Pergamon

0277-5387(94)00483-8

Polyhedron Vol. 14, No. 13-14, pp. 1771–1777, 1995

0277-5387/95 $9.50+0.00

INTERACTIONS OF NICKEL(II) WITH ADENOSINE, URIDINE AND CYTIDINE MONOPHOSPHATES. A CALORIMETRIC STUDY

LUIS A. HERRERO and ANGEL TERRÓN*

Departament de Química, Universitat de les Illes Balears, E-07071 Palma de Mallorca, Spain

(*Received* 21 *October* 1994; *accepted* 18 *November* 1994)

Abstract—The thermodynamic parameters of the interaction between nickel(II) and 3′AMP, 2′AMP, 5′UMP and 5′CMP in aqueous solution were determined calorimetrically (ionic strength adjusted to 0.1 with tetramethylammonium bromide, 25°C at physiological pH). The experimental conditions were carefully selected to avoid polynuclear complex formation. The calorimetric data confirm the existence of the [(Ni(NMP)$_2$]$^{2-}$ complexes for 3′AMP and 2′AMP, the latter previously studied by Frey and Stuehr by potentiometry. The [(Ni(NMP)$_2$]$^{2-}$ complex formation constant (log $K_2 = 1.96$ and 2.16 for the 3′AMP and 2′AMP derivatives, respectively) and complex formation enthalpies ($\Delta H_1 = +4.5$ kJ mol^{-1} and $\Delta H_2 = -10.0$ kJ mol^{-1} for the 3′AMP complexes, and $\Delta H_1 = +2.4$ kJ mol^{-1} and $\Delta H_2 = -22.3$ kJ mol^{-1} for the 2′AMP complexes) were determined. The values of ΔH_1 for the complexes Ni(5′UMP) and Ni(5′CMP) are, respectively, 8.3 and 8.7 kJ mol^{-1}. The degree of macrochelation indicated by Sigel after very careful potentiometric studies is confirmed by the calorimetric data, and follows the order: [Ni(5′AMP)]$_{cl}$ > [Ni(2′AMP)]$_{cl}$ > [Ni(3′AMP)]$_{cl}$. These results confirm the formation of the ML$_2$ complex in solution and agree very well with the hypothesis of 'stacking' between the purine bases promoted for metal ions.

The bioinorganic chemistry of nickel is a topic of increasing interest.[1,2] The interactions of nickel(II) with nucleotides in solution have been reviewed recently.[3] The study of nickel(II)–3′AMP and nickel(II)–2′AMP and nickel(II) formation constants with pyrimidine nucleotides have been determined by potentiometric methods.[4–7] Only previous calorimetric studies with 5′AMP have been published[8,9] but none, to our knowledge, with 2′AMP or 3′AMP.

There are no X-ray diffraction studies on nickel(II) and 3′AMP or 2′AMP compounds and very few crystalline structures with related nucleotides have been described so far.[10] There is a solid state structure for [Ni(5′AMP)(H$_2$O)$_5$]·H$_2$O where nickel(II) is bound to N(7) and *via* a coordination water molecule also to the phosphate group. Other crystalline structures, in which the metal ion is coordinated to two nucleotides in *cis* position, have also been described for nickel(II) with 5′GMP and 5′IMP. The coordination sites are the same as in the former complex. The latter structures suggest a relevant base stacking stabilization between the purine bases in *cis* position, despite a butterfly-like arrangement of the two purine residues.

Frey and Stuehr[4] established the formation of the [Ni(2′AMP)$_2$]$^{2-}$ complex potentiometrically. Sigel[5,6] studied the relationship between macrochelation and the first stability constants for the nickel(II) and 5′AMP, 3′AMP and 2′AMP compounds; in the 2′AMP case the *anti* conformation of the nucleotide is preferred and would allow an interaction with N(3) of the adenine ring, but not with the N(7) for steric reasons; macrochelation is more difficult in the 3′AMP complex because the unfavourable *syn*

---

* Author to whom correspondence should be addressed.

conformation is required. Sigel also measured the self-association of different nucleotides.[10,11,12]

The aim of our study is to confirm, by a different technique, the existence of the $[Ni(2'AMP)_2]^{2-}$ complex and the analogous 3′AMP complex, to determine directly thermodynamic values, and consider the influence of adenosine base stacking stabilization in these complexes.

EXPERIMENTAL

The nucleotides were obtained from Sigma and Serva. The purity was checked by elution through a liquid chromatography column Pharmacia basic system I with a UV detector; in all cases a single fraction was detected. The nucleotide solutions were freshly prepared by dissolving the required amount of the solid in deionized, distilled water and the concentration in the solution was tested by spectrophotometry in a UV–vis Perkin–Elmer 552 spectrophotometer. The nickel(II) nitrate (analytical grade) was purchased from Merck, and the nickel concentration in solution was determined by weighing and by atomic absorption spectroscopy using a Perkin–Elmer 703 spectrophotometer. The tetramethylammonium bromide (analytical grade) was purchased from Merck and Aldrich. All the solutions were prepared with deionized and then distilled water and the ionic strength (I) was adjusted to 0.1 with tetramethylammonium bromide. The pH in equilibrium was determined by means of a Crison micropH 2002 with an accuracy of 0.001.

The calorimetric measurements were carried out in a LKB 2001 batch calorimeter connected to an IBM PS2 for data acquisition by means of the BATCHCAL program previously described.[13] All the measurements were taken at 25.00±0.01°C. The nucleotide solutions were placed in the reference cell to correct the heat of dilution in all cases; the metal heat of dilution was obtained experimentally and then subtracted from the reaction heat.

The measurements of nucleotide enthalpy protonation were carried out, mixing 3.00 mM HCl (4 cm^3) and ~8.0 mM of the respective nucleotide (2 cm^3) at $I = 0.1$ (using tetramethylammonium bromide) in the calorimeter reaction cell. The calorimeter reference cell contained 2 cm^3 of the nucleotide solution of the same concentration and 4 cm^3 of tetramethylammonium bromide to correct for the enthalpy dilution of the nucleotide. The values of the enthalpy of reaction minus the heat of dilution allow the enthalpy of protonation of the nucleotide to be calculated very easily.

RESULTS AND DISCUSSION

Definition of equilibrium constants

The main equilibria considered for the nickel(II)–2′AMP and nickel(II)–3′AMP systems are:

$$Ni^{II} + NMP^{2-} \rightleftharpoons [Ni(NMP)] \quad K_1 \text{ and } \Delta H_1 \qquad (1)$$

$$[Ni(NMP)] + NMP^{2-} \rightleftharpoons [(Ni(NMP)_2]^{2-} \quad K_2 \text{ and } \Delta H_2 \qquad (2)$$

$$Ni^{II} + NMPH^{-} \rightleftharpoons [Ni(NMPH)]^{+} \quad K'_1 \text{ and } \Delta H'_1 \qquad (3)$$

$$NMP^{2-} + H^{+} \rightleftharpoons NMPH^{-} \quad K_{pr} \text{ and } \Delta H_{pr} \qquad (4)$$

where H_2NMP is $H_23'AMP$ or $H_22'AMP$, respectively.

In order to carry out the calculations of this theoretical model the SAFCOR program[8] was used. The program considers the following values: log K_1, ΔH_{pr} (protonation heat of the nucleotides), pK_{pr} and the experimental heat values (Q = experimental heat of dilution), the initial metal (M_0) and nucleotide (L_0) concentrations, as well as the reaction and reference cells pH (pH and pH_r respectively). The values of K_1 and K_{pr} were taken from the literature and ΔH_{pr} was measured experimentally: log $K_1 = 2.00 \pm 0.03$ for 3′AMP, 1.95 ± 0.03 for 2′AMP, $pK_{pr} = 6.01 \pm 0.02$ and 5.83 ± 0.02, respectively (values from the literature[6] after correction of the background electrolyte), and $\Delta H_{pr} = -3.0 \pm 0.4$ kJ mol^{-1} and 0.0 ± 0.3 kJ mol^{-1}, respectively (values obtained experimentally by calorimetry).

The third equilibrium can be neglected in the experimental conditions of the study.[8]

For the pyrimidine nucleotides 5′UMP and 5′CMP only equilibria (1) and (4) may be considered. The values of K_1 and K_{pr} were taken from the literature[7] (after correction of the background electrolyte) and ΔH_{pr} was measured experimentally: log $K_1 = 2.00 \pm 0.06$ for 5′CMP and 2.03 ± 0.05 for 5′UMP, $pK_{pr} = 6.25 \pm 0.02$ and 6.21 ± 0.01, respectively, and $\Delta H_{pr} = 3.0 \pm 0.3$ kJ mol^{-1} and $\Delta H_{pr} = +3.4 \pm 0.4$ kJ mol^{-1}, respectively.

The SAFCOR program and data treatment

The SAFCOR program[8] calculates the best ΔH_1 and ΔH_2 and log K_2, compares the theoretical and experimental heats for each experimental point and estimates the 3σ deviation, considering not only the experimental error but the error of the parameters introduced in the program as well.

The experimental data for the nickel(II)–2′AMP and nickel(II)–3′AMP systems for correlations between Q_1 and X are presented in Fig. 1, where:

$$Q_1 = \Delta H_1 + X\Delta H_2$$

$$Q_1 = QR/(C_{ML} + C_{ML2})$$

$$X = C_{ML2}/(C_{ML} + C_{ML2})$$

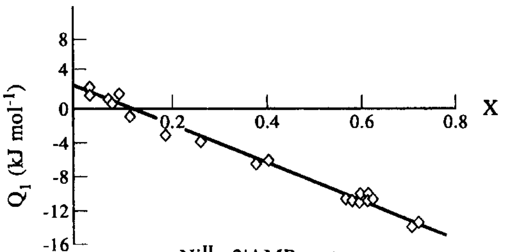
$$QR = Q - QP$$

Here Q is the reaction heat, QP is the heat associated with eq. (4) and C_{ML} and C_{ML2} are the amounts of [Ni(NMP] and $[(Ni(NMP)_2]^{2-}$ at equilibrium, respectively, where NMP is 2′AMP or 3′AMP in each case.

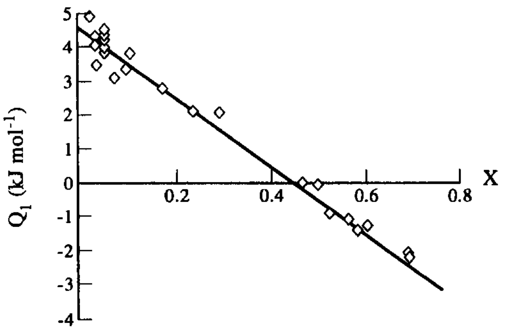
The pyrimidine nucleotides are more easy to study because no 1:2 complexes form, and equilibrium (2) need not to be considered.

Thermodynamic parameters and structural considerations

Table 1 contains the values obtained for ΔH_1, ΔH_2 and log K_2 for 2′AMP, 3′AMP, 5′CMP and 5′UMP with the 3σ deviation in comparison with the 5′AMP values.[8] The entropies have been calculated from the experimental results considering $\Delta G = \Delta H - T\Delta S$ and $\Delta G = -2.30RT \log K$. The experimental measurements are collected in Tables 2–5 and Fig. 1 shows the correlation between Q_1 and X for the nickel(II)–2′AMP and nickel(II)–3′AMP systems.



Ni^{II} - 2′AMP system

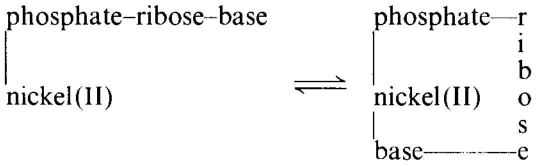


Ni^{II} - 3′AMP system

Fig. 1. Correlation between Q_1 and X.

All these results point to the formation of the $[(Ni(NMP)_2]^{2-}$ species in agreement with the previous potentiometric results of Frey and Stuehr for the 2′AMP system.[4] The log K_2 value obtained by calorimetry of 2.16 ± 0.07 for the nickel(II)–2′AMP system is in very good agreement with that previously measured by potentiometry (log $K_2 = 2.20$, $I = 0.1$ at 15°C).[4]

The ΔH_1 values for the three adenosine monophosphates present great differences. It is necessary to consider the different steric contribution to stabilize a macrochelate complex of nickel(II) bound to the phosphate group and to the N(7) or N(3) ring base in solution, and the different degree of macrochelation for each nucleotide: $71 \pm 4\%$ for 5′AMP and $13 \pm 13\%$ for 3′AMP or 2′AMP.[5,6]



There are two different species in solution $[Ni(NMP)]_{op}$, where nickel(II) is bound only to the phosphate group and the closed macrochelate complex $[Ni(NMP)]_{cl}$; $[Ni(2'AMP)]_{cl}$ can afford the stable *anti* conformation, but the $[Ni(3'AMP)]_{cl}$ complex must present, for steric hindrance, the very unstable *syn* conformation. In any case, Sigel established[5,6] that it is clear that the degree of formation of the closed species follows the order: $[Ni(5'AMP)]_{cl} > [Ni(2'AMP)]_{cl} > [Ni(3'AMP)]_{cl}$. Thus, the apparent differences in the ΔH_1 values can be related to the different degree of macrochelation of the nucleotide. In the nickel(II)–5′CMP and nickel(II)–5′UMP systems, the coordination takes place only through the phosphate group, and the enthalpies measured by calorimetry (ΔH_1 $+8.7 \pm 0.9$ and $+8.3 \pm 0.7$ kJ mol^{-1}, respectively, at $I = 0.1$ and 25°C) are coincident (Table 1). The value of $+8.5 \pm 0.8$ can be tentatively assigned to the enthalpy of interaction between nickel(II) and the phosphate group from a nucleoside monophosphate. This endothermic interaction is stabilized due to the entropic value (+58 J mol^{-1} K^{-1}). The formulae of the nucleotides are indicated in Fig. 2.

The different nature of the nickel complexes with adenosine monophosphates indicated by Sigel[5,6] also appears in the calorimetric data. It is possible

Table 1. Comparison between experimental data for the [Ni(NMP) and $[Ni(NMP)_2]^{2-}$ complexes

NMP	ΔH_1 (kJ mol^{-1})	ΔS_1 (e.u.)	Log K_2	ΔH_2 (kJ mol^{-1})	ΔS_2 (J mol^{-1} K^{-1})	$\Delta H_2 - \Delta H_1$ (kJ mol^{-1})	$\Delta S_2 - \Delta S_1$ (J mol^{-1} K^{-1})
5′AMP[a]	-10.0 ± 1.0	15	2.34 ± 0.14	-21.6 ± 2.0	-28	-11.6	-43
3′AMP	$+4.5 \pm 0.3$	52	1.96 ± 0.08	-10.0 ± 1.3	$+4$	-14.5	-48
2′AMP	$+2.4 \pm 0.6$	46	2.16 ± 0.07	-22.3 ± 1.4	-34	-24.7	-80
5′UMP	$+8.3 \pm 0.7$	58					
5′CMP	$+8.7 \pm 0.9$	58					

[a] Data from ref. 8.

Table 2. Nickel(II)–5′UMP system. Amount of [Ni(5′UMP)] complex and enthalpies obtained

Measurement	C_{ML} (mol × 10^7)	Q (mJ)	ΔH_1 (kJ mol^{-1}
1	103	62.2	8.5
2	103	60.0	8.2
3	103	59.3	8.2
4	63.4	37.3	8.3
5	63.4	37.3	8.4
6	63.4	37.3	8.4
7	43.0	25.5	8.4
8	43.0	25.1	8.3
9	43.0	25.7	8.2

Table 3. Nickel(II)–5′CMP system. Amount of [Ni(5′CMP)] complex and enthalpies obtained

Measurement	C_{ML} (mol × 10^7)	Q (mJ)	ΔH_1 (kJ mol^{-1})
1	98.4	62.3	8.7
2	98.4	60.5	8.5
3	98.4	60.3	8.5
4	60.6	37.3	8.9
5	60.6	36.8	8.8
6	60.6	37.6	9.0
7	37.4	22.9	8.6
8	37.4	22.5	8.5
9	37.4	23.7	8.8

to consider the formation of the complexes in two steps:

$$Ni^{II} + NMP^{2-} \rightleftharpoons [Ni(NMP)]_{op} \quad \Delta H_{1op} \qquad (5)$$

$$[Ni(NMP)]_{op} \rightleftharpoons [Ni(NMP)]_{cl} \; \Delta H_{1cl} \qquad (6)$$

Where NMP is used as the abbreviation for 5′AMP, 3′AMP or 2′AMP, respectively. The enthalpy of the pure interaction nickel(II) phosphate represented as ΔH_{1op} can be estimated as $+8.5 \pm 0.8$ as indicated above, whilst ΔH_{1cl} is the enthalpy value due to the interaction of the metallic ion with the ring base, possibly through the N(3) for 2′AMP and 3′AMP and through the N(7) with 5′AMP.[5,6] For the latter, and considering the 71% degree of macrochelation[5,6] a value for ΔH_{1cl} [enthalpy of interaction Ni^{II}–N(7)] of -26.0 kJ mol^{-1}, very similar to the single value in the literature for Ni^{II}–N(7) in 9-methylpurines (-20.8 kJ mol^{-1})[14] is obtained.

The ΔH_1 enthalpy values for [Ni(2′AMP)] and [Ni(3′AMP)] (Table 1) are less positive than the [Ni(5′UMP] and [Ni(5′CMP)] values but not negative like the [Ni(5′AMP)] enthalpy. The small amount of macrochelate in the equilibrium, eq. (6), produces the exothermic Ni^{II}–N(7) ring interaction, diminishing the endothermic ΔH_{1op} contribution of eq. (5). In agreement with the potentiometric studies of Sigel,[5,6] the interaction of nickel(II) with 2′AMP is less endothermic than the interaction with 3′AMP (+2.4 and +4.5 kJ mol^{-1}, respectively), indicating a major degree of macrochelation for 2′AMP than for 3′AMP. Considering tentatively, in a first broad approach, that the Ni^{II}–N(3) ring interaction is similar to the Ni^{II}–N(7) enthalpy, a degree of macrochelation of 23% for 2′AMP and 15% for 3′AMP is obtained. Although these percentages are not clearly quantitative, they indicate that the order of macrochelation is: $[Ni(5'AMP)]_{cl} > [Ni(2'AMP)]_{cl} > [Ni(3'AMP)]_{cl}$. Thus, the order of macrochelation obtained by Sigel is confirmed by calorimetry. The degree of macrochelation implies different entropic values, and it is clear again that in the [Ni(3′AMP)] complex (Table 1) a small amount of $[Ni(3'AMP)]_{cl}$ is present ($\Delta S = 52$ e.u. in comparison with $\Delta S = 58$ e.u. for [Ni(5′CMP)] and [Ni(5′UMP)]). The [Ni(2′AMP)] entropic value of 46 e.u. indicates a greater degree of macrochelation than for the 3′AMP complex, and less than for the [Ni(5′AMP)] complex. Thus, not only the enthalpy values, but also the entropic values are in agreement with the order of macrochelation

Table 4. Experimental data for the nickel(II)–2′AMP system[a]

Measurement	Mo (mol dm^{-3} × 10^4)	Lo (mol dm^{-3} × 10^4)	pH (mol dm^{-3} × 10^4)	pHr (mol dm^{-3} × 10^4)	Q (ml)
1	33.3	8.93	7.35	7.50	2.2
2	33.3	7.60	7.28	7.45	1.1
3	33.3	12.8	7.32	7.50	−2.0
4	33.3	23.6	7.30	7.50	−8.8
5	33.3	35.6	7.40	7.57	−20.0
6	6.67	53.2	7.22	7.23	−10.8
7	16.7	53.2	7.18	7.23	−26.2
8	16.7	125	7.25	7.30	−78.5
9	6.67	125	7.27	7.30	−31.8
10	64.7	10.2	7.20	7.40	1.1
11	64.7	10.2	7.20	7.40	2.0
12	64.7	10.2	7.20	7.40	1.7
13	64.7	10.2	7.20	7.40	2.0
14	33.3	20.9	7.13	7.26	−4.5
15	6.67	194	7.23	7.25	−16.1
16	16.7	194	7.21	7.25	−118.0
17	33.3	3.53	6.89	7.07	0.9
18	19.6	135	7.28	7.33	−93.0
19	33.3	3.53	6.89	7.07	0.7
20	33.3	3.53	6.89	7.07	0.7
21	33.3	3.53	6.89	7.07	1.1
22	32.3	128	7.19	7.31	−138.7
23	19.6	128	7.25	7.31	−83.8
24	12.8	128	7.27	7.31	−57.3
25	32.3	135	7.23	7.31	−146.0

[a] Mo, total ion concentration; Lo, total nucleotide concentration; pH, reaction cell; pHr, reference cell pH; Q, reaction heat (subtracted the heat dilutions).

proposed by Sigel using the potentiometric technique.

To discuss the enthalpy and entropic values of the ML_2 complexes is more difficult. Not only must the macrochelation, the nickel phosphate and nickel ring interaction be considered, but also the intramolecular stacking stabilization between the bases.[8,11,12] It can be considered, as a first approach, that the enthalpy for the nickel(II)–NMP interaction is the same in the first and second NMP ligand. The difference $\Delta H_2 - \Delta H_1$ (Table 1) is very similar for 5′AMP and 3′AMP, and can be assigned tentatively to the stacking interaction between the bases, with a high degree of macrochelation and *cis* configuration for the 5′AMP derivatives[8] and a minor degree of macrochelation for the 3′AMP derivatives. However, the 2′AMP enthalpy difference $\Delta H_2 - \Delta H_1 = -24.7$ kJ mol^{-1} and entropic difference $\Delta S_2 - \Delta S_1 = -80$ e.u. can only be explained due to the coordination of nickel(II) through the N(3) ring (different from the N(7) for the 5′AMP case) and a promotion of the degree of macrochelation with the entrance of the second ligand: the latter would be an exothermic process and at the same time a process that diminishes the entropy, and the degree of macrochelation would be facilitated by stacking between the purine bases. This hypothesis is in agreement with the values $\Delta H_2 = -22.3$ kJ mol^{-1} for the $[(Ni(2'AMP)_2]^{2-}$ complex and -21.6 kJ mol^{-1} for the $[(Ni(5'AMP)_2]^{2-}$ complex, being very similar indicating a structural *cis* macrochelate configuration; and -10.0 kJ mol^{-1} for the $[(Ni(3'AMP)_2]^{2-}$ complex where macrochelation is not favoured due to steric hindrance[5,6] and the enthalpy value is consequently much less exothermic.

CONCLUSIONS

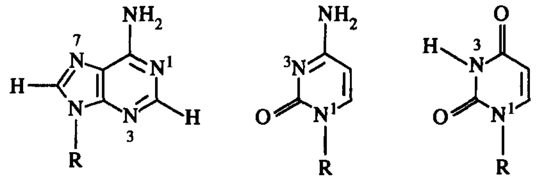
The calorimetric data confirm the existence of the $[(Ni(NMP)_2]^{2-}$ complexes for 3′AMP and 2′AMP, the latter previously studied by Frey and Stuehr by potentiometry.

The degree of macrochelation indicated by Sigel[5,6] after very careful potentiometric studies is confirmed by the calorimetric data, and follows the order $[Ni(5'AMP)]_{cl} > [Ni(2'AMP)]_{cl} > [Ni(3'AMP)]_{cl}$.

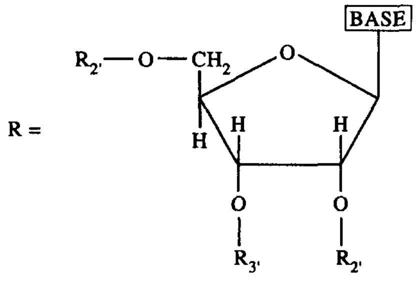
Table 5. Experimental data for the nickel(II)–3′AMP system[a]

Measurement	Mo (mol dm^{-3} × 10^4)	Lo (mol dm^{-3} × 10^4)	pH (mol dm^{-3} × 10^4)	pHr (mol dm^{-3} × 10^4)	*Q* (ml)
1	33.3	126	7.52	7.54	−0.8
2	16.7	126	7.57	7.54	−1.0
3	6.67	126	7.55	7.54	−2.5
4	32.3	31.5	7.18	7.21	10.8
5	16.7	14.9	7.18	7.24	3.6
6	6.67	14.9	7.19	7.24	1.8
7	33.3	4.37	6.96	7.04	2.8
8	16.7	40.1	7.21	7.35	5.8
9	19.6	276	7.28	7.32	−23.0
10	16.7	54.4	7.22	7.25	7.4
11	19.6	192	7.31	7.35	−11.9
12	33.3	16.8	7.10	7.17	7.1
13	64.7	11.0	7.13	7.30	8.8
14	33.3	12.8	6.98	7.10	5.0
15	64.7	7.57	7.07	7.21	6.3
16	33.3	5.83	7.00	7.12	3.3
17	64.7	7.57	7.07	7.21	5.4
18	19.6	276	7.28	7.32	−21.8
19	16.7	165	7.23	7.27	−8.1
20	6.67	165	7.25	7.27	−4.3
22	65.7	11.0	7.10	7.30	8.8
23	65.7	11.0	7.10	7.30	9.8
24	64.7	11.0	7.13	7.30	9.2

[a] Mo, total ion concentration; Lo, total nucleotide concentration; pH, reaction cell; pHr, reference cell pH; *Q*, reaction heat (subtracted the heat dilutions).



AMP^{2-} CMP^{2-} UMP^{2-}



2'-AMP^{2-} : $R_{2'} = -PO_3^{2-}$; $R_{3'} = R_{5'} = -H$

3'-AMP^{2-} : $R_{3'} = -PO_3^{2-}$; $R_{2'} = R_{5'} = -H$

5'-AMP^{2-}, 5'-CMP^{2-} and 5'-UMP^{2-} : $R_{5'} = -PO_3^{2-}$; $R_{2'} = R_{3'} = -H$

Fig. 2. Chemical structures of the pyrimidine and adenine nucleotide monophosphates.

Acknowledgement—This work has received financial support from the Spanish Authorities DGICYT project number PB-91-0806-C02-02.

REFERENCES

1. H. Sigel and A. Sigel, *Metal Ions in Biological Systems*, Vol. 23. Marcel Dekker Inc., New York (1988).
2. J. R. Lancaster Jr, *The Bioinorganic Chemistry of Nickel*. VCH, New York (1988).
3. R. B. Martin, *Met. Ions Biol. Systems* 1988, **23**, 315.
4. C. M. Frey and J. E. Stuehr, *J. Am. Chem. Soc.* 1972, **94**, 8898.
5. H. Sigel, *J. Chem. Soc., Chem. Soc. Rev.* 1993, **22**, 255.
6. S. S. Massoud and H. Sigel, *Eur. J. Biochem.* 1989, **179**, 451.
7. S. S. Massoud and H. Sigel, *Inorg. Chem.* 1988, **27**, 1447.
8. L. A. Herrero and A. Terrón, *Eur. J. Biochem* 1991, **202**, 401.
9. M. R. Melardi, J. Galea and G. Ferroni, *Bull Soc. Chim. Belg.* 1979, **88**, 1015.
10. A. Terrón, *Comments Inorg. Chem.* 1993, **14**, 63.
11. K. H. Scheller and H. Sigel, *J. Am. Chem. Soc.* 1983, **105**, 5981.
12. P. R. Mitchell and H. Sigel, *Eur. J. Biochem.* 1978, **88**, 149.
13. M. T. Oms, A. M. Calafat, R. Forteza, J. J. Fiol, A. Terrón, V. Cerdá and V. Moreno, *Thermochim. Acta* 1989, **141**, 141.
14. J. Arpalahti and H. Lönnberg, *Inorg. Chim. Acta* 1983, **80**, 25.