Interaction of Ni(II) with nucleotides and dipeptides — A quantitative study

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Interaction of Ni(II) with purine nucleotides (5'-guanosine monophosphate and 5'-inosine monophosphate) and dipeptides (DL-alanyl-alanine and DL-alanyl-phenylalanine) in a 1:1 and 1:1:1 ratios has been investigated by potentiometric pH measurements at 25, 35 and 45°C. The formation constants for various systems are determined by computer program. The stabilization of these complexes is expressed in terms of $\Delta \log K$. The stacking interaction associated with nucleotides and dipeptides have been identified with parameters like $K_1$, $K_2$, $\%$ of (MLA), and $\Delta G^\circ$. The pH profiles of various species are generated in order to identify the stable species. The thermodynamic parameters associated with the above systems are also evaluated for a more comprehensive understanding of these reactions in solution.

The non-covalent interactions of biological molecules provide the flexibility and specificity required in most important bioprocesses. These non-covalent interactions especially intramolecular stacking interactions between the purine moiety of nucleotides and the aromatic rings of secondary ligands, viz., 2, 2'-bipyridine amino acids etc., have been the subject matter of several investigations. The influence of aromatic ring stacking has been proved to be responsible for the enhancement of stability in ternary complexes in solution. At biological pH, these interactions determine the efficiency and specificity of such processes as interaction occurring in proteins, enzymes, DNA and RNA. Therefore, more and more information is needed for better understanding. The present paper, which provides useful information regarding the stability and thermodynamic aspects of these interactions, is an endeavour in this direction. The Ni(II) was chosen since the bioinorganic chemistry of Ni(II) is gaining importance because of its antineoplastic and carcinogenetic activity of its complexes and also the data with this metal is relatively scant. The paper details the study on the interaction of Ni(II) with purine nucleotides viz GMP, IMP (5'-monophosphates of guanosine and inosine respectively) and dipeptides (DL-alanyl-alanine and DL-alanyl-phenylalanine) (Chart-1). Since GMP differs from IMP by way of having an additional exocyclic substituent amino (NH₂) at C(2) position, this study also provides an opportunity to assess the influence of exocyclic substituent on the stabilities. Among the dipeptides, alanyl-alanine being the simplest was chosen as a reference to probe the influence of amino acid part of these interactions.

Materials and Methods

The disodium salts of 5'-GMP and 5'-IMP, DL-alanyl-alanine and DL-alanine-phenylalanine were obtained from Sigma Chemical Company, USA. For every titration fresh solid ligand was weighed out. Stock solutions of analytically pure metal nitrates were prepared and their concentrations determined by EDTA titrimetry. The experimental method involved the potentiometric titration of ligands in the absence and presence of metal ions at 25.0, 35.0, 45.0 ±0.10°C against standard carbonate free NaOH. The ionic strength was maintained constant at 0.10 M...
using KNO₃ (from BDH, Germany) as the supporting electrolyte using low concentration of the reactants (1×10⁻³M). During the experiment, oxygen free N₂ was passed through the titration cell to avoid the adverse effect of atmospheric CO₂. Each experiment was repeated twice to get concurrent readings.

Calculations
The acid dissociation constants of various ligands based on experimental values are determined by the computer program PKAS¹⁴ and are presented in Table 1.

Binary systems
In Ni(II)-GMP / IMP (1 : 1) binary systems, different types of complexes were observed.
In the case of Ni(II)-GMP, the following equations were used (charges are omitted for clarity):

M + H₂L ⇌ MHL + H⁺ ... (1)
MHL ⇌ ML + H⁺ ... (2)

for Ni(II)-IMP Systems Eq. (3) was used,

M + H₂L ⇌ ML + 2H⁺ ... (3)

The formation constants for Ni(II)-dipeptide systems were determined using Eq. (4),

M + HA ⇌ MA + H⁺ ... (4)

Ternary systems
The formation constants of Ni-GMP-dipeptide, Ni-IMP-dipeptide were determined using Eq. (5) and Eq. (6) respectively.

M + H₃L ⇌ MHL + H⁺

MHL + HA ⇌ MLA + 2H⁺ ... (5)

For Ni-IMP-dipeptide
M + H₂L + HA ⇌ MLA + 3H⁺ ... (6)

where M = Metal ion, H₂L = GMP/IMP, HA-dipeptides.

All the formation constants were subjected to refinement considering all possible species present in the solution, i.e., H₂L⁻, H₃L⁻ L₃⁻, HA, A⁻, ML, ML₂, MA, HA₂, MAL excluding hydroxo and polynuclear species using computer program BEST¹⁴. The error limits in these constants were minimised (sigma fit = 0.001 to 0.00001).

BEST was also used to generate complete species distribution curves at various pH values.

Thermodynamic constants
For this investigation as well as for a large number of similar investigations in the literature, the standard state is defined as 0.10 M (KNO₃) solution so that the activity of any species become unity when it is highly dilute in 0.10 M (KNO₃) i.e. the activity of any reacting ionic or non-ionic species x becomes

\[ \lim_{x \to 0} \frac{a_x}{[X]} = 1 \]

in the pure ionic medium. On this basis all the constants determined are very close to the thermodynamic constants¹⁵. These equilibrium constants differ somewhat in magnitude from constants obtained for a standard state of zero concentration of electrolyte, however, experience has shown the values of \( \Delta H_f^0 \) are about the same for the two concentrations.

Values of enthalpy of formation \( \Delta H_f^0 \) were obtained at each temperature interval by the relationship,

\[ \Delta H_f^0 = \frac{2.303 \ RT_1 \ T_2 \ \log (K_2 - K_1)}{(T_2 - T_1)} \]  

Values of \( \Delta G_f^0 \) and \( \Delta S_f^0 \) for various reactions involved were calculated by the relationship,

\[ \Delta G_f^0 = -RT \ \ln K \]  

\[ \Delta S_f^0 = \frac{\Delta H_f^0 - \Delta G_f^0}{T} \]

where

\( \Delta H_f^0 \) = change in the standard enthalpy of formation.

\[ R \] = gas constant

\[ T \] = absolute temperature
Results and Discussion

Determination of pK values of GMP and IMP

The titration curves of GMP and IMP (Fig. 1a) showed inflections at \( a = 1 \) (where \( a \) = moles of base added per moles of ligand) followed by buffer region, indicating the stepwise dissociation of its protons. The \( pK_a \) and \( pK_{2a} \) were assigned to the dissociations of phosphate secondary hydrogen and N(1)H respectively and the constants are compiled in Table 1.

**Determination of pK values of dipeptides**

The potentiometric titration curves of mono protonated alanyl-alanine (Fig. 1c) and ananyl-phenyl alanine resulted in a inflection at \( a = 1 \) followed by a buffer region at higher pH. The \( pK_a \) and \( pK_{2a} \) of these ligands are assigned to dissociations of carboxyl and amino groups respectively, and the constants are compiled in Table 1.

**Binary systems**

Ni(II)-GMP (1:1) system

The titration curve of Ni(II) system showed an inflection at \( a = 1 \) followed by buffer region. Accordingly, it was assumed that a protonated and a normal complex was formed in the buffer region between \( a = 0-1 \) and \( a = 1-2 \) respectively. The constants \( K^M_{ML} \) and \( K^{ML}_{ML} \) were determined using

<table>
<thead>
<tr>
<th>Ligand</th>
<th>25°C</th>
<th>35°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( pK_a )</td>
<td>( pK_{2a} )</td>
<td>( pK_a )</td>
</tr>
<tr>
<td>IMP</td>
<td>6.36</td>
<td>9.00</td>
<td>6.30</td>
</tr>
<tr>
<td>Ala-alα</td>
<td>3.64</td>
<td>8.25</td>
<td>3.67</td>
</tr>
<tr>
<td>Ala-phe</td>
<td>3.24</td>
<td>7.92</td>
<td>3.21</td>
</tr>
</tbody>
</table>

**Table 1**—Ionization constants* of the ligands and Formation** constants of binary and ternary complexes of Ni(II) ion; \( I = 0.10 \) M (KNO₃)

<table>
<thead>
<tr>
<th>System</th>
<th>Composition of the complex</th>
<th>( K^M_{ML} )</th>
<th>( K^{ML}_{ML} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni-GMP</td>
<td>1:1</td>
<td>3.38</td>
<td>3.31</td>
</tr>
<tr>
<td>Ni-IMP</td>
<td>1:1</td>
<td>4.35</td>
<td>4.24</td>
</tr>
<tr>
<td>Ni-ala-alα</td>
<td>1:1</td>
<td>4.71</td>
<td>4.66</td>
</tr>
<tr>
<td>Ni-ala-phe</td>
<td>1:1</td>
<td>3.91</td>
<td>3.85</td>
</tr>
<tr>
<td>Ni-GMP-ala-alα</td>
<td>1:1:1</td>
<td>3.50</td>
<td>3.45</td>
</tr>
<tr>
<td>Ni-GMP-ala-phe</td>
<td>1:1:1</td>
<td>8.94</td>
<td>8.82</td>
</tr>
<tr>
<td>Ni-IMP-ala-alα</td>
<td>1:1:1</td>
<td>8.86</td>
<td>8.72</td>
</tr>
<tr>
<td>Ni-IMP-ala-phe</td>
<td>1:1:1</td>
<td>9.05</td>
<td>8.91</td>
</tr>
</tbody>
</table>

*Accurate to ± 0.02 pK units; **Accurate to ± 0.03 pK units
Eq. (1) and (2) respectively. The constants thus calculated are presented in Table 1.

Ni(II)-IMP (1:1) system

In the titration curve of Ni(II) system, no inflection was observed (Fig. 1b). Accordingly, formation of normal complex was considered in the buffer region $a = 0-2$, which gave constant values. The constant $K_{ML}^M$ was determined with the help of Eq. (3). The stability constants are listed in Table 1.

Ni(II) - dipeptide (1:1) system

The potentiometric titration curve of Ni(II)-ala-ala (Fig. 1d) and Ni(II)-ala-phe in 1:1 ratio resulted in an inflection at $a = 1$ followed by a buffer region. The titration curve of binary system exactly coincided with that of free ligand in the region between $a = 0$ and $a = 1$. This indicates that the formation of complex occurred only in the region after $a = 1$. Hence $K_{ML}^M$ was calculated in the region $a = 1-2$ using Eq. (4). The constants are given in Table 1.

Ternary systems

Ni(II) - GMP - dipeptide (1:1:1) system

The potentiometric titration curves of mixed ligand systems of Ni(II)-GMP-ala-ala and Ni(II)-GMP-ala-
Table 2—Intramolecular aromatic ring stacking in ternary complexes (Temp=25°C)

<table>
<thead>
<tr>
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<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Δ log K</td>
<td>0.41</td>
<td>1.39</td>
<td>0.63</td>
<td>1.13</td>
</tr>
<tr>
<td>ΔΔlog K</td>
<td>0.98</td>
<td>0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K1</td>
<td>8.54</td>
<td>2.16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% of (MLA)ₐₐ</td>
<td>89.5</td>
<td>68.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−ΔG°ₐₐ</td>
<td>5.59</td>
<td>2.85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

phe showed an inflection at a = 1 and a = 2 followed by a buffer region. The titration curves coincided with that of free ligand curve of dipeptide in the region between a = 0 and a = 1 and with that of binary Ni(U)-GMP in the region a = 1 and a = 2. Therefore, the formation of ternary complex in the said region was ruled out. Accordingly, it was assumed that the formation of ternary complex took place in buffer region between a = 2-4. The formation constant

\[ K_{MLA}^{M} \] was determined using Eq (5). The constants are compiled in Table 1.

**Ni(II)-IMP-dipeptide (1:1:1) system**

The potentiometric titration curves of mixed ligand systems of Ni(II)-IMP-ala-ala (Fig.1e) and Ni(II)-IMP-ala-phe showed an inflection at a = 1 followed by a buffer region. Normal ternary complex formation was assumed between a = 1 and 4 and the formation constant \[ K_{MLA}^{M} \] was determined using Eq. (6). The constants are presented in Table 1.

The dissociation constants and formation constants pertaining to the interaction of nucleotides and dipeptides with various metal ions are also provided in Table 1. Although the ionisation constants and formation constants for binary complexes of dipeptides were available, they were re-evaluated to avoid possible errors in the evaluation of various parameters reported.

In GMP and IMP, N(7) also acts as a potential metal binding site in addition to phosphate oxygen and N(I). However, N(7) was not considered in this work since the titration curves of binary and ternary systems overlapped with that of free ligand curve in the region of N(7) involvement. Similar observations were made earlier with other systems. It is of interest to note that the interaction of metal ions with nucleotides is highly pH dependent viz. N(7) and phosphate oxygen are found to be more favoured sites in acidic medium and the preference for N(I) site increases as pH increases. Therefore, for assessing the extra stabilization in ternary complex, a quantity \[ \Delta \log K \] has been introduced. The \[ \Delta \log K \] is defined as,

\[ \Delta \log K = \log \beta_{MLA}^M - \log K_{ML}^M - \log K_{MA}^M \]

The \[ \Delta \log K \] values of nucleotides with different dipeptides increase in the order ala-ala < ala-phe indicating the dependence of stabilisation of ternary complex on the aromatic ring size of secondary ligands. Similar observations were also made earlier. The less positive \[ \Delta \log K \] values of ala-ala in both GMP and IMP systems can be explained on the basis of intramolecular interaction. Ala-ala has limited tendency for hydrophobic interactions and being an aliphatic ligand cannot take part in stacking interaction.

In order to rationalise the stacking interaction, additional parameters like \[ \Delta \Delta \log K, K_1, \% \] of (MLA)ₐₐ and \[ \Delta G°ₐₐ \] have been evaluated. The \[ \Delta \Delta \log K \] is expressed as

\[ \Delta \Delta \log K = \Delta \log K (M-GMP/IMP-ala-phe) - \Delta \log K (M-GMP/IMP-ala-ala) \]

Alanyl-alanine is taken as a reference for zero based scale of stacking interaction and extent of stacking is computed for other system.

Further, in solution, intramolecular equilibrium may exist between two isomers in open and stacked (or closed) respectively. \[ K_1 \] is the dimensionless constant for intramolecular equilibrium which is independent of absolute concentration of ternary complexes and expressed as,
"K_{t} = [M (GMP/IMP) (dipep)] / M (GMP / IMP) (dipep)_{eq}]

This can be calculated using the equation,

\[ K_{t} = 10^{A \Delta \log K} - 1 \]

The % of stacked isomer could be calculated from \( K_{t} \) values,

\[ \% \text{ of MLA}_{\text{MLA}} = (K_{t} / 1 + K_{t}) \times 100 \]

The free energy of \( \Delta G^0 \) change in KJ/mol associated with stacking interaction is calculated from,

\[ \Delta G^0 = -RT \Delta \Delta \log K \]

A detailed discussion of these parameters can be found elsewhere and values of the above parameters are listed in Table 2. Though, the values are only rough estimates, they clearly show that ternary complexes of ala-phe are more stabilized due to stacking interaction compared to the other dipeptide (ala-ala). This is further reflected in species distribution curves of the systems; for example formation of the complex Ni(II)-IMP-ala-phe (50%) (Fig. 3) and Ni(II)-GMP-ala-phe (40%) reaches maximum at physiological pH 7.5 followed by the ternary complex of Ni(II)-IMP-ala-ala (35%) (Fig. 2) and Ni(II)-GMP-ala-ala (25%).

A better picture emerged from the thermodynamic data (Table 3). The data is comparable with the literature wherever similar systems are involved. However, the data for the ternary systems are reported for the first time. The non-covalent interactions are manifested in the entropy and enthalpy values associated with the above systems. The stability of ternary complexes are reflected in the more negative and more positive entropy values. The less stacking interaction in GMP system is reflected in the more positive values of \( \Delta S^0 \) as compared to IMP system. The relatively high entropy values for both the systems is due to free COO group. The more negative enthalpy values are due to differences in the bonding of metal-aquo and metal-ligand species. The transition from metal-aquo to metal-ligand is significant as different donor atoms are involved.

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References