Potentiometric and \(^1\)H NMR studies of binary and ternary complexes of thiamine hydrochloride with copper (II), zinc (II), nickel (II) or cobalt (II) and various secondary ligands

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The interaction of thiamine hydrochloride with Cu (II), Zn (II), Ni (II), Co (II) and different secondary ligands like imidazole, tryptophan, phenylalanine, pyridine, 2,5-dicarboxylic acid, thiodipropionic acid and iminodiacetic acid have been studied potentiometrically. The interaction of these ligands with thiamine and tryptophan or thiamine and imidazole with Zn (II) in \(D_2O\) solution have also been studied by \(^1\)H NMR and the changes observed confirm the inter-ligand interactions between thiamine and TRP or thiamine and imidazole. The electronic spectra also confirm the inter-ligand interactions between thiamine and TRP or phenylalanine.

Thiamine hydrochloride (Vit B\(_1\)) occurs largely in cells and its active co-enzyme form thiamine pyrophosphate whose interaction with metal ions is biologically important as it has a wide-range of coordination sites\(^6,7\). Hence the complexing properties of thiamine hydrochloride (T.HCl) have been studied with various bivalent metal ions and different secondary ligands\(^8,9\). In our earlier paper we have reported the stabilities of ternary complexes of metal-thiamine-thymine and uracil systems\(^5\). In the present note, we are presenting the stabilities of T.HCl with imidazole, tryptophan, phenylalanine, thiodiacetic acid (TDA), thiodipropionic acid (TDPA), pyridine, 2,5-dicarboxylic acid (PYDCA) and iminodiacetic acid (IMDA).

**Experimental**

Chromatographically pure samples of thiamine hydrochloride, imidazole, tryptophan, phenylalanine, TDA, TDPA, PYDCA, IMDA ligands were obtained from E. Merck. For each titration T.HCl is directly weighed in its solid form into the titration cell. Standard solutions of secondary ligands were prepared and metal ion solutions were prepared in aqueous medium and standardised with disodium salt of EDTA as described by Schwarzenbach\(^6\). Carbonate free sodium hydroxide was prepared and standardised by titration with pure potassium hydrogen phthalate as given by Schwarzenbach and Bidermann\(^9\).

The experimental method consists of the potentiometric titration of metal ligand system with sodium hydroxide in a double jacketed cell at \(35\pm 0.1^\circ C\). In the course of titration, the ionic strength of the solution was maintained constant at \(0.1 \, M\) using \(KNO_3\). The formation constants of binary and ternary complexes were determined by titration of the aqueous solutions (50 ml) containing M (II) + L or A in 1:1 and M (II) + L + A in 1:1:1 ratio against carbonate free standard NaOH 1. 0.001 \(M\) metal nitrate, 0.001 \(M\) ligand L or A and 0.1 \(M\) \(KNO_3\). 0.001 \(M\) metal nitrate, 0.001 \(M\) ligand L, 0.001 \(M\) ligand A and 0.1 \(M\) \(KNO_3\).

Presaturated nitrogen was passed through experimental solution throughout the course of titration. The change in \(pH\) of the solution was determined with a Digisun pH-meter (model No. 707) equipped with combined glass electrode. The electrode system was calibrated by a direct titration.

The species considered to be present in the solution are LH\(^+\), L, AH\(^-\) and A or ML. The NMR spectra of complexes were recorded on Varian 270 MHz spectrometer. The spectra were recorded with reference to TSP locking the \(D_2O\).

The UV spectra of ligands and metal ligand complexes (1:1 and 1:1:1) were recorded on Hitachi U

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Table 1—Dissociation constants and stability constants of binary and ternary metal (II) complexes

<table>
<thead>
<tr>
<th>Ligand</th>
<th>pK_a</th>
<th>pK_z</th>
<th>Copper</th>
<th>Nickel</th>
<th>Cobalt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Binary</td>
<td>Ternary</td>
<td>Binary</td>
</tr>
<tr>
<td>Thiamine Hydrochloride</td>
<td>4.97</td>
<td>2.98</td>
<td>2.30</td>
<td>2.52</td>
<td>2.36</td>
</tr>
<tr>
<td>TDA</td>
<td>3.01</td>
<td>4.53</td>
<td>6.70</td>
<td>7.97</td>
<td>10.04</td>
</tr>
<tr>
<td>TDPA</td>
<td>3.92</td>
<td>3.35</td>
<td>6.19</td>
<td>2.76</td>
<td>4.51</td>
</tr>
<tr>
<td>IMDA</td>
<td>2.89</td>
<td>10.56</td>
<td>13.68</td>
<td>7.97</td>
<td>10.04</td>
</tr>
<tr>
<td>PYDCA</td>
<td>2.49</td>
<td>5.68</td>
<td>7.96</td>
<td>4.78</td>
<td>8.61</td>
</tr>
<tr>
<td>Imidazole</td>
<td>7.19</td>
<td>4.25</td>
<td>5.10</td>
<td>2.69</td>
<td>3.25</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>9.15</td>
<td>8.09</td>
<td>11.21</td>
<td>4.96</td>
<td>5.12</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>9.10</td>
<td>7.78</td>
<td>11.00</td>
<td>4.64</td>
<td>4.78</td>
</tr>
</tbody>
</table>

Structure I—Tentative structure of metal (II) - thiamine hydrochloride

3410 spectrometer using 1.0 x 10^4 ligands and metal ion solutions. The pH of the solutions was adjusted to 4.5 for 1:1 (Zn-T.HCl) and 7.0 pH for 1:1:1 Zn-T.HCl-TRP or phenylalanine by adding required amount of NaOH.

The acid dissociation constants of the ligands were calculated by direct algebraic method. The acid dissociation constants (K_{na}) were calculated using Eq (1).

\[ K_{na} = \frac{[H^+]([a-n+1]T_l+[H^+]-[OH^-])}{T_l-([a-n+1]T_l+[H^+]-[OH^-])} \]  

where 'a' is the mol of base added per mole of ligand and T_l is total ligand concentration.

The binary and ternary binding constants and % species were calculated using BEST PKa and SPE programme\(^11\) and the distribution plots were plotted using Sigma plot version 3.0.

Results and discussion

The titration curve of 1:1:1 metal ion T.HCl-A (A=TDA, TDPA, PYDCA, IMDA) showed inflection at m=3 and simultaneous complex formation was assumed and their stability constants calculated. The results are shown in Table 1. (The plot shows the titrating curves of Cu (II) - T.HCl-A. Similar curves were obtained for other metal ions studied). The coordination sites are shown in Structure I.

For the mixed ligand complexes of Cu (II)-thiamine hydrochloride-IMD, inflection was observed at m=1 [where L = T.HCl. M = Cu (II), Zn (II), Ni (II), Co (II)] and stepwise complex formation was assumed and the stability constants were calculated accordingly.

Theophenides et al.\(^12\)\(^-\)\(^16\) concluded based on the studies on the interaction of metal ions with thiamine, that N_I of pyrimidine ring is coordinated to the metal ion. Other investigators\(^17\)\(^-\)\(^18\) found that group II B metal ions would preferentially bind through N-3 position or the amino group of T.HCl. The metal binding site depends upon solvent, pH, nature of cations and anions.

The NMR spectra of Zn (II) - T.HCl taken in D_2O indicated downfield shift of pyrimidine ring protons (C_2-CH_3 & C_6-H) and supports the involvement of N_I in coordination to M^2+. Gallov\(^19\) and co-workers in their PMR studies of the complexes of TPP with divalent metal ions indicated that both pyrophosphate group and the N_I of pyrimidine ring are involved in coordination. Based on NMR studies of Gallov\(^19\) and our studies we conclude that thiamine hydrochloride binds metal through N_I of pyrimidine ring.

Proton dissociation constants and formation constants for binary and ternary complexes are reported in Table 1. The relative stability of the ternary complexes MLA as compared to that of the binary complexes MA and ML have been quantitatively explained in terms of the parameters \Delta \log K given by the equation.
Table 2—Δ log K values of various complexes

<table>
<thead>
<tr>
<th>Complex</th>
<th>Copper (II)</th>
<th>Zinc (II)</th>
<th>Nickel (II)</th>
<th>Cobalt (II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M (II) – T.HCl – TDA</td>
<td>-0.72</td>
<td>-0.48</td>
<td>-1.38</td>
<td>-0.96</td>
</tr>
<tr>
<td>M (II) – T.HCl – TDPA</td>
<td>-0.05</td>
<td>-0.54</td>
<td>-0.27</td>
<td>-0.22</td>
</tr>
<tr>
<td>M (II) – T.HCl – PYDCA</td>
<td>+0.43</td>
<td>+1.53</td>
<td>+0.93</td>
<td>+0.55</td>
</tr>
<tr>
<td>M (II) – T.HCl – IMDA</td>
<td>+0.23</td>
<td>-0.05</td>
<td>+1.01</td>
<td>-0.06</td>
</tr>
<tr>
<td>M (II) – T.HCl – Imidazole</td>
<td>+0.85</td>
<td>+0.56</td>
<td>+0.99</td>
<td>+0.48</td>
</tr>
<tr>
<td>M (II) – T.HCl – Tryptophan</td>
<td>+0.23</td>
<td>+0.16</td>
<td>+0.10</td>
<td>+0.07</td>
</tr>
<tr>
<td>M (II) – T.HCl – Phenylalanine</td>
<td>+0.53</td>
<td>+0.14</td>
<td>+0.10</td>
<td>+0.13</td>
</tr>
</tbody>
</table>

Fig. 2—Percentage species distribution curves of Cu(II)-PYDCA-T.HCl (1:1:1) and its corresponding binary and free ligands.

\[ \Delta \log K = \log K_{\text{ML}} - \log K_{\text{MA}} \]

or

\[ \Delta \log K = \log K_{\text{ML}} - \left( \log K_{\text{M}} + \log K_{\text{ML}} \right) \]

The Δ log K values obtained are listed in Table 2. The formation constants of the binary thiamine hydrochloride are compared with TOP and TPP. The stability constants of T.HCl are less than TOP and TPP. The Δ log K values of ternary complexes (Table 2) involving TDA, TDPA, IMDA are negative as expected from statistical grounds, whereas the stability constants of ternary complexes of M (II) – T.HCl-L (L=PYDCA, IMD, TRP or phenylalanine) are more stable than the corresponding binary complexes. The positive Δ log K values for these systems can be attributed to the stacking interactions between the pyrimidine ring of thiamine hydrochloride and aromatic side chain of amino acids or pyridine ring of PYDCA or imidazole ring. The Cu (II) – T.HCl-A systems are more stable than the other metal (II) – T.HCl-A system, the higher stabilities of Cu (II) ternary systems may be due to Jahn-teller distortion. The Δ log K values of M (II) – T.HCl-PYDCA are more positive than other systems. This can be attributed to pronounced stacking in acidic media as M (II) thiamine PYDCA complexation is taking place in lower buffer region.

Figure 1 shows the species distribution diagram of the various species involved as a function of pH. The Cu (II) – T.HCl (1:1) complex formation is maximum at pH 5.0 whereas Cu (II) – PYDCA (1:1) complex formation is maximum (79%) at pH 3.8 and the latter decreases slowly as ternary 1:1:1 Cu (II) – T.HCl-PYDCA formation takes place. Ternary complex formation is negligible up to pH 4.0 and reaches maximum at 6.5 pH. The absence of stacking interaction with T.HCl and TDA or TDPA etc., are reflected in their species distribution, which is not shown in Fig. 1.

The 'H NMR chemical shifts for free thiamine hydrochloride, tryptophan, imidazole and its complexes with Zn (II) in D2O solution were recorded. The 'H NMR spectra of Zn (II) thiamine in D2O shows a downfield shift (0.10) of C4-H, C2=CH2 of pyrimidine ring indicating that it is coordinated to metal ion through N1 of pyrimidine ring. C2-H, C4-H and C3-H signals of imidazole show large downfield shift upon coordination to Zn (II). Similarly the CH2 and CH signals of the aliphatic chain of tryptophan shifted to downfield (0.2 and 0.1 ppm) upon coordination to Zn (II). There is not much change in the signal position of ring protons. The 'H NMR measurements were made to detect the intramolecular stacking in the mixed ligand complexes containing T.HCl and tryptophan or imidazole. The proton signals of T.HCl and TRP in (1:1:1). Zn (II) – T.HCl-TRP are shifted to upfield (5.37 and 3.87) those of (1:1) metal ligand system (5.60 and 3.90). The upfield shift of T.HCl and TRP clearly shows the existence of an intramolecular stacking. It is also observed that the
upfield shift of imidazole signals (C\textsubscript{2}-H, C\textsubscript{3}-H and C\textsubscript{5}-H) (8.30, 7.25 ppm) and C\textsubscript{2}-CH\textsubscript{3} and C\textsubscript{6}-H signals (2.60, 8.06) of pyrimidine ring of T.HCl in the ternary (1:1:1) Zn (II) – T.HCl-IMD complex compared to corresponding (1:1) Zn (II) – T.HCl (2.60, 8.06) and Zn (II) – IMD (8.35, 7.25) indicates the stacking interaction between imidazole and pyrimidine ring of T.HCl in the ternary complexes.

The presence of charge transfer interaction between T.HCl and the indole part of the tryptophan in the ternary complexes is supported by electronic spectra of the complexes in solution\textsuperscript{18}. The spectra [Zn-T.HCl-TRP] shows different number of bands compared to Zn (II) – TRP and Zn (II) – thiamine. The additional band at 215 nm in the ternary complex is due to charge transfer between indole of tryptophan and pyrimidine part of thiamine. Similar spectra were recorded for ternary Zn (II) – T.HCl-phenylalanine. The spectra in the region 220-280 nm gave only two broad bands at 232 and 268 nm.

References
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