Complexation of Nickel(II) with Guanosine 5′-Monophosphate and Inosine 5′-Monophosphate: A Potentiometric and Calorimetric Study

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The constants (K_s) and enthalpies (ΔH_s) for stacking interactions between purine nucleoside monophosphates were determined by calorimetry; the values thus obtained were guanosine as follows: $K_s = 2.1 \pm 0.3$ M^{-1} and $\Delta H_s = -41.8 \pm 0.8$ kJ/mol for adenosine 5′-monophosphate (5′AMP); $K_s = 1.5 \pm 0.3$ M^{-1} and $\Delta H_s = -42.0 \pm 1.5$ kJ/mol for guanosine 5′-monophosphate (5′GMP); and $K_s = 1.0 \pm 0.2$ M^{-1} and $\Delta H_s = -42.3 \pm 1.1$ kJ/mol for inosine 5′-monophosphate (5′IMP). The interaction of nickel(II) with purine nucleoside monophosphates was studied using potentiometric and calorimetric methods, with 0.1 M tetramethylammonium bromide as the background electrolyte, at 25 °C. The presence in solution of the complexes [Ni(5′GMP)$_2$]$^{2-}$ and [Ni(5′IMP)$_2$]$^{2-}$ was observed. The thermodynamic parameters obtained were log $K_{ML} = 3.04 \pm 0.02$, log $K_{ML2} = 2.33 \pm 0.02$, $\Delta H_{ML} = -18.4 \pm 0.9$ kJ/mol and $\Delta H_{ML2} = -9.0 \pm 1.9$ kJ/mol for 5′GMP; and log $K_{ML} = 2.91 \pm 0.01$, log $K_{ML2} = 1.92 \pm 0.01$, $\Delta H_{ML} = -16.2 \pm 0.9$ kJ/mol and $\Delta H_{ML2} = -0.1 \pm 2.3$ kJ/mol for 5′IMP. The relationships between complex enthalpies and the degree of macrochelation, as well as the stacking interaction between purine bases in the complexes are discussed in relation to previously reported calorimetric data.

Introduction

The bioinorganic chemistry of nickel is a topic of increasing interest.[1,2] The study of the interactions of nickel(II) with nucleotides offers an unique opportunity for understanding various properties of nickel(II)−metal complexes, such as the carcinogenicity of some nickel compounds[3] and the anti-neoplastic activity recently detected in some nickel complexes.[4]

Few crystalline structures involving guanosine 5′-monophosphate (5′GMP) and inosine 5′-monophosphate (5′IMP) have been reported so far.[5] There are known solid state structures of the type [Ni(5′NMP)(H$_2$O)$_5$]·nH$_2$O, where NMP is 5′GMP or 5′IMP in which the nickel(II) ion is bound to N(7) (Figure 1) and also, via a coordinated water molecule, to the phosphate group. Other crystalline structures in which the metal ion is coordinated to two nucleotides in the cis position have also been reported for nickel(II) complexes with 5′GMP and 5′IMP (Figure 2). The coordination sites are the same as in the former complex. These structures suggest a relevant base stacking stabilizing effect between the purine bases in the cis position, despite the butterfly-like arrangement of the two purine residues. Recently, Sadler reported the platinum(II) analogues (with the water not directly coordinated to the metal ion) of these nickel complexes.[5e] These structures are, from a purely geometric point of view, very similar to those previously described for the interaction of cis platinum with nucleotides.[6]
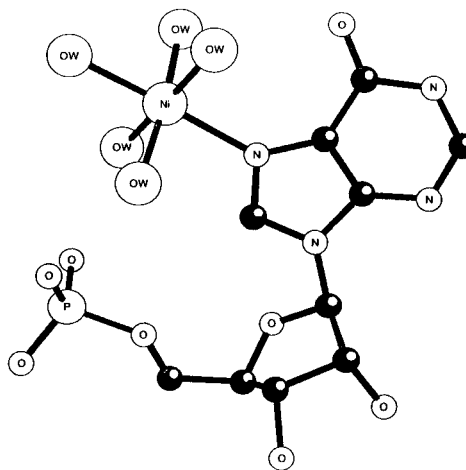

Figure 1. Ball and stick representation of the molecular structure of the complex [Ni(5′IMP)(H$_2$O)$_5$] (from ref 5b).

Interaction with the metal can also alter the hydrogen bond and stacking interactions between the bases in RNA and DNA molecules, as pointed out by Barton.[6] In a recent paper, Marzilli also studied the potential occurrence of atropisomerism in complexes of the type Pt(5′GMP)$_2$ in solution by use of NMR.[7]

The interactions of Ni(II) with nucleotides in solution were reviewed recently.[8] Frey and Stuehr[8b] established the formation of the complexes [Ni(5′AMP)$_2$]$^{2-}$ (5′AMP = adenosine 5′-monophosphate) and [Ni(2′AMP)$_2$]$^{2-}$ (2′AMP = adenosine 2′-monophosphate) potentiometrically. Sigel et al.[8c,d] studied the relationship between macrochelation and the first stability constant for the Ni(II) and purine nucleoside monophosphate

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(6) (a) Lippard, S. J *Metals in Medicine.* In *Bioinorganic Chemistry*; Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J. S., Eds.; University Science Books: Mill Valley, CA, 1994, p 455. (b) Barton, J. *Metal/ Nucleic Acid Interactions.* In *Bioinorganic Chemistry*; Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J. S., Eds.; University Science Books: Mill Valley, CA 1994, p 505.
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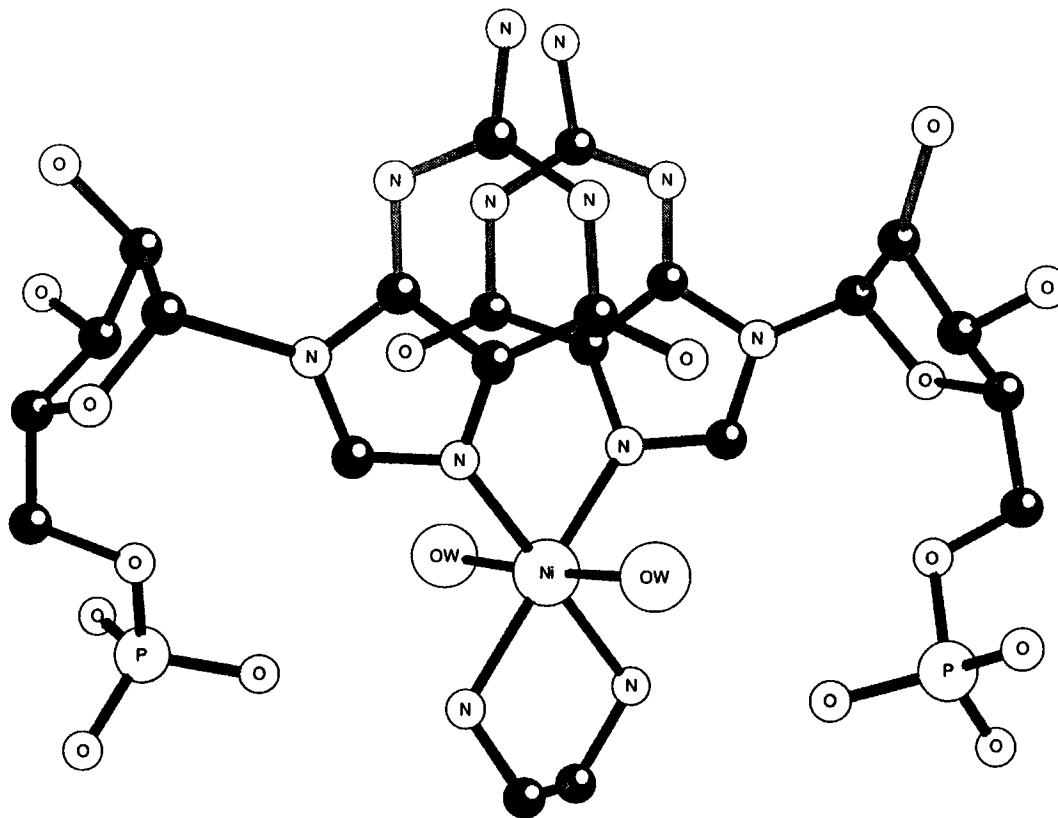
Figure 2. Ball and stick representation of the molecular structure of the complex [Ni(5′GMPH)₂(en)(H₂O)₂] (en = ethylenediamine) (from ref 5c).
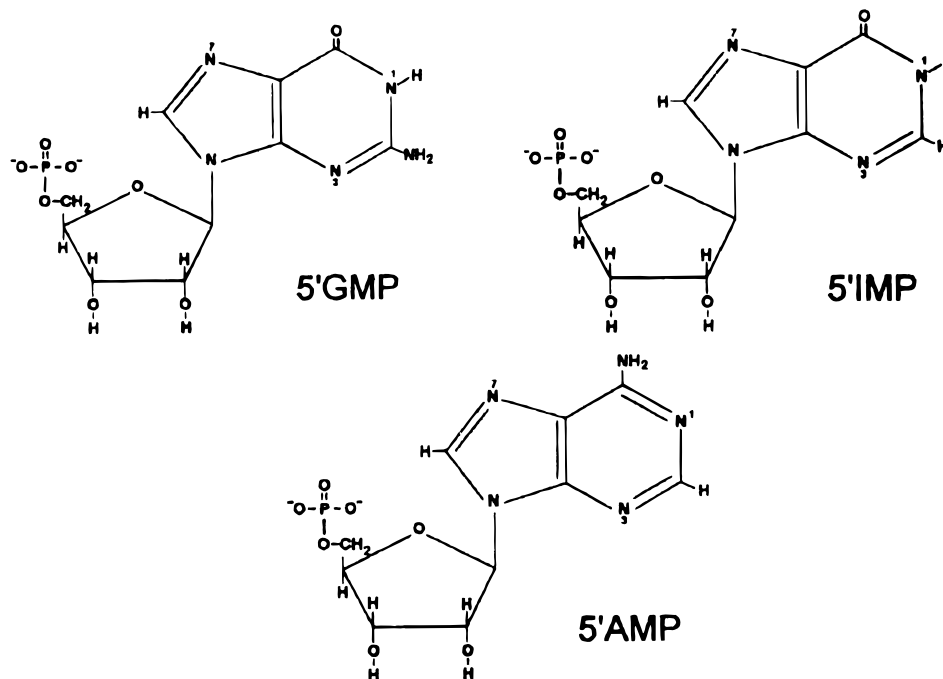
Figure 3. Formulas and abbreviations for the purine nucleoside monophosphates.

complexes. They also assessed the self-association of various nucleotides by NMR.[9]

Calorimetric data for the complexes of nickel(II) with 5′AMP, 3′AMP (3′AMP = adenosine 3′-monophosphate), 2′AMP,

5′CMP (5′CMP = cytidine 5′-monophosphate), and 5′UMP (5′UMP = uridine 5′-monophosphate) have been reported[10] but not, to our knowledge, for those with 5′GMP or 5′IMP.

The purpose of this work was to confirm, by using a different technique, the occurrence of the [Ni(5′NMP)₂]²⁻ complexes for

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guanosine and inosine 5′-monophosphates in solution, to directly determine thermodynamic parameters, and to discuss the influence of purine base stacking stabilization in these complexes.

Experimental Section

Materials. The acids H$_2$5′GMP and H$_2$5′IMP used in the potentiometric experiments were purchased from Sigma; the disodium salts of adenosine 5′-monophosphate, guanosine 5′-monphosphate, and inosine 5′-monophosphate (Figure 3) used in the calorimetric experiments were obtained from Serva. Nickel(II) nitrate (analytical grade) was purchased from Merck. Tetramethylammonium bromide (analytical grade) was supplied by Aldrich. The concentrations of the nucleotide solutions were determined by weighing and then confirmed by UV spectrophotometry. All solutions were prepared in deionized, distilled water; the ionic strength was adjusted to 0.1 with tetramethylammonium bromide. The NaOH solution (Merck) used in the potentiometric titrations was determined with potassium hydrogen phthalate (Merck).
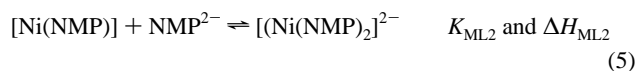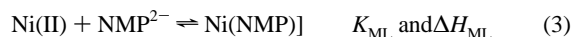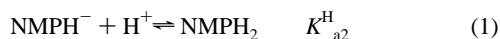
Potentiometric Titrations. These were carried out by using a CRISON microH 2002 potentiometer and a glass electrode. Potentials were acquired by means of an IBM PC running the VALGRAN software package.[11] NaOH titrant was added automatically form a CRISON MICROBU2031 microburet. Titrations were conducted in a jacketed titration vessel under an N$_2$ atmosphere in order to remove carbon dioxide; the temperature was maintained at 25 ± 0.1 °C by circulating water through the jacket. Magnetic stirring was applied.

Determination of Acidity Constants. The deprotonation constants for the acid nucleotides were determined by titrating a known volume (about 50 mL) of H$_2$NMP solution with NaOH. The initial concentration of nucleotide ranged from 6 × 10^{-4} to 2 × 10^{-3} M.

The stability constants K_{MLH} and K_{ML} for the [Ni(NMPH)]$^-$ and [Ni(NMP)] complexes were determined by titrating a known volume of H$_2$NMP solution containing nickel(II) nitrate. The initial concentration of nucleotide was between 2 × 10^{-4} and 6 × 10^{-4} M. A concentration ratio of metal ion to nucleotide higher than 25:1 was used in all cases to avoid the formation of [Ni(NMP)$_2$]$^{2-}$ complexes.

The K_{ML2} constants for the [Ni(NMP)$_2$]$^{2-}$ complexes were determined by titrating a known volume of nucleotide solution in the presence of 1.16 × 10^{-3} M nickel(II) nitrate. The initial nucleotide concentration in solution varied from 6.45 × 10^{-3} to 1.20 × 10^{-2} M, and the concentration of NaOH titrant was 9.37 × 10^{-2} M in all cases.

The main equilibria considered for the Ni(II)−5′GMP and Ni(II)−5′IMP systems were

$$\text{NMPH}^- + \text{H}^+ \rightleftharpoons \text{NMPH}_2 \qquad K^{\text{H}}_{a2} \qquad (1)$$

$$\text{NMP}^{2-} + \text{H}^+ \rightleftharpoons \text{NMPH}^- \qquad K^{\text{H}}_{a1} \ (K_{pr}) \text{ and } \Delta H_{pr} \qquad (2)$$

$$\text{Ni(II)} + \text{NMP}^{2-} \rightleftharpoons \text{Ni(NMP)]} \qquad K_{ML} \text{ and} \Delta H_{ML} \qquad (3)$$

$$\text{Ni(II)} + \text{NMPH}^- \rightleftharpoons [\text{Ni(NMPH)}]^+ \qquad K_{MLH} \text{ and } \Delta H_{MLH} \quad (4)$$

$$[\text{Ni(NMP)}] + \text{NMP}^{2-} \rightleftharpoons [(\text{Ni(NMP)}_2]^{2-} \qquad K_{ML2} \text{ and } \Delta H_{ML2}$$
$$(5)$$

where H$_2$NMP is H$_2$5′GMP or H$_2$5′IMP, respectively.

The parameters determined and equilibria considered for each determination are shown below. The linear equations used to fit experimental data were derived from the mass balances in each case

in order to establish a relationship between the measured constants and known parameters or others obtained during the potentiometric titration.

In the following expressions, A_t, is the total concentration of acid protons for each experimental point; K_w, the ionic product of water (13.72 ± 0.01 under our experimental conditions); and c_{nuc}, the overall concentration of nucleotide. The K_{a2} values used were those previously determined by Sigel et al.[8c]

(a) Determination of pK_{a1}. The equilibria considered were (1) and (2). From the mass balance, we obtained the following expression:

$$y/[(1-y)[\text{H}^+]] = K_{a1} + \{[(2-y)[\text{H}^+]]/(1-y)\}K_{a1}K_{a2} \quad (6)$$

$$y = \{A_t + (K_w/[\text{H}^+]) - [\text{H}^+]\}/c_{nuc}$$

(b) Determination of K_{ML} and K_{MLH}. The equilibria considered were (1) − (4) and the equation to fit was

$$(\gamma - \delta)/c_M = K_{ML} - K_{MLH}\gamma \qquad (7)$$

where

$$\gamma = K_{a1}\beta[\text{H}^+]$$

$$\delta = 1 - \{K_{a1}K_{a2}[\text{H}^+]^2\}$$

$$\beta = c_{nuc} - \alpha$$

$$\alpha = A_t - [\text{H}^+] + (K_w/[\text{H}^+])$$

(c) Determination of K_{ML2}. The equilibria considered were (1) − (5), and the equation for the linear correlation between K_{ML2} and the experimental parameters was

$$z/\{(1-z)K_{ML}[\text{NMP}^{2-}]\} - \{K_{ML}K_{a1}[\text{H}^+]\}/K_{ML} - 1 =$$
$$\{K_{ML2}(2-z)[\text{NMP}^{2-}]\}/(1-z) \quad (8)$$

where

$$z = \{c_{nuc} - [\text{NMP}^{2-}] - K_{a1}[\text{NMP}^{2-}][\text{H}^+](1 + K_{MLH}[\text{M}^{2+}])\}/M_o$$

M_o being the overall metal ion concentration, [NMP^{2-}] the free deprotonated nucleotide concentration, and [M^{2+}] the free nickel(II) concentration at equilibrium.

Under our working conditions, the measured standard potential for the glass electrode was given by

$$E = E'^\circ + g \log [\text{H}^+]$$

where

$$E'^\circ = E^\circ + g \log \gamma_{\text{H}^+} + E_j$$

E° = the standard potential (mV) for the electrode

E_j = the liquid junction potential

γ_{H^+} = the proton activity coefficient

g = the Nernst constant

E'° can be assumed constant for one potentiometric titration and was determined for each titration.

For calculations, all experimental results were fitted simultaneously for each system. In each calculation, a rough estimate standard potential of the glass electrode and experimental data were used. Standard potentials were optimized by using the program MINIPOT[11b] and POTCAL, POTPK, POTMLH, and POTML2 (adaptations of MINIPOT to our system that allowed simultaneous treatment of data for several potentiometric titrations[11c]). The linear regression for the equations ((6), (7), or (8) for each system) and the constants K_{a1}, K_{ML}, K_{MLH}, and K_{ML2} were determined. The final standard potentials minimized
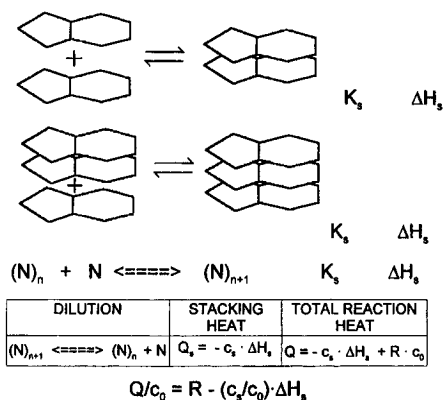
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Figure 4. Stacking interactions.

Figure 5. Correlation diagram for the Ni(II)−5′GMP system ($r =$ 0.95).

combined quadratic differences between the theoretical and experimental potentials [$\sum(E_t - E)^2$]. The deviations of the calculated parameters from the linear regression equation are given as 3σ values.

Calorimetric Measurements. Calorimetric measurements were carried out on a LKB 20001 batch microcalorimeter connected to an IBM PC for data acquisition by means of the program BATCHCAL.[12] All measurements were made at 25.00 ± 0.01 °C. The pH at equilibrium was measured by using a CRISON micropH 2002 pH-meter accurate to within ±0.001.

The heats of protonation of 5′GMP^{2-} and 5′IMP^{2-} were determined by addition in individual experiments of 4 mL of 3.27 mM HCl solutions to 2 mL of nucleotide solutions (8.35 mM for 5′GMP and 9.21 mM for 5′IMP). The initial pH of the nucleotide solutions was 7.

Constants and heats of autostacking were determined by dilution with 2 or 4 mL of NMP solutions of pH ≈ 7 in 4 or 2 mL, respectively, of background electrolyte. The nucleotide concentrations used were between 8.5 and 250 mM in order to test the proposed theoretical model over a wide range of concentrations. The ionic strength was adjusted to 0.1 with tetramethylammonium bromide. When the nucleotide concentration exceeded 35 mM, the ionic strength was inevitably greater than 0.1 M.

The formation enthalpies of the complexes [Ni(NMP)] and [Ni(NMP)$_2$]$^{2-}$, ΔH_{ML} and ΔH_{ML2}, were determined by mixing, in individual experiments, 4 mL of nucleotide solutions with 2 mL of nickel(II) nitrate solutions in a calorimetric cell. The nickel concentrations in the reaction cell at equilibrium were between 0.35 and 3.5 mM; higher concentrations were never used in order to avoid the formation of dimeric hydroxonickel complexes.[13] The nucleotide concentration at equilibrium in the reaction and reference cells varied from 0.5 to 20 mM. Nucleotide solutions were placed in the reference cell to correct the dilution heat in all cases: the dilution heat for the metal solution was obtained experimentally and then subtracted from that for the reaction cell.

Calorimetry was also used to determine the autostacking constant and enthalpy of stacking for the three nucleotides. Stacking parameters were determined by using the isodesmic model (Figure 4), where stacking is considered a noncooperative phenomenon and K_s and ΔH_s are taken to be the same for each new base association. These thermodynamic parameters can be determined calorimetrically by measuring the dilution heats of nucleotide solutions at variable concentrations. The total dilution value was experimentally fitted to an expression where (Figure 4) the enthalpy of stacking was present as a function of c_s (the amount (mol), that underwent dissociation), R was a constant that differed for each nucleotide, and c_0 was the initial molar concentration of nucleotide. One can derive a linear expression whose slope is the enthalpy of stacking over a wide range of nucleotide concentrations.
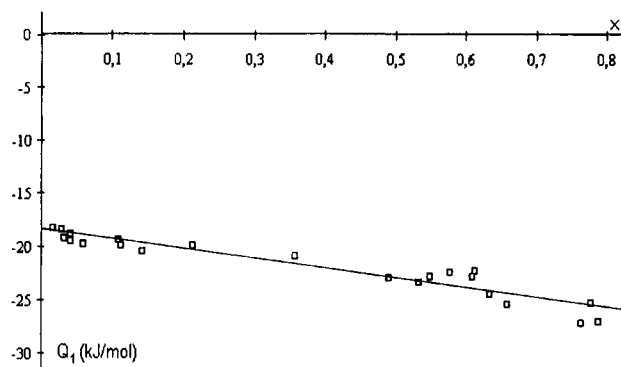
The program STACKING[11c] calculates ΔH_s, K_s, the error (given as 3σ) from experimental heats, and c_0 by linear regression of the equation

$$Q/c_0 = (-c_s/c_0)\Delta H_s + R \qquad (9)$$

and optimization of initial estimates for K_s.

Thermodynamic Parameters for the Complexes. The calorimetric determinations required the knowledge of the following parameters: log K_{ML}, log K_{ML2}, ΔH_{pr} (the protonation heat for the nucleotides), pK_{pr} (pK_{a1}) and the experimental heat values (Q = experimental heat − dilution heat), the initial metal (M_0) and nucleotide (L_0) concentrations, and the pH in the reaction and reference cells (pH and pH$_r$, respectively). The main equilibria considered for the Ni(II)−5′GMP and Ni(II)−5′IMP systems were (2), (3), and (5). Equilibrium 4 was neglected under experimental calorimetric conditions because of the small value of K_{MLH} relative to K_{ML} and the low NMPH$^-$ concentration at the working pH. All of these parameters were either found by experiment or determined under the same conditions as those for the calorimetric measurements.

The total heat was a contribution of equilibria 2, 3, and 5, and the dilution heats

$$Q = (C_{ML} + C_{ML2})\Delta H_{ML} + C_{ML2}\,\Delta H_{ML2} + C_H\,\Delta H_p \quad (10)$$

$$Q_R = Q - C_H \Delta H_P$$

$$Q_I = \Delta H_{ML} + X \Delta H_{ML2} \qquad (11)$$

$$Q_I = Q_R/(C_{ML} + C_{ML2})$$

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

$$Q_R = Q - QP$$

Q_R is the reaction heat; Q_P is the heat associated with eq 2; and C_{ML} and C_{ML2} are the amounts of [Ni(NMP)] and [Ni(NMP$_2$)]$^{2-}$ complexes respectively, at equilibrium (NMP being 5′GMP or 5′IMP in each case). Experimental values were obtained from linear regression of eq 11. The values thus obtained and those previously reported for 5′AMP[10a] are listed in Table 2. The correlations diagrams are shown in Figures 5 and 6.

Entropies were calculated from $\Delta G = \Delta H - T\Delta S$ and $\Delta G = -2.30RT \log K$.

Results and Discussion

Thermodynamic Parameters of the Ligands. We used tetramethylammonium bromide instead of alkaline salts as the background electrolyte for calorimetric measurements. The heat of interaction of sodium ion (or other alkaline ions) with the phosphate group of nucleotides can mask calorimetric determinations. For this reason, pK_{a1} values (pK_{pr}) for the nucleotides were determined in 0.1 M tetramethylammonium bromide; the results (Table 1) are quite consistent with previously reported values.[8c,d] The protonation enthalpies for the nucleotides (NMP^{2-}) were determined by calorimetry; the results suggest

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Table 1. Thermodynamic Parameters for the Purine Nucleoside Monophosphates at 25 °C and $I = 0.1$ in Tetramethylammonium Bromide[a]

NMP	K_s, M^{-1}	ΔH_s, kJ/mol	ΔS_s, J/(mol K)	pK_{pr}	ΔH_{pr}, kJ/mol	ΔS_{pr}, J/(mol K)
5′AMP	2.1 ± 0.3	-41.8 ± 0.8	-134 ± 4	6.22 ± 0.01	2.0 ± 0.4	126 ± 1
5′GMP	1.5 ± 0.3	-42.0 ± 1.5	-138 ± 6	6.23 ± 0.01	3.0 ± 0.3	129 ± 2
5′IMP	1.0 ± 0.2	-42.3 ± 1.1	-142 ± 4	6.22 ± 0.01	2.4 ± 0.4	127 ± 2

[a] Errors are given as 3σ. K_s, ΔH_s, and ΔS_s, equilibrium constant and enthalpy and entropy of stacking; pK_{pr}, ΔH_{pr}, and ΔS_{pr}, pK_{a1} and enthalpy and entropy of protonation for equilibrium 2.
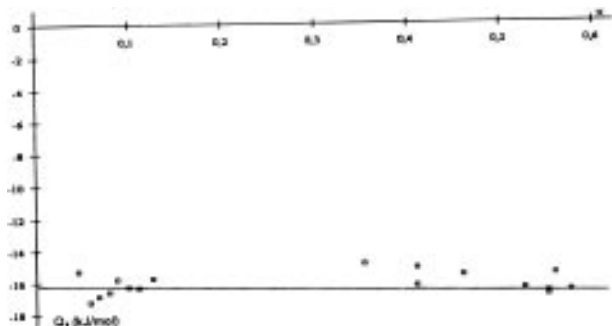

Figure 6. Correlation diagram for the Ni(II)−5′IMP system ($r = 0.96$).

an endothermic process (Table 1). The value for 5′AMP is consistent with the calorimetric value of Antonelli.[14]

The autostacking constants obtained were also consistent with those previously obtained by NMR[9] (see Table 1). The value 2.1 ± 0.1 M^{-1} for 5′AMP coincides with that reported by Sigel;[9d] also the values for 5′GMP and 5′IMP are in very good agreement with those obtained by Neurohr at 30 °C.[9f] It is significant that two different techniques such as NMR and batch calorimetry povided coincident values for hydrophobicity constants. K_s decreased in the following sequence 5′AMP > 5′GMP > 5′IMP. Also, ΔH_s was about +42 kJ/mol for the three nucleotides. Hence, the stacking process is exothermic and has a negative entropy as determined from thermodynamic data.

Thermodynamic Parameters and Structural Considerations. The formation constants for the nickel(II) complexes obtained by potentiometry and the measured enthalpies and entropies are given in Table 2. K_{ML} values are in very good agreement with those recently reported by Sigel et al.[8c]

Sigel used potentiometry to study the degree of macrochelation of different purine nucleotides[8c] and discussed the equilibria between complexes where the metal was only bound to the phosphate group (open form) and complexes where the metal was bound to the purine ring and the phosphate group (closed form)


(12)
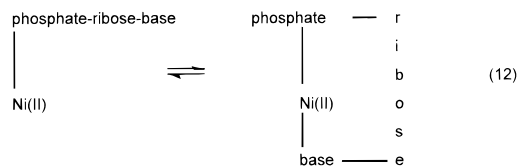
A plot of ΔH_{ML} or ΔS_{ML} against the degree of macrochelation revealed that the higher the degree of macrochelation is, the lower are the enthalpy and entropy. Therefore, the macrocycle formation is energetically favorable and entropically unfavorable, so no chelate effect is present, probably because of the large number of bonds in the macrocycle (Figure 7).

In order to study the consequences of macrochelation on the formation enthalpies of the [Ni(NMP)] complexes in detail, we

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Table 2. Thermodynamic Parameters for the Nickel(II)−Purine Nucleoside Monophosphate Complexes at 25 °C and $I = 0.1$ (Adjusted with Tetramethylammonium Bromide)[a]

	5′AMP	5′GMP	5′IMP
K_{MLH}		23 ± 2	13 ± 1
log K_{ML}	2.55 ± 0.02 [10a]	3.04 ± 0.02	2.91 ± 0.01
log K_{ML2}	2.34 ± 0.14 [10a]	2.33 ± 0.02	1.92 ± 0.01
ΔH_{ML}, (kJ/mol)	-10.0 ± 1.0 [10a]	-18.4 ± 0.9	-16.2 ± 0.9
ΔH_{ML2}, (kJ/mol)	-21.6 ± 2.0 [10a]	-9.0 ± 1.9	-0.1 ± 2.3
ΔS_{ML}, (J/(K mol)	15 [10a]	-4 ± 3	1 ± 3
ΔS_{ML2}, (J/(K mol)	-27.8 [10a]	14 ± 7	36 ± 8

[a] Errors are given as 3σ.
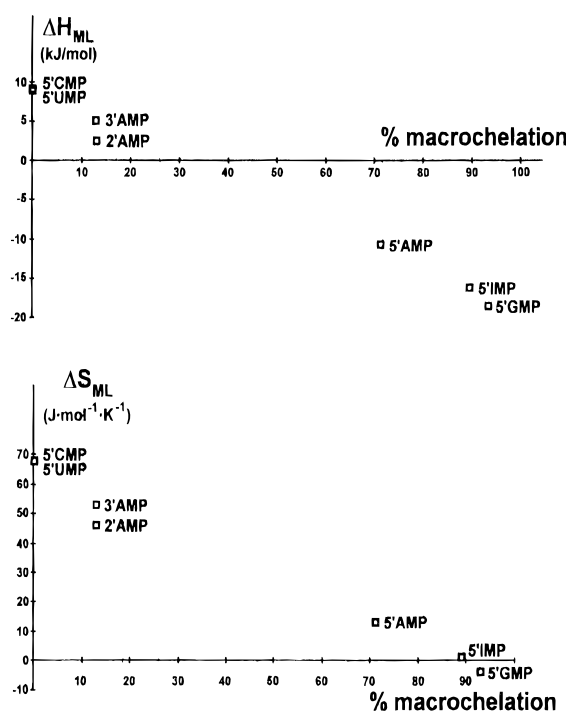
Figure 7. Variation of the enthalpy and entropy (ΔH_{ML} and ΔS_{ML}, with data from this work and refs 10a,c) and the degree of macrochelation as calculated by Sigel (from ref 8c).

can split the overall reaction in two steps. In the first, the open complex is formed:

$$\text{Ni(II)} + \text{NMP}^{2-} \rightleftharpoons [(\text{Ni(NMP)}_{op}] \qquad \Delta H_{op} \qquad (13)$$
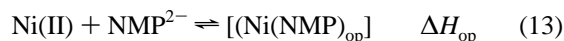
In the second, the open complex is partially converted into the closed complex:

$$[(\text{Ni(NMP)}_{op}] \rightleftharpoons [(\text{Ni(NMP)}_{cl}] \qquad \Delta H_{cl} \qquad (12)$$

The degree of macrochelation, r, is defined as the ratio of the concentration of the closed complex $[\text{Ni(NMP)}_{cl}]$ to the overall concentration of the complex [Ni(NMP)] at equilibrium:

$$r = [(\text{Ni(NMP)}_{cl}]/\{(\text{Ni(NMP)}_{op}] + [(\text{Ni(NMP)}_{cl}\} \quad (14)$$

Therefore, the formation enthalpy for the complex can be expressed as the combined enthalpies of the previous two

Table 3. Enthalpies and Entropies As Related to the Degree of Macrochelation (r from Ref 8c)

complex	r	ΔH_{ML}, kJ/mol	ΔH_{op}, kJ/mol	ΔH_{cl}, kJ/mol	ΔS_{ML}, J/(mol K)	ΔS_{op}, J/(mol K)	ΔS_{cl}, J/(mol K)
Ni(5′AMP)	0.71 ± 0.03	-10.0 ± 1.0	$+8.5 \pm 0.8$	-26.0 ± 3.6	$+15 \pm 4$	$+58 \pm 4$	-61 ± 13
Ni(5′IMP)	0.89 ± 0.01	-16.2 ± 0.9	$+8.5 \pm 0.8$	-27.8 ± 2.2	$+1 \pm 3$	$+58 \pm 4$	-64 ± 7
Ni(5′GMP)	0.93 ± 0.01	-18.6 ± 1.1	$+8.5 \pm 0.8$	-28.9 ± 2.1	-4 ± 3	$+58 \pm 4$	-67 ± 7
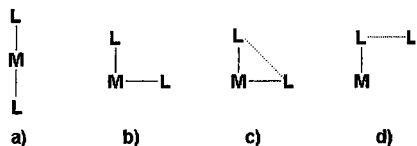
Figure 8. Possible solution structures for the complexes [(Ni(NMP)$_2$]$^{2-}$, where NMP denotes purine nucleoside monophosphate.

Table 4. Differences in Thermodynamic Parameters between ML$_2$ and ML Complexes

system	$\log K_{ML2} - \log K_{ML}$	$\Delta H_{ML2} - \Delta H_{ML}$, kJ/mol	$\Delta S_{ML2} - \Delta S_{ML}$, J/(mol K)
Ni(II)−5′AMP	-0.21	-11.6	-43
Ni(II)−5′GMP	-0.71	$+9.4$	$+18$
Ni(II)−5′IMP	-0.99	$+16.1$	$+35$

reactions, taking into account that the second step only takes place in part:

$$\Delta H_{ML} = \Delta H_{op} + r\Delta H_{cl} \qquad (15)$$

In this way, one can estimate the enthalpic and entropic contribution for the bonds between nickel(II) ion and the purine rings in these complexes, possibly through N(7) as in the solid state. Since we had determined ΔH_{ML} and estimated previously ΔH_{op} for the interaction of the nickel(II)−phosphate group ($+8.5 \pm 0.8$ kJ/mol),[10c] r values had previously been calculated by Sigel,[8c,d] and eq 15 was used to calculate ΔH_{cl}, which turned out to be about -27 kJ/mol for the three purine nucleotide (Table 3). Therefore, the interaction is energetically favorable in these complexes. This value is of the same magnitude as that previously reported for the interaction of nickel(II) with 2,9-methyl purines ($\Delta H = -20.8$ kJ/mol [15]).

The same treatment can be applied to the entropy by using the following equation:

$$\Delta S_{ML} = \Delta S_{op} + r\Delta S_{cl} \qquad (16)$$

The entropies obtained for the interaction of nickel(II)−purine ring in these complexes were also very similar for the three nucleotides and revealed that the process is entropically unfavorable (Table 3).

The results of the potentiometric and calorimetric measurements confirm the occurrence in solution of complexes of the type [(Ni(NMP)$_2$]$^{2-}$, similar to those found in the solid state (see Figure 2), for the ligands 5′GMP and 5′IMP.

In [(Ni(NMP)$_2$]$^{2-}$ complexes there must be some macrochelation for the first and second ligands. Determining the degree of macrochelation for these complexes is not easy, however. The enthalpy of interaction of nickel(II) with the phosphate group or N(7) purine ring in the first nucleotide ligand

(equilibrium 3) must clearly be different from that for the entry of the second ligand (equilibrium 5); pure electrostatic interactions are different and so is the electrophilic character of hexaaquanickel(II) from that of [(Ni(NMP)(H$_2$O)$_4$]. In any case, the degree of macrochelation seems to be lower for the second ligand than for the first, if entropy variations are assumed to be roughly identical.

After the second ligand is incorporated, stacking phenomena can gain significance. On the basis of possible geometries (Figure 8) for the complexes, one has four different situations: (a) the two nucleotides bond to the metal ion in the trans position; (b) the two ligands are in a cis conformation, with no hydrophobic interaction; (c) the two nucleotides are in a cis position, with a relevant stacking interaction; and (d) the second ligand is not directly coordinated to the metal ion and interacts only hydrophobically with the other ligand. The X-ray patterns point to structures of the type "b", "c", or "d", no solid-state structure of type "a" has to our knowledge been reported for nucleoside monophosphates.

The differences between the first and second ligand entries can be quantitatively assessed via the differences $\log K_{ML2} - \log K_{ML}$, $\Delta H_{ML2} - \Delta H_{ML}$, and $\Delta S_{ML2} - \Delta S_{ML}$ (Table 4). For the 5′AMP derivatives, $\log K_{ML2}$ is similar to $\log K_{ML}$; also, the differences $\Delta H_{ML2} - \Delta H_{ML} < 0$ and $\Delta S_{ML2} - \Delta S_{ML} < 0$ suggest the occurrence of a c type structure with a hydrophobic interaction between the purine rings. Such an interaction could be responsible for the enthalpic and hydrophobic differences, which can be assigned on a rough approximation to the thermodynamic stacking parameters for the [Ni(5′AMP)$_2$]$^{2-}$ complex. These values are lower than those for the autostacking parameters of 5′AMP ($\Delta H = -42$ kJ/mol and $\Delta S = -134$ J/(K mol)) and inconsistent with a "d" type structure, for which enthalpy and entropy differences should be similar to those between the autostacking values.

For Ni(II)−5′GMP and Ni(II)−5′IMP, the differences $\log K_{ML2} - \log K_{ML} < 0$, $\Delta H_{ML2} - \Delta H_{ML} > 0$, and $\Delta S_{ML2} - \Delta S_{ML} > 0$ clearly show that the entry of the second ligand is unfavorable in relation to the first ligand and that the process is energetically unfavorable and entropically favorable. These thermodynamic data are seemingly inconsistent with a predominant c or d type structure, but consistent with an a or b type structure involving no interaction between the ligands. However, a weak stacking effect can be masked by the degree of macrochelation since both effects are present.

The autostacking interaction sequence for the purine rings in the nucleotides (5′AMP > 5′GMP > 5′IMP) is similar to that for the interaction between the nucleotides in the [Ni(NMP)$_2$]$^{2-}$ complexes ([Ni(5′AMP)$_2$]$^{2-}$ > [Ni(5′GMP)$_2$]$^{2-}$ or [Ni(5′IMP)$_2$]$^{2-}$).

(15) Arpalahti, J.; Lönnberg, H. *Inorg. Chim. Acta.* **1983**, *80*, 25.