Interactions of Divalent Metal Ions with Inorganic and Nucleoside Phosphates. IV. Thermodynamics of the Nickel(II)–AMP System

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Abstract: Potentiometric measurements as a function of temperature were carried out in the Ni^{2+}–AMP system in order to resolve literature differences concerning the temperature dependence of the secondary phosphate ionization of AMP and the heats of ionization of the Ni^{2+} complexes. The secondary phosphate ionization is described by the quadratic relation $pK_{a2} = -9.617 + 2236.254/T + 0.02794T$ over the temperature range 5–40° in 0.1 M KNO_3. The enthalpies of complexation of Ni^{2+} with AMP were found to be -3.1 and -3.3 kcal/mol for NiAMP and $Ni(AMP)_2$, respectively.

In enzymatic reactions in which nucleotides participate as cofactors, the reactive species is usually a divalent metal ion complex of the nucleotide. As a consequence, considerable attention has been focused upon the stabilities and enthalpies of these complexes. A large fraction of this work is summarized in recent reviews.[1,2] We are directing our attention in this paper to the proton and Ni^{2+} complexes with adenosine 5′-monophosphate (AMP^{2-}). Both types of complexes involve literature discrepancies with regard to the thermodynamics of the interactions.

There is general agreement that the two pK's of AMP are at approximately 3.8 (base) and 6.2 (secondary phosphate). For the dependence of the secondary phosphate ionization (K_{a2}) upon temperature, however, two laboratories have published conflicting reports. Khan and Martell[3] obtained a value of $\Delta H_{a2} = 1.9 \pm 0.1$ kcal/mol, while Phillips and coworkers[4] reported $\Delta H_{a2} = -0.85$ kcal/mol (no error limits stated). Both studies presented pK_{a2} as varying linearly with $1/T$, although in opposite directions (Figure 1). On the other hand, inorganic orthophosphate and several orthophosphate esters show a quadratic temperature dependence around room temperature.[5–10] This difference in behavior, if real, could be taken to imply some unusual influence of the adenosine moiety on the phosphate ionization.

In addition, Frey and Stuehr[11] recently reported that Ni^{2+} forms a 1:2, as well as a 1:1 complex with AMP. The value of the stability constant for $Ni(AMP)_2$ is comparable in magnitude to that for NiAMP. Since previous thermodynamic studies of the NiAMP system

did not take the 1:2 complex into account, the stability constant and enthalpy change reported for NiAMP are presumably incorrect. Consequently, we are reporting here a study of the temperature dependences of the equilibrium constants for the proton dissociation and complexation of AMP with Ni^{2+}.

Experimental Section

Materials. Solutions of AMP were prepared daily from the free acid purchased from Sigma. Stock solutions of $Ni(NO_3)_2$ were standardized with EDTA. Titrations were carried out with 0.1 M KOH (Fisher Certified), the concentration of which was periodically checked against primary standard potassium hydrogen phthalate. The buffers for pH standardization were obtained either from Beckman Instruments or from the National Bureau of Standards.

Methods. Equilibrium constants were determined at several temperatures in the range 5–40° in a glass cell thermostated with a Lauda K2/R refrigerated water circulator. Temperatures were measured to ±0.02° with a calibrated thermometer. The experimental solutions were mechanically stirred and nitrogen was bubbled through to prevent CO_2 absorption. All pH measurements were made with an Orion 90–01 reference electrode and a Fisher 13–639–3 glass electrode in conjunction with a Corning Model 104 four-channel pH meter–electrometer. The electrodes were equilibrated at each temperature and standardized against NBS primary standard phthalate, phosphate, and borate buffers (pH values at 25° of 4.01, 6.86, and 9.18, respectively), purchased from Beckman Instruments. At the extremes of the temperature range some difficulties were encountered in obtaining complete agreement among the three buffers. In these cases the instrument was adjusted as accurately as possible with the buffers and final calibration was achieved by carrying out titrations of acetic acid. Since the pH of acetic acid can be accurately predicted[12] over the entire titration, it was a simple matter to compare the measured to the predicted values and thus to generate a correction table at each temperature. Even at the lowest and highest temperatures, the corrections were small (generally <0.03 pH unit); in the intermediate temperature range they were usually negligible.

Additions of KOH were made with a 2-ml Gilmont microburet. The ionic strength of all solutions was maintained at 0.1 M by KNO_3. This medium was chosen, even though potassium ions interact significantly with nucleotides, in order to provide comparisons with earlier measurements, the majority of which have been carried out in 0.1 M KNO_3.[1,2] We have pointed out[13] that, when the cation of the supporting electrolyte (S^+) interacts with the ligand under study, a measured binding constant K' is related to the "true" binding constant, K, by the relation

$$K' = K/[1 + K_{SL}(S^+)] \qquad (1)$$

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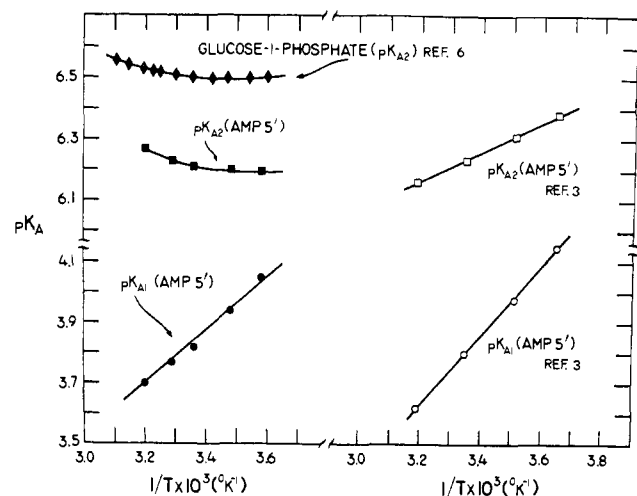(12) H. S. Harned, and B. B. Owen, "Physical Chemistry of Electrolyte Solutions," 3rd ed, Reinhold, New York, N. Y., 1955, p 676.
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Figure 1. Temperature dependence of pK_{a1} for AMP and of pK_{a2} for AMP and G1P.

Results and Discussion

The titration curve of AMP, in agreement with earlier work, indicates two pK's corresponding to the ionization of the adenine ring and the phosphate moiety, respectively. The pK_a values at $25°$ and their corresponding thermodynamic parameters are listed in Table I. The temperature dependences, obtained in this and

Table I. Thermodynamic Parameters for the Ionizations of AMP in $0.1\ M$ KNO_3 at $25°$

n	pK_{an}[a]	ΔG_{an},[b] kcal/mol	ΔH_{an},[c] kcal/mol	ΔS_{an}, cal/(deg mol)
1	3.82 ± 0.01	5.22 ± 0.02	4.2 ± 0.2	-3.4 ± 1.0
2	6.21 ± 0.01	8.47 ± 0.01	-1.1 ± 0.2[d]	-32 ± 1
	$pK_{a2} = -9.617 + 2236.254/T + 0.02794T$			
	$\Delta H_{a2} = 10228 - 0.1278T^2$			

[a] Average error from a minimum of three determinations. [b] Computed from pK_a. [c] Error estimated from variance of slope of least-squares line. [d] At $25°$; value is quite temperature dependent.

where K' is the measured stability constant, K is the value obtained in the absence of an interaction with the supporting electrolyte, and K_{SL} is the stability constant for the supporting electrolyte cation-ligand interaction. The pK_a values were determined at each temperature by at least two titrations of 15-ml aliquots of $5 \times 10^{-3}\ M$ AMP. The variation in the values of the pK_a was no more than ± 0.01 at any temperature. The stability constants of the two complexes formed between Ni^{2+} and AMP were determined from the titration of solutions containing $5 \times 10^{-3}\ M$ AMP and varying metal ion concentrations. The ratio of overall ligand to metal ion concentration was varied from $1:2$ to $3:1$, i.e., sixfold. The values of the stability constants at a given temperature varied by no more than $5-10\%$.

Treatment of Data. Hydrogen ion concentrations at $0.1\ M$ ionic strength were computed from the measured activities using $\gamma_H = 0.83$. The pK_a's were not sufficiently separated to be evaluated independently; they were computed by the algebraic procedure described by Albert and Serjeant.[14]

The equilibrium constants pertaining to the NiAMP system are defined as follows (charges omitted for simplicity).

$$K_{a1} = (H)(HL)/(H_2L)$$

$$K_{a2} = (H)(L)/(HL) \quad (2)$$

$$K_{ML} = (ML)/(M)(L)$$

$$\beta_2 = K_{ML} \cdot K_{ML_2} = (ML_2)/(M)(L)^2$$

Frey and Stuehr[11] have shown that for such a system the following equation may be derived from eq 2 and the mole balances $((M^0), (L^0),$ and (H^0) being the total metal ion, total ligand, and total proton concentrations respectively).

$$Y = K_{ML}X + \beta_2 \quad (3)$$

$$Y = A/\{[2(M^0) - A(L)](L)\}$$

$$X = [(M^0) - A(L)]/\{[2(M^0) - A(L)](L)\}$$

$$A = \left[\frac{(L^0)}{(L)}\right] - \left[1 + \frac{(H)}{K_{a2}} + \frac{(H)^2}{K_{a1}K_{a2}}\right]$$

$$(L) = \frac{2(L^0) - K - (H) + (OH)}{(H)/K_{a2} + 2(H^2)/(K_{a1}K_{a2})}$$

$$K = \text{concentration of KOH added}$$

A graph of Y vs. X yields a straight line with slope $= K_{ML}$ and intercept $= \beta_2$.

in other laboratories, are shown in Figure 1. The temperature dependence of pK_{a1} appears linear in the temperature region studied with $pK_{a1} = 3.82 \pm 0.01$ at $25°$ and $\Delta H_{a1} = 4.2 \pm 0.3$ kcal/mol. These results are in quite good agreement with those of Khan and Martell[3] at $25°$ in the same medium: $pK_{a1} = 3.80 \pm 0.01$ and $\Delta H_{a1} = 4.9 \pm 0.1$ kcal/mol.

On the other hand, as can be seen from Figure 1, we find that the temperature dependence of pK_{a2} is not linear as indicated by the earlier studies. Our results are given to ± 0.01 unit by the equation $pK_{a2} = -9.617 + 2236.254/T + 0.02794T$. At $25°$ we obtain $pK_{a2} = 6.21 \pm 0.01$ and $\Delta H_{a2} = -1.1 \pm 0.2$ kcal/mol. While our pK_{a2} value at $25°$ agrees quite well with that of Khan and Martell ($pK_{a2} = 6.23 \pm 0.01$, $I = 0.1\ M\ KNO_3$), the temperature dependence does not. We find that pK_{a2} varies nonlinearly with $1/T$, consistent with the behavior of several orthophosphate esters in the temperature range under consideration. The curve for glucose 1-phosphate, G1P, shown in Figure 1, may be considered as typical.[6] One sees that the morphologies of the curves for G1P and AMP, as well as the location of the minimum, are similar. We conclude, therefore, that there is nothing unusual about the ionization of the phosphate group in AMP. The pK_{a2} values reported by Phillips and coworkers[4] at $I = 0.1\ M$ $(n\text{-Pr})_4NBr$ are consistently 0.2 unit higher than our own for two reasons. First, they measured "mixed" pK values, which are larger by $-\log \gamma_H$ (≈ 0.08 unit at $I = 0.1$). Second, their supporting electrolyte cation does not appreciably interact with AMP. By eq 1, their values should be larger still by $\log [1 + K_{KAMP}(K^+)]$ which is 0.11 unit if $K_{KAMP} \cong 3$ at $I = 0.1$. Nevertheless, our ΔH_{a2} value of -1.1 ± 0.2 kcal/mol at $25°$ is in good agreement with the value of -0.85 kcal/mol obtained by Phillips and coworkers.[4] The latter value, however, was based on pK_{a2}'s at only three temperatures, which apparently were not sufficient to detect curvature in the temperature dependence. The agreement in the ΔH values indicates that the quantity $1 + K_{KAMP}(K^+)$ in eq 1 is not appreciably temperature dependent. We had previously[13] come to the same conclusion with regard to the analogous interactions with ATP.

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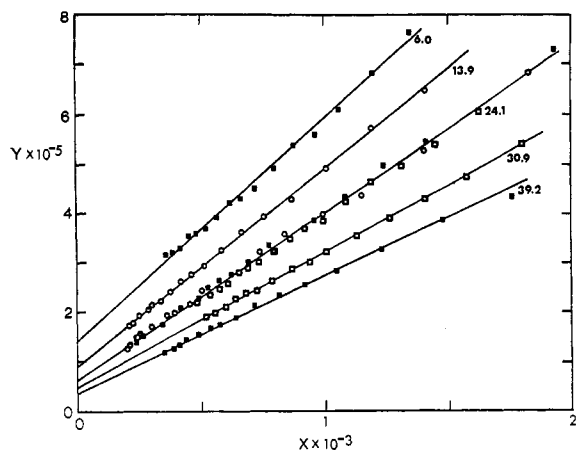
Figure 2. Experimental data graphed according to eq 3 for Ni-AMP at the indicated temperatures. $(M^0)/(L^0)$ ratios as follows: ■, 1:1; □, 2:1; ○, 1:2.

The stability constants of the two complexes formed between Ni^{2+} and AMP were obtained from the titration curves of solutions containing ratios of ligand to metal ion which varied from 3 to 0.5. Figure 2 shows some of the experimental results for NiAMP at several temperatures. The best slope and intercept of each straight line were determined by a least-squares analysis. We are able to confirm the earlier report[11] from this laboratory that a $Ni(AMP)_2$ complex of a stability comparable to that of NiAMP is formed. The results at 25° are listed in Table II and the experimental temperature de-

Table II. Thermodynamic Parameters for the Formation of NiAMP and $Ni(AMP)_2$ in 0.1 M KNO_3 at 25°

n	K_{ML_n},[a] M^{-1}	ΔG_{ML_n},[b] kcal/mol	ΔH_{ML_n},[c] kcal/mol	ΔS_{ML_n}, cal/(mol deg)
1	306 ± 10	−3.39 ± 0.03	−3.1 ± 0.3	1 ± 1
2	200 ± 15	−3.14 ± 0.04	−3.3 ± 0.4	−0.5 ± 1.5

[a] Average error from a minimum of three determinations. [b] Computed from K_{ML_n}. [c] Error estimated from variance of slope of least-squares line.

pendences are shown in Figure 3. The enthalpy changes for the metal complexation steps were obtained by graphing log K vs. $1/T$. Uncertainties in the ΔH values were estimated from the variance of slope of the least-squares lines. The complexation enthalpies are, within experimental error, identical.

The previous determination[3] of the temperature dependence of the NiAMP stability constant indicated a much smaller ΔH value (−1.0 kcal/mol), with K_{ML} = 725 at 15°. Reference to Figure 2, on the other hand, shows that the slopes (K_{ML}) do in fact vary quite significantly with temperature. If, however, one were to utilize in Figure 2 only experimental points near the origin, without taking K_{ML_2} into account, he would compute K_{ML} to be ~700–800 at 15°. We have no ex-
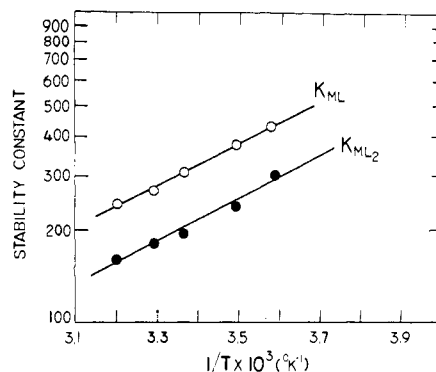

Figure 3. Temperature dependence of K_{ML} and K_{ML_2} for Ni^{2+} complexes with AMP.

planation for the relative insensitivity to temperature reported in the earlier determination.

The values of K_{ML} and K_{ML_2} at 25° are 306 ± 15 and 200 ± 15 M^{-1}. It is somewhat surprising that the two stability constants for the interaction of Ni^{2+} with a doubly charged anionic ligand should be so similar. We suggested previously[11] that the bis complex might be stabilized by a stacking interaction between the two nucleotides in the complex. This would be possible if the Ni^{2+} ion were simultaneously bound to the phosphate of one nucleotide and a ring nitrogen of the other. Such a structure now seems less probable since we have found that the values of the enthalpies and entropies for the formation of the two complexes are, within experimental error, identical. It seems unlikely that the thermodynamic parameters should have virtually the same values if two different types of ligand binding occurred. As long ago as 1955, Poulsen and Bjerrum[15] pointed out that, for normal transition metal ion complexes, "heats of successive steps are the same within the certainty of the experiment." The majority of such data for transition metal ions tabulated in a recent compilation[16] is consistent with this generalization. We conclude, therefore, that there is no substantial difference in the nature of the bonding for the two Ni^{2+} complexes of AMP.

Finally, we would like to remark that the value of the overall equilibrium constant $\beta_2 = K_{ML}K_{ML_2}$ (6.1 × 10⁴ M^{-2} at 25°) for Ni^{2+} is comparable to the K_{ML} values of higher nucleoside phosphates such as ATP. The bis complex is significant at ordinary concentrations $(C^0 > 10^{-3}\ M)$ even at 1:1 ratios of AMP to Ni^{2+}. When experiments are carried out under conditions such that the nucleotide is present in excess over the metal ion, then $Ni(AMP)_2$ can be the predominant complex.

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