Inorg. Chem. **1980**, *19*, 505–510

Contribution from the Department of Chemistry,
Case Western Reserve University, Cleveland, Ohio 44106

Interactions of Divalent Metal Ions with Inorganic and Nucleoside Phosphates. 8. Thermodynamics and Kinetics of the Ni(II)–2′-AMP, –3′-AMP, and –5′-AMP Systems

J. C. THOMAS, C. M. FREY, and J. E. STUEHR*

Received August 17, 1979

The kinetics of Ni(II) interactions with four adenosine monophosphates were investigated by the temperature-jump technique. One relaxation effect for Ni–2′-AMP, –5′-AMP, and –5′-dAMP and two relaxation effects for Ni–3′-AMP attributable to metal–ligand reactions were found and studied in detail. The relaxation times were found to be concentration and pH dependent. The results were interpreted by a mechanism involving complexation of both protonated and free ligands and subsequent slow formation of back-bound complexes. The rate constants obtained for this mechanism are consistent with those obtained for the Ni(II) systems involving phosphate interactions only. The rate constants for the initial steps of complexation are nearly the same for the four nucleotides. There are considerable differences among the rate constants for bridged complex formation.

In the previous paper[1] of this series, we presented kinetic results for the interactions of divalent nickel with several monophosphate systems. The ligands chosen permitted complexes with Ni^{2+} involving only the phosphate moiety. The observed pH-dependent relaxation time was interpreted on the basis of a mechanism involving parallel complexation pathways via free and protonated ligands.

The adenine nucleotides are particularly interesting in that their complexes with Ni^{2+} can involve, in addition to the phosphate interactions, binding to the adenine part of the molecule.[2] The ease of formation of a back-bound complex involving simultaneous interaction with adenine as well as phosphate sites varies with the isomer 2′, 3′, or 5′. These differences are clearly manifested in their stability contants with Ni^{2+}, which increase in the order 3′ < 2′ < 5′. We report here a temperature-jump study of complexation kinetics of the three AMP isomers with Ni^{2+}. A preliminary report of some of the results appeared some years ago in a review article.[3]

Experimental Section

Solutions of the nucleotides purchased from Sigma Chemical Co. were prepared daily. Stock solutions of Ni^{2+} were prepared from reagent grade nitrate or chloride obtained from J. T. Baker Chemical Co. The pK_a's of the nucleotides and their binding constants with Ni(II) were determined from pH titration of the nucleotides (or salts) without and with the presence of the metal ion, respectively. The procedure is described in previous papers.[4]

A temperature-jump spectrometer[5] (Messanlagan Studiengesellschaft) was used for most of the kinetic experiments. They were carried out at 15 °C in the presence of 0.1 M KNO_3. Spectrophotometric observation was carried out by coupling the reactions to the pH indicators bromocresol purple or chlorophenol red. Details of the experimental procedure are given in the preceding paper.[1] Blank solutions, consisting of metal and indicator or ligand and indicator, showed only fast proton-transfer effects.

The concentrations of ligand and metal ion ranged from 4×10^{-4} to 1×10^{-2} M and the ligand:metal ratio was near unity in most cases. Reactions were followed at 528–570 nm, depending on the indicator and pH. Some kinetic experiments were also carried out on a Durrum D110 stopped-flow spectrophotometer in the search for a possible slower effect. Experiments carried out in the presence of pH indicators exhibited only optical changes corresponding to the T-jump effect but nothing slower.

The kinetic data were analyzed and fitted to mechanisms by a nonlinear regression procedure. The equilibrium concentrations of the species were calculated from the equilibrium constants and known concentrations by a Univac 1108 computer.

Table I. Equilibrium Constants for Four Ni(II)-AMP Systems[a]

	pK_{a_1}[e]	pK_{a_2}[f]	K_{ML}	K_{MHL}[b]	K_{ML_2}
2′-AMP[b]	3.74	6.021	150	5	25
3′-AMP[b]	3.74	5.792	120	5	0
5′-AMP[c]	3.89	6.197	400	15	160
5′-dAMP[d]	3.99	6.267	390	15	196

[a] At 15 °C and 0.1 M KNO_3. [b] This work. [c] Reference 6. [d] L. Loew and J. Stuehr, unpublished results. [e] Base ionization. [f] Secondary phosphate ionization.

Results

pK_a's and metal ion binding constants for Ni(II)–2′-AMP and –3′-AMP were calculated from the titration results by a nonlinear regression method. Values obtained from different runs agreed well (±~10% for stability constants, ±0.005 unit for pK_a). Thermodynamic results are listed in Table I.

Kinetic results at 15 °C and $I = 0.1$ over the pH range 5.0–6.3 were obtained for four Ni–nucleotide systems. They are discussed separately.

Ni–5′-AMP, –5′-dAMP. One relaxation time in the millisecond time range (in contrast to ca 0.1 ms for the three monophosphate systems Ni–RibP, Ni–P_i, and Ni–5′-CMP; see preceding paper[1]) was observed for these two nucleotides (Table II). The relaxation time was found to be dependent on the pH of the solution and reactant concentrations as shown in Figure 1. Three distinct differences are noted between these systems and the three other monophosphate systems reported in the accompanying paper:[1] (1) For solutions of comparable reactant concentrations, the observed relaxation effects are slower by about a factor of 10. (2) The pH dependency of Ni–5′-AMP and Ni–dAMP is opposite to that of Ni–RibP etc., and the relaxation times at a given concentration are *lower* at higher pH. (3) Graphs of τ^{-1} vs. $(\bar{M} + \bar{L} + \overline{HL})$ for Ni–5′-AMP, at a given pH, are linear at low concentrations but gradually level off at higher concentrations (Figure 1); no comparable curvature is observed for the Ni–RibP and related systems. The limiting slope obtained from this plot is only half that of a similar plot for the Ni–RibP system and the intercept is an order of magnitude lower than that for Ni–RibP.

The strong pH dependence once again indicates protonated-ligand complexation pathways as was found in the inorganic phosphates. The curvature clearly indicates the presence of additional steps compared to the simple phosphate systems. In addition, we should account mechanistically for the complex $Ni(AMP)_2$ which is known to exist[6] in this system. Thus, the

---

(1) J. C. Thomas, C. M. Frey, and J. E. Stuehr, *Inorg. Chem.*, preceding paper in this issue.
(2) R. Phillips, *Chem. Rev.*, **66**, 501 (1966).
(3) C. M. Frey and J. E. Stuehr in "Metal Ions in Biological Systems", Vol. I, H. Sigel, Ed., Marcel Dekker, New York, 1974, p 51.
(4) Thomas Briggs, Ph.D. Thesis, Case Western Reserve University, 1976.
(5) (a) A. F. Yapel, Jr., and R. Lumry, *Method Biochem. Anal.*, **20**, 169 (1970); (b) M. Eigen and L. DeMaeyer, *Tech. Org. Chem.*, **8**, 895 (1963).
(6) (a) C. M. Frey and J. E. Stuehr, *J. Am. Chem. Soc.*, **94**, 8898 (1972); (b) J. L. Banyasz and J. E. Stuehr, *ibid.*, **96**, 6481 (1974).

0020-1669/80/1319-0505$01.00/0

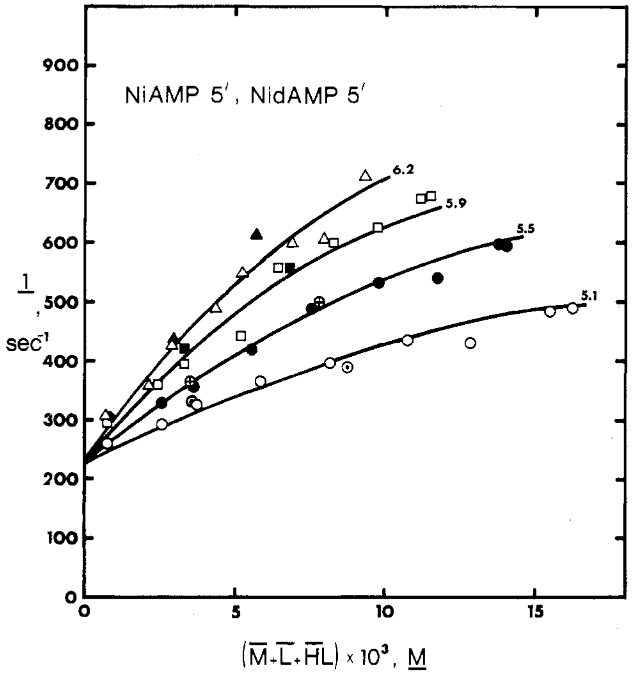
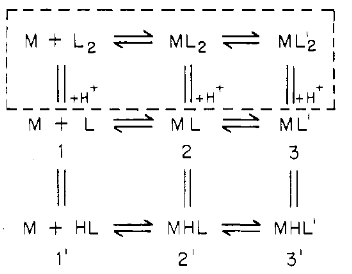


Figure 1. Concentration and pH dependence of the relaxation time (τ_2) for the Ni(II)–5′-AMP and –5′-dAMP systems at 15 °C and $I = 0.1$. Solid lines through the points were calculated from eq 1 for (τ_-) with the rate constants in Table III. ▲, ■, ⊕, and ⊙ correspond to 5′-dAMP data.

Scheme I



mechanism that best describes the experimental data involves complexation with the deprotonated and monoprotonated phosphate group and subsequent ring interaction forming bridged complexes. The mechanism is represented in Scheme I, where ML and MHL are the phosphate bound complexes and ML′ and MHL′ represent bridged complexes involving simultaneous binding with the phosphate and adenine moieties. The portion of Scheme I enclosed in the dotted lines includes possible contributions from bis complex formation. These species are thought not to contribute significantly to the rate but are included for completeness. Proton transfer reactions involving AMP and the indicator are treated as preequilibria. Solution of this scheme results in a quadratic equation, the two roots of which are the reciprocal relaxation times:

$$1/\tau_{\pm} = 0.5\{(a_{11} + a_{22}) \pm [(a_{11} + a_{22})^2 - 4(a_{11}a_{22} - a_{12}a_{21})]^{1/2}\} \quad (1)$$

The coefficients a_{ij} are fairly complicated functions of the rate constants and equilibrium concentrations. For Scheme I, the explicit values for a_{ij} are given in the Appendix. It is worth noting that, as for the inorganic phosphate systems, the effective forward and reverse rate constants can be expressed as the sum of pH-independent and -dependent terms, e.g., $k_{1f} = k_{12} + k_{12}'K_A[H]$, $k_{2f} = k_{23} + k_{23}'K_B[H]$, etc.

Of the two relaxation times given by eq 1, τ_+^{-1} (corresponding to the positive sign) begins at a high value and increases rapidly with concentration. This is the behavior observed for the inorganic and related phosphates in the accompanying paper.[1] The smaller root corresponding to the negative sign, τ_-^{-1}, increases with concentration and levels off at high concentrations. This is the behavior found experimentally for 5′-AMP; thus the data were fitted to τ_-. Results corresponding to the positive root were observed for the 2′ and 3′ systems (see below) but not for the 5′ isomer, presumably because the equilibrium greatly favors ML′ over ML, resulting in a small amplitude for the faster step.

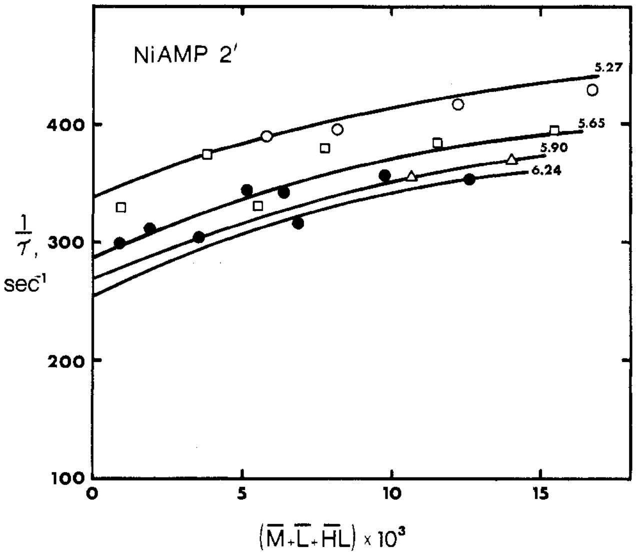


Figure 2. Concentration and pH dependence of the relaxation time for the Ni(II)–2′-AMP system.

There are in principle four unknown equilibrium constants (K_A, K_B, K_C, K_{23}) and eight unknown rate constants to be determined. In practice, the four reverse constants can be eliminated by incorporating the appropriate equilibrium constants. The stability constants for Ni–phosphate interactions (K_{12} and K_{12}') can be taken as the K_{ML} and K_{MHL} values of Ni–RibP, i.e., 80 and 3 M, respectively. K_{23}, K_B, and K_C can then be calculated from the relationships $K_{ML}^{\Sigma} \equiv K_{12}(1 + K_{23})$ and $K_B \equiv (K_{12}'/K_{12})K_A$ ($K_C \equiv (K_{23}'/K_{23})K_B$), where K_{ML}^{Σ} is the experimental value in Table I. As a consequence, only four rate constants are left to be determined. A nonlinear least-squares computer program as described for the other systems[1] was used to determine the constants. The best fit constants for Ni–5′-AMP are shown in Table III. The rate constants for the phosphate interactions in AMP are nearly identical with those found for other systems. The experimental results for Ni–dAMP are nearly identical with those of 5′-AMP and can be quantitatively reproduced, within experimental error, with the constants obtained for 5′-AMP, as shown in Table II and Figure 1.

Ni–2′-AMP, –3′-AMP. Two relaxation effects were observed for the Ni(II)–3′-AMP system, one at about 0.2 ms (τ_1) and the other at about 3 ms (τ_2), Figure 2. The amplitude of the τ_1 effect was about half to one-third that of the τ_2 effect. Both effects were clearly observable for the same solutions (Table II). The effects became smaller at lower concentrations and pH. The uncertainty for τ_1 is ±20% while that for τ_2 is less than 10%. Both effects are pH and concentration dependent. However, τ_1 is faster at lower pH values while τ_2 is faster at higher pH. The concentration dependence is clearer for τ_1 than for τ_2. The behavior of the τ_1 effect is very similar to that observed for the Ni(II)–RibP system, while the τ_2 effect is in the same time range as for the Ni(II)–5′-AMP system. *This nucleotide system represents the first for which both predicted relaxation times have been completely characterized as a function of pH and concentration.*

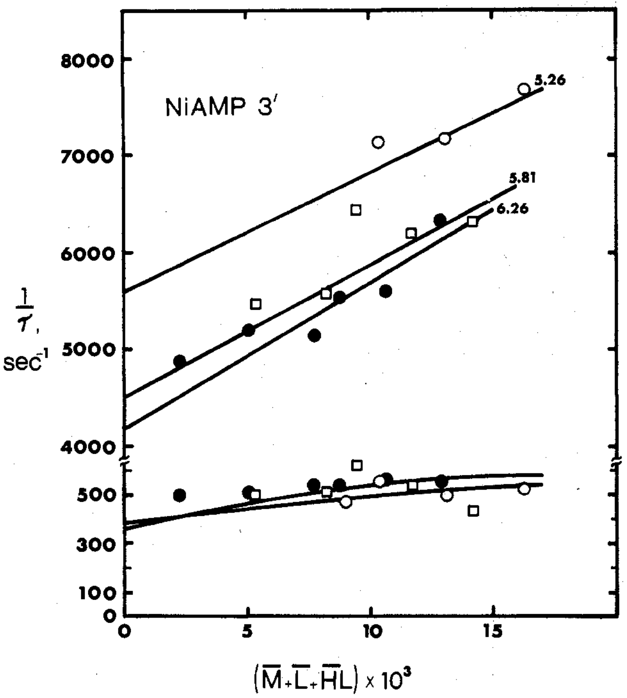


Figure 3. Concentration and pH dependence of the two relaxation times (τ_1, τ_2) for the Ni(II)–3′-AMP system. Solid lines through the points were calculated from eq 1 for τ_+ (upper curves) and τ_- (lower curves).

For Ni(II)–2′-AMP only one relaxation effect was consistently observed, with a relaxation time of about 2 ms (Figure 3). A fast effect in the 0.1 ms range was occasionally observed; it was too small in amplitude to be studied in detail. The concentration dependence and time scale of the slower effect correspond to τ_2 of the Ni(II)–3′-AMP system, although the pH dependence is in the opposite direction. The uncertainty of the relaxation time is on the order of ±10%.

The experimental τ_1^{-1} and τ_2^{-1} values were assigned to τ_+^{-1} and τ_-^{-1} values in Scheme I, respectively, as described above for the 5′-AMP system, with the rate constants k_{12}, k_{12}', k_{23}, k_{23}' determined by nonlinear regression (see Table III). $1/\tau$ values calculated with these constants are given in Table II. For Ni-2′-AMP, the τ_2 values are not particularly sensitive to k_{12} and k_{12}'. However, for 3′-AMP, for which both τ_1 and τ_2 have been observed, k_{12} and k_{12}' can be evaluated with a high degree of precision. The fast root of Scheme I as calculated from the rate constants in Table III is listed in Table II as $\tau_{+(\text{calcd})}^{-1}$.

It should be noted that although all four systems can be fitted to the same mechanism, the predicted behavior of the fast root differs significantly from system to system. For 2′-AMP and 3′-AMP, where the two reaction steps are more widely separated than for Ni–5′-AMP (as determined by k_{12} and k_{23}), the two roots are relatively uncoupled. As a consequence, τ_+^{-1} behaves very much like τ^{-1} of Ni–RibP, where only the first step in Scheme I exists. For Ni–5′-AMP and 5′-dAMP, on the other hand, the two roots are more closely coupled and τ_+^{-1} does not quite parallel τ^{-1} of Ni–RibP, especially in its dependence on pH. The pH dependence of τ_+^{-1} for Ni–5′-AMP could be reversed if k_{12}' were lower than 1.2×10^4 M^{-1} s^{-1}, the other constants being the same.

While the work described in this paper was being completed, Taylor and Diebler[7] published their results on the Ni–5′-AMP system under different experimental conditions. Our results basically agree with theirs (see Table III), when one takes into account the temperature difference between the two studies. Their work was carried out under the condition $[M]_o \gg [L]_o$, resulting in the ML_2 species being negligible. We have tested their Ni–5′-AMP data (sent to us through private communication) via eq 1 and the present constants (adjusted for the temperature difference) and found a good fit. They also reported some measurements in Ni(II)–3′-AMP in 0.1 M $NaClO_4$ and a buffer at pH 6; one effect, corresponding to our τ_2, was observed but not characterized in detail.

Discussion

All the rate and equilibrium constants determined from the kinetic analyses are listed in Table III. Also shown are the ligand penetration rate constants for the two-step ligand substitution mechanism[8]

$$Ni^{2+} + L^{2-} \overset{K_{os}}{\rightleftharpoons} Ni\cdot OH_2\cdot L \overset{k_{1p}}{\rightleftharpoons} Ni\text{–}L$$

$$k_{obsd} = K_{os}k_{1p}$$

where k_{obsd} would be equivalent to our k_{12} or k_{12}'. K_{os} values of the outer sphere equilibrium constants are 10 and 2 for the L^{2-} and HL^- reactions, respectively.[9,10]

Phosphate-Binding Steps. Although many rate constants are just those expected for Ni^{2+} on the basis of charge type and known solvent exchange rates, there are some interesting features in the numerical values of the constants. Values of k_{12}, the rate constant for the phosphate moiety of the nucleotides, generally agree well with the corresponding values for the other three phosphate systems. The ligand penetration rate constants are consistent with previous studies involving Ni^{2+}. The dissociation rate constant k_{21} is 2700 ± 700 s^{-1} for the three nucleotide systems, reflecting the similarity in bonding among the three phosphate moieties. The close agreement with the corresponding constants for the phosphates alone is strong evidence that the first step in the nucleotide complexation is indeed binding to the phosphate.

k_{12}' values for 5′-HAMP and 5′-HdAMP with Ni^{2+} are somewhat lower than would be expected for a 2–1 charge interaction, and as found for k_{12}' for Ni–HRibP. This result is consistent with the existence of a hydrogen bond between the protonated phosphate moiety and the purine ring (probably to N7). Such an interaction could hinder the complex formation by reducing the probability of ligand penetration by restricting the rotation of the phosphate groups. This is in agreement with NMR results,[11] which showed the shift of the H8 proton of 5′-AMP and 5′-dAMP to be very sensitive to the secondary phosphate ionization. Although it was argued that this could not account for the even larger deshielding effect brought about on further ionization, and other mechanisms (involving magnetic and electrostatic field effects) were proposed, there is no reason for these to be mutually exclusive. Examination of space filling molecular models of 5′-AMP shows that it is possible to form a hydrogen bond between the 5′-phosphate and the N7. This hypothesis of a hydrogen bond in 5′-HAMP is consistent with the secondary phosphate p*K*: the pK_a's of 5′-AMP and 5′-dAMP are 0.2–0.5 unit higher than those for 2′-AMP and 3′-AMP (Table I) where no similar deshielding effect of H8 was found. The kinetic results of 2′-AMP and 3′-AMP, which showed no lowering of k_{12}', also support this conclusion.

Back-Bound Complexes. The rate constants always appear in pairs in eq 2 as $k_{12} + k_{12}'K_A[H]$ and $k_{23} + k_{23}'K_B[H]$; see

---

(7) R. S. Taylor and H. Diebler, *Bioinorg. Chem.*, **6**, 247 (1976).

(8) F. Basolo and R. G. Pearson, "Mechanism of Inorganic Reactions", Wiley, New York, 1967.

(9) J. Banyasz and J. Stuehr, *J. Am. Chem. Soc.*, **95**, 7226 (1973).

(10) C. M. Frey, J. Banyasz, and J. Stuehr, *J. Am. Chem. Soc.*, **94**, 9198 (1972).

(11) M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, *J. Am. Chem. Soc.*, **90**, 1042 (1968).

Table II. Kinetic Data for the Interaction of Ni(II) with 2'-AMP, 3'-AMP, 5'-AMP, and 5'-dAMP[a]

$10^3[\text{M}]_0$, M	$10^3[\text{L}]_0$, M	pH[b]	$\tau_1(\text{exptl})^{-1}$, s^{-1}	$\tau_+(\text{calcd})^{-1}$, s^{-1}	$\tau_2(\text{exptl})^{-1}$, s^{-1}	$\tau_-(\text{calcd})^{-1}$, s^{-1}
Ni-2'-AMP						
10.0	10.0	5.27		6458	413	457
10.0	10.0	5.61		5346	385	400
10.0	10.0	5.895		4728	370	371
10.0	10.0	6.25		4301	354	353
10.0	10.0	6.565		4122	308	345
7.0	7.0	5.27		5916	417	439
7.0	7.0	5.61		4832	385	384
7.0	7.0	5.895		4257	359	357
7.0	7.0	6.233		3885	357	340
4.5	4.5	5.27		5394	396	419
4.5	4.5	5.67		4206	381	358
4.5	4.5	6.25		3458	316	322
2.93	3.34	5.26		5077	408	404
2.93	3.34	5.67		3886	330	342
2.93	3.34	6.23		3196	344	309
2.0	2.13	5.66		3678	388	330
2.0	2.13	6.255		2967	305	295
1.0	1.06	6.22		2750	314	279
0.45	0.501	5.68		3242	370	299
0.45	0.501	6.26		2586	340	266
Ni-3'-AMP						
10.0	10.2	5.277	7687	7523	526	540
10.0	10.2	5.827	6324	6420	435	562
10.0	10.2	6.297	6332	6096	556	571
8.0	7.67	5.288	7174	7155	500	519
8.0	7.67	5.811	6200	6111	541	538
8.0	7.67	6.303	5600	5765	571	541
6.0	6.02	5.264	7140	6891	556	495
6.0	6.02	5.811	6450	5792	625	511
6.0	6.02	6.215	5530	5495	540	519
5.0	5.17	5.223	[c]		476	479
5.0	5.17	5.808	5574	5629	513	496
5.0	5.17	6.183	5130	5344	541	503
3.0	3.15	5.811	5467	5235	500	457
3.0	3.15	6.277	5200	4922	512	463
1.2	1.26	6.283	4878	4514	500	410
Ni-5'-AMP						
10.0	11.0	5.10		7857	490	480
10.0	11.0	5.52		6793	596	598
10.0	11.0	5.90		6204	678	664
10.0	11.0	6.27		5886	714	690
10.0	10.2	5.10		7846	484	477
10.0	10.2	5.47		6894	597	584
10.0	10.2	5.89		6227	678	665
8.0	8.16	5.10		7718	430	452
8.0	8.16	5.45		6783	541	544
8.0	8.16	5.86		6100	625	618
8.0	9.2	6.32		5711	606	648
6.5	6.63	5.10		7606	435	430
6.5	6.63	5.50		6545	533	523
6.5	6.63	5.91		5906	597	584
6.5	6.63	6.30		5602	606	607
4.63	4.9	5.01		7743	396	379
4.57	4.84	5.46		6433	488	465
4.55	4.82	5.94		5674	556	523
4.56	4.65	6.42		5365	548	546
3.45	3.14	5.03		7539	364	356
3.45	3.14	5.54		6122	418	436
3.5	3.18	5.82		5680	440	469
3.44	3.13	6.32		5281	488	493
2.0	2.13	5.15		6946	324	335
1.98	2.11	5.45		6108	357	366
1.98	2.11	5.95		5335	392	402
1.96	2.06	6.36		5079	426	418
1.36	1.43	5.05		7177	292	300
1.36	1.43	5.46		5979	331	335
1.37	1.44	5.96		5228	357	363
1.3	1.33	6.30		5001	357	368
0.4	0.384	5.15		6645	260	264
0.4	0.384	5.50		5703	303	274
0.4	0.384	5.91		5095	301	281
0.4	0.384	6.32		4829	305	287

Table II (*Continued*)

$10^3[M]_0$, M	$10^3[L]_0$, M	pH[b]	$\tau_1(\text{exptl})^{-1}$, s^{-1}	$\tau_+(\text{calcd})^{-1}$, s^{-1}	$\tau_2(\text{exptl})^{-1}$, s^{-1}	$\tau_-(\text{calcd})^{-1}$, s^{-1}
			Ni–dAMP			
5.0	5.13	5.10		7442	389	407
5.0	5.13	5.51		6352	500	491
5.0	5.13	5.91		5747	556	543
5.0	5.13	6.32		5442	615	567
3.63	5.99	6.25		5369	656	526
2.1	2.16	6.11		5218	505	425
2.0	2.0	5.10		7066	331	332
2.0	2.0	5.51		5956	367	378
2.0	2.0	5.90		5373	421	407
2.0	2.0	6.30		5102	435	426

[a] At 15 °C and $I = 0.1$ M KNO_3. [b] a_H converted to C_H by $\gamma_H = 0.83$. [c] Effect too small to be characterized.

Table III. Rate and Equilibrium Constants for Ni–2′-AMP, -3′-AMP, -5′-AMP, and -5′-dAMP[a]

	Ni–2′-AMP	Ni–3′-AMP	Ni–5′-AMP, -5′-dAMP
	Phosphate Complexes		
K_{12},[b] M^{-1}	90 ± 20	54 ± 15	80
$10^{-5}k_{12}$, M^{-1} s^{-1}	1.8 ± 0.5	1.8 ± 0.3	2.3 ± 0.1
k_{21},[c] s^{-1}	2000	3330	2870
$10^{-4}k_{1p}$, s^{-1}	1.8	1.8	2.3
$10^{-4}k_{12}'$, M^{-1} s^{-1}	3.5 ± 0.7	3.6 ± 0.6	2.0 ± 0.2
$10^{-4}k_{21}'$,[c] s^{-1}	1.1	1.2	1.0
K_{12}', M^{-1}	3.3	3	2 ± 1
$10^{-4}k_{1p}'$,[d] s^{-1}	1.7	1.8	1.0
	Back-Bound Complexes		
K_{23}[e]	0.67	1.2	4
k_{23}, s^{-1}	175 ± 50	520 ± 160	1510 ± 50
k_{32}, s^{-1}	260	430	380
k_{23}', s^{-1}	440 ± 20	520 ± 160[f]	1670 ± 50
k_{32}', s^{-1}	860	780	260
K_{23}'	0.5	0.66	6.5

[a] At 15 °C and $I = 0.1$ M KNO_3. [b] Values obtained from minimization procedure; where no uncertainty is indicated, the constant was fixed. [c] Calculated from the forward rate and the appropriate equilibrium constants. [d] Calculated from the relationships $k_{12} = K_{os}k_{1p}$ and $k_{12}' = K_{os}'k_{1p}'$. [e] Calculated from the relationships $K_{ML} = K_{12}(1 + K_{23})$ and $K_{MHL} = K_{12}'(1 + K_{23}')$. [f] Data were not sensitive to k_{23}', which was consequently set equal to k_{23}.

Appendix. The quantity $K_B[H]$ is between 0.05 and 0.5 over the pH range studied. Thus unless k_{23}' is much larger than k_{23}, the data will be less sensitive to the k_{23}' term.

There has been a great deal of effort directed over the years to identifying the site of the Ni^{2+} adenine interaction in the adenine coenzymes. Ultraviolet[12] and NMR spectroscopy[13-16] seemed to implicate the N7 position on the adenine; this has been confirmed in a recent paper by Collins et al.[17] In that X-ray study of the $(H_2O)_5Ni(5'\text{-AMP})\cdot H_2O$ molecule, they found that Ni^{2+} binds directly to the N7 of adenine. The remaining five primary coordination sites of Ni^{2+} were found to be occupied by water molecules. Binding of Ni^{2+} to phosphate occurred via a water bridge in a kind of outer-sphere complex in the solid state. The relative slowness of the complexation with the phosphate group in the present work, however, clearly establishes the existence of *direct* bonding between Ni^{2+} and the phosphate in aqueous solution.

The differences in k_{23} among the 2′-, 3′-, and 5′-AMP probably reflect the differences in the steric arrangement between the phosphate and the adenine base. Rate constants k_{23} for back-bound complexes are 1510, 520, and 175 s^{-1} for 5′-, 3′-, and 2′-AMP, respectively. The large value for the 5′ isomer suggests that the structure of the Ni–5′-AMP complex in solution is similar to the conformation in the crystal. Examination of space-filling models of Ni complexes with these three nucleotides, and consideration of the possible binding sites, leads us to propose the following for the ML′ complexes: for Ni–5′-AMP and Ni–2′-AMP, Ni^{2+} can bind to both the phosphate oxygen and N7 nitrogen in an anti conformation. Such a structure cannot be formed for Ni–3′-AMP by using models, but the alternative binding site to N3 is readily accessible. For Ni–2′-AMP and Ni–3-AMP, there are very limited C–O, P–O bond rotations by which these chelates can be formed, resulting in high steric hindrance for the ML′ formation, compatible with the low k_{23} values we observed.

The species ML′ and MHL′ are expected to be very similar structurally, and we might expect k_{23} and k_{23}' values to be similar. This is borne out for 3′-AMP, 5′-AMP, and 5′-dAMP. The factor of 3 difference between k_{23} and k_{23}' for Ni–2′-AMP appears to be real (i.e., beyond combined errors) although the cause is not apparent from this study. A possible explanation might be that the monoprotonated phosphate group is held in a favorable conformation for MHL′ complex formation through hydrogen bonding to the base nitrogen (N3?), favoring the formation of MHL′ compared to ML′.

The rate constants for the Ni(II)–adenine ring interaction should be useful in providing information about the details of this interaction. When more such data are available for other metal ion nucleotide systems, comparisons of the rate constants could lead to insight as to their spatial relationships.

Possible ML_2 Contributions. Recent studies[5] have shown that Ni^{2+} forms ML_2 as well as ML complexes with 5′-AMP. Since only one slow relaxation effect was observed in the T-jump experiments, we are left with three possibilities for the formation of ML_2: the reaction is too rapid or too slow to be observed by T-jump measurements or the effect is too small, as stated earlier. The stopped-flow experiments showed no positive indication of a slow effect. Therefore, we tested various mechanistic pathways involving ML_2 as shown by the dashed area in Scheme I. The expanded mechanisms involving $ML + L = ML_2$ or $M + L_2 \rightleftharpoons ML_2 \rightleftharpoons ML'_2$ also produced good fits to our data, with rate constants not greatly different from those shown in Table III. The value for k_{12} dropped to $\sim 1.4 \times 10^5$ M^{-1} s^{-1} (i.e., the same as that for Ni–RibP). We conclude that any of the above ML_2 pathways are practically possible, but that they cannot be shown to make significant contributions to the relaxation time.

Conclusions

We have shown that adenosine monophosphates (2′, 3′, 5′, and deoxy-) form bridged complexes with Ni^{2+} by binding

---

(12) P. W. Schneider, H. Brintzinger, and H. Erlenmeyer, *Helv. Chim. Acta*, **47**, 992 (1964).

(13) N. A. Berger and G. L. Eichhorn, *Biochemistry*, **10**, 1847 (1971).

(14) G. L. Eichhorn, P. Clark, and E. D. Becker, *Biochemistry*, **5**, 245 (1966).

(15) A. W. Missen, D. F. S. Natusch, and L. J. Porter, *Aust. J. Chem.*, **25**, 129 (1972).

(16) L. G. Marzilli, W. C. Trogler, D. P. Hollis, T. J. Kistenmacher, C. H. Chang, and B. Z. Hanson, *Inorg. Chem.*, **14**, 2568 (1965).

(17) A. D. Collins, P. DeMeester, D. M. L. Goodgame, and A. C. Skapski, *Biochim. Biophys. Acta*, **402**, 1 (1975).

simultaneously to the phosphate and base groups. We conclude on the basis of the numerical values of rate constants for complex formation that these are inner-sphere interactions. Rate constants for the phosphate moiety of the nucleotide are in agreement with the results for simple phosphate systems (RibP, etc.). The rate constants for the base interaction depend on the position of the phosphate group, 5′ appearing to be the most favorable position for chelate formation.

Acknowledgment. This work was supported by the National Institutes of Health in the form of present grants to J.E.S.

Appendix

The close-to-equilibrium rate equations for Scheme I are

$$\mathrm{d}\delta\mathrm{M}/\mathrm{d}t = -k_{12}(\mathrm{M}\delta\mathrm{L} + \mathrm{L}\delta\mathrm{M}) + k_{21}(\delta\mathrm{ML}) - k_{12}'(\mathrm{M}\delta\mathrm{HL} + \mathrm{HL}\delta\mathrm{M}) + k_{21}'(\delta\mathrm{MHL})$$

$$\mathrm{d}(\delta\mathrm{ML}' + \delta\mathrm{MHL}')/\mathrm{d}t = k_{23}(\delta\mathrm{ML}) - k_{32}(\delta\mathrm{ML}') + k_{23}'(\delta\mathrm{MHL}) - k_{32}'(\delta\mathrm{MHL}')$$

All concentration variables except two may be eliminated by use of the mole balance relationships involving M, L, H and the preequilibrium relationships involving the rapid proton transfer reactions. The resulting pair of first-order differential equations may be put into the determinantal form

$$\begin{vmatrix} a_{11} - 1/\tau & a_{12} \\ a_{21} & a_{22} - 1/\tau \end{vmatrix} = 0$$

where the a_{ij} coefficients are

$$a_{11} = (k_{12} + k_{12}'[\mathrm{H}]K_\mathrm{A})\left(\frac{[\mathrm{M}]}{1+\beta} + [\mathrm{L}]\right) + (k_{21} + k_{21}'K_\mathrm{B}[\mathrm{H}])\frac{1}{1+\gamma}$$

$$a_{12} = \left(\frac{k_{21} + k_{21}'K_\mathrm{B}[\mathrm{H}]}{P}\right)\left\{1 + K_\mathrm{C}[\mathrm{H}]\left(\frac{K_\mathrm{A}[\mathrm{L}]}{1+\alpha} + 1 + K_\mathrm{A}[\mathrm{H}]\right) + \frac{K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}']}{1+\alpha} + K_\mathrm{A}[\mathrm{H}]K_\mathrm{C}\frac{[\mathrm{ML}] + [\mathrm{ML}']}{1+\alpha}\right\}$$

$$a_{21} = \left(\frac{k_{23} + k_{23}'K_\mathrm{B}[\mathrm{H}]}{B}\right)\left\{\frac{K_\mathrm{A}[\mathrm{L}]}{1+\alpha} + 1 + K_\mathrm{A}[\mathrm{H}] + \frac{K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}']}{1+\alpha}\right\} + \frac{K_\mathrm{C}[\mathrm{ML}](K_\mathrm{B} - K_\mathrm{A})[\mathrm{H}]}{(1+\alpha)B}a_{11}$$

$$a_{22} = \left(\frac{k_{23} + k_{23}'K_\mathrm{B}[\mathrm{H}]}{B}\right)\left\{(1 + K_\mathrm{C}[\mathrm{H}])\frac{K_\mathrm{A}[\mathrm{L}]}{1+\alpha} + 1 + K_\mathrm{A}[\mathrm{H}] + (1 + K_\mathrm{A}[\mathrm{H}])\frac{K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}']}{1+\alpha} + P(k_{32} + k_{32}'K_\mathrm{C}[\mathrm{H}])\right\} + \frac{K_\mathrm{C}[\mathrm{ML}'](K_\mathrm{B} - K_\mathrm{A})[\mathrm{H}]}{(1+\alpha)B}a_{12}$$

where

$$\alpha = [\mathrm{In}]/(K_\mathrm{In} + [\mathrm{H}])$$

$$\beta = [K_\mathrm{A}[\mathrm{H}](1 + (K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}'])/(1+\alpha)) + K_\mathrm{B}[\mathrm{H}](K_\mathrm{A}[\mathrm{L}]/(1+\alpha) + K_\mathrm{A}[\mathrm{H}])]/[1 + K_\mathrm{A}[\mathrm{L}]/(1+\alpha) + K_\mathrm{B}[\mathrm{H}] + (K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}'])/(1+\alpha)]$$

$$\gamma = [K_\mathrm{A}[\mathrm{H}]((K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}'])/(1+\alpha)) + K_\mathrm{B}[\mathrm{H}]K_\mathrm{A}[\mathrm{L}]/(1+\alpha) + K_\mathrm{B}[\mathrm{H}](1 + K_\mathrm{A}[\mathrm{H}])]/[K_\mathrm{A}[\mathrm{L}]/(1+\alpha) + 1 + K_\mathrm{A}[\mathrm{H}] + (K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}'])/(1+\alpha)]$$

$$B = (1 + K_\mathrm{C}[\mathrm{H}])P + \frac{K_\mathrm{C}[\mathrm{ML}']}{1+\alpha}(1 + K_\mathrm{A}[\mathrm{H}])(K_\mathrm{B} - K_\mathrm{C})[\mathrm{H}]$$

$$P = (1 + K_\mathrm{B}[\mathrm{H}])\left\{\frac{K_\mathrm{A}[\mathrm{L}]}{1+\alpha} + 1 + K_\mathrm{A}[\mathrm{H}]\right\} + (1 + K_\mathrm{A}[\mathrm{H}])\frac{K_\mathrm{B}[\mathrm{ML}] + K_\mathrm{C}[\mathrm{ML}']}{1+\alpha}$$

and

$$K_\mathrm{A} = [\mathrm{HL}]/[\mathrm{H}][\mathrm{L}] \qquad K_\mathrm{B} = [\mathrm{MHL}]/[\mathrm{ML}][\mathrm{H}]$$

$$K_\mathrm{C} = [\mathrm{MHL}']/[\mathrm{ML}'][\mathrm{H}] \qquad K_\mathrm{IN} = [\mathrm{In}][\mathrm{H}]/[\mathrm{HIn}]$$

Registry No. Ni^{2+}, 14701-22-5; 2′-AMP, 130-49-4; 3′-AMP, 84-21-9; 5′-AMP, 61-19-8; 5′-dAMP, 653-63-4.