{-1}. Linear interpolation then yields for the 0 and 1− formal net charged species, $\Delta\epsilon$ = 1.07 and 1.15 mM^{-1}, respectively.

It has been suggested that when a metal ion is inner sphere coordinated to the base portion of the nucleotide, the innermost or α-phosphate group is only outer sphere coordinated or not coordinated at all in an intramolecular complex [2]. Thus the combined formal net charge on the nickel ion and the inner coordination sphere is 1− for ATP^{4-}, 0 for ADP^{3-}, and 2+ for AMP^{2-}. That the combined formal net charge in the Ni^{2+} complex of AMP^{2-} is the same as that of aqueous Ni^{2+} is indicated by a plethora of crystal structures of transition metal ion–nucleoside monophosphate complexes [17] including [18] Ni^{2+} and AMP^{2-} which show inner sphere nucleic base and only outer sphere phosphate coordination in an intramolecular complex. Comparison of the $\Delta\epsilon$ values in Table I for the nucleotide complexes with those interpolated in the previous paragraph of the same formal net charge yields α values of 0.29 for ATP^{4-} (only slightly increased over that deduced above), 0.66 for ADP^{3-}, and 0.85 for AMP^{2-}. It is evident that the α values reflecting inner sphere adenine coordination to Ni^{2+} increase as the number of phosphate residues decreases. This result indicates that increasing the number and hence denticity of phosphate residues reduces the ability of the nucleic base portion of a nucleotide to be inner sphere coordinated in a monomeric complex.

It is useful to compare the fraction of nucleotide complexes in which the nucleic base is inner sphere coordinated to the metal ion, α, as derived above with that expected based on a comparison of the stability constants for an adenine nucleotide and the corresponding cytosine nucleotide or appropriate monoprotonated phosphate, neither of which forms a macrochelate and which bind transition metal ions solely at the phosphate residues. Such an analysis suggests that a third complex exists in addition to the cytosine nucleotide-like complex and the macrochelate with the perturbed absorption spectrum. Complexation of metal ion, M, to a nucleotide, PB, with the phosphate, P, and base portion, B, separately designated may be represented as

$$M + PB \underset{}{\overset{K_1}{\rightleftharpoons}} MPB \overset{K_2}{\rightleftharpoons} PMB$$

$$MPB \overset{K_3}{\rightleftharpoons} MPB'$$

Species MPB refers to a nucleotide that has undergone complexation at the phosphate residues only, PMB to the macrochelate that yields a perturbed adenine absorption spectrum, and MPB′ to a complex the structure of which will be discussed. Equilibrium constants are defined in the usual way to give K_1 = [MPB]/[M][PB], K_2 = [PMB]/[MPB], and K_3 = [MPB′]/[MPB].

Whether determined potentiometrically or spectrophotometrically nucleotide stability constants, K_s,

TABLE II. Parameters for Ni^{2+} Complexes of Adenine Nucleotides.

	ATP^{4-}	ADP^{3-}	AMP^{2-}
α	0.29	0.66	0.85
K_s/K_1	2.40	4.90	4.88
K_2	0.70	3.23	4.14
K_3	0.70	0.67	–
Mol Fractions:			
MPB	0.42	0.20	0.2
PMB	0.29	0.66	0.8
MPB′	0.29	0.14	0.0

reported in the literature are overall stability constants given by

$$K_s = K_1(1 + K_2 + K_3) \quad (2)$$

and the fraction of spectrally perturbed macrochelate is

$$\alpha = K_2/(1 + K_2 + K_3)$$

Combination of the last two equations yields $K_2 = \alpha K_s/K_1$ from which K_2 may be calculated.

Since a small difference between large numbers often appears, and there is some variation among stability constants reported from different laboratories, for consistency we consider Ni^{2+} stability constants from a single laboratory reported for 15 °C and 0.1*M* ionic strength [12]. From the following values for K_s and K_1 their ratios are collected in Table II. For ATP^{4-}, $K_s = 10^{4.79}$ and for CTP^{4-} or $HOP_3O_9^{4-}$, $K_1 = 10^{4.41}$. For ADP^{3-}, $K_s = 10^{4.18}$ and for CDP^{3-} or $HOP_2O_6^{3-}$, $K_1 = 10^{3.49}$. For AMP^{2-}, $K_s = 390$ and for CMP^{2-} or ribose-5′-phosphate, $K_1 = 80$. Multiplication of the K_s/K_1 ratios in the second row of Table II by the previously derived α values in the first row yields the K_2 values in the third row and from them the K_3 values in the fourth row. The AMP^{2-} complex represents a special case because the largest α value that is accommodated by the K_s/K_1 ratio used is 0.80 and the experimental value of 0.85 ± 0.07 is marginally greater. Use of net 1+ or zero charged interpolated $\Delta\epsilon$ values would yield smaller α values. Finally, the last three rows of Table II show the mol fractions of the three species implied by the equilibrium constants for each of the three adenine nucleotides.

The mol fraction of the adenine base inner sphere coordinated Ni^{2+} complex, PMB, increases as the number of phosphate residues decreases (Table II), while the mol fractions of the phosphate only bound complex, MPB, and that of species MPB′ decrease. The increase in PMB mol fractions from ATP^{4-} through ADP^{3-} to AMP^{2-} Ni^{2+} complexes is reflected by the increasing values of K_2 through the series. K_2 is a complex constant as it contains contributions not only from inner sphere coordination of the base but presumably also from loss of inner sphere α phosphate coordination to possible outer sphere complexation. The species MPB′ possesses a stability similar to but slightly less than that of MPB for ATP^{4-} and ADP^{3-} ($K_3 = 0.7$). Species MPB′ does not occur with cytosine nucleotides and does not possess an inner sphere coordinated adenine base, which would give rise to a difference spectrum. It may represent a complex in which the α-phosphate has been released from inner sphere coordination and is only outer sphere coordinated, and in which the adenine base might be outer sphere coordinated to the Ni^{2+}, which is inner sphere coordinated to the β (ADP^{3-}) or β and γ (ATP^{4-}) phosphate residues.

For Mg^{2+} the near identity of the stability constants [12] for ATP^{4-} ($10^{4.05}$) and CTP^{4-} or $HOP_3O_9^{4-}$, and for ADP^{3-} ($10^{3.21}$) and CDP^{3-} or $HOP_2O_6^{3-}$, gives $K_s/K_1 = 1$ and implies that both K_2 and $K_3 << 1$. Thus PMB and MPB′ are not present in significant amounts. This conclusion is consistent with the value of $\alpha = 0$ deduced from the spectrophotometric results reported in this paper. It is also anticipated that Ca^{2+} does not form an intramolecular macrochelate.

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