Stability and thermodynamics of Zn(II)- cysteinemethylester and histidinemethylester system –relevance to zinc core in transcription factor IIIA

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The requirements for zinc in the regulation of gene expression is exemplified by transcription factor III A (TF III A) which is a zinc – cysteine protein, containing zinc fingers that bind to DNA. The zinc – core in this protein consisting of Zn – Cysteine – Histidine residues has an important role in the stabilization. Given the ubiquitous occurrence of zinc fingers in biological systems, the stability and thermodynamic parameters associated with the zinc – core have been assessed. The various factors that are responsible for the formation and stabilization of the core are identified.

The last decade has seen remarkable developments in establishing zinc – dependent interrelationships between enzyme activities, protein structures and folding, and products of gene expression. The requirements for zinc in the regulation of gene expression is exemplified by transcription factor III A (TF III A), a zinc protein comprising of zinc fingers, that bind to DNA. Zinc fingers are regions of protein containing four residues of histidine and / or cysteine that are coordinated to zinc in a tetrahedral configuration (Structure I) and form a loop that can take part in protein-nucleic acid interactions. The role of zinc though not very clear, it is required for DNA - binding activity. In proteins, the coordination polyhedron of structural zinc is dominated by cysteine thiolates while that of catalytic zinc is dominated by histidine ligands. However, coordination of both cysteine and histidine ligands around zinc in “zinc fingers” adds a new dimension to the functionality of zinc. The metal ion probably stabilizes the nucleic acid – binding domain of the protein in order to maintain the proper topology for nucleic acid association. In view of this the stability of zinc core in these proteins assumes significance.

Accordingly, an interaction of zinc with cysteinemethylester (Cysme) and histidinemethylester (Hisme) has been investigated in detail as a model for zinc core in transcription factor III A. In the present manuscript a detailed study of potentiometric pH measurements were carried out to determine the stability factor and to identify the various species present in solution, in particular at biological pH 6-8. The study was extended to different temperatures to evaluate the thermodynamic parameters associated with these interactions as they provide a comprehensive understanding of such reactions that occur in vivo. Further, since Zn(II) has neither intrinsic color nor unpaired electrons, nuclear magnetic resonance (NMR) is a useful but limited technique for investigating metal ligand coordination in zinc complexes. Thus this technique was applied only to identify the bonding modes in the system.

Materials and Methods
Cysteinemethylester hydrochloride and histidinemethylester dihydrochloride were obtained from the Sigma chemical company (USA). AnalAR grade Zinc nitrate was obtained from E.Merck, Darmstadt (West Germany). They were used as supplied.

The experimental method consisted of potentiometric titration of ligands in the absence and presence of zinc under controlled experimental conditions. For every titration, fresh solid ligand (Cysme and
Hisme) was weighed out into a double wall jacketed reaction cell to avoid possible hydrolysis. The zinc ion was standardized volumetrically by titration with the sodium salt of EDTA in the presence of a suitable indicator as outlined by Schwarzenbach. Carbonate-free sodium hydroxide was prepared by the method of Schwarzenbach and Biedermann and was standardized by titration with potassium hydrogenphthalate. The ionic strength was maintained constant by using 0.10M KNO₃ as supporting electrolyte and relatively low concentrations of the ligand and metal ion (1×10⁻³M) were used. During the course of titrations a stream of oxygen free nitrogen was passed through the reaction cell to eliminate the adverse effect of atmospheric carbon dioxide. A Digison model - DI - 707 digital pH meter fitted with combined microglass-electrode was used to determine hydrogen ion concentrations. The electrode system was calibrated by direct titration of acetic acid and an observed pH meter reading was compared with the actual hydrogen ion concentration. The pH regions below 3.5 and above 10.5 were calibrated with measurements in HCl and NaOH solutions, respectively. Each experiment was repeated at least twice for accuracy. Further details can be found elsewhere.

The protonation constants of ligands, cysteinemethylester and histidinemethylester were determined by computer program PKAS using experimental data. The constants are given in Table 1. Although complexes ML, MA, and MLA account for the metal - binding by cysteinemethylester and histidinemethylester, the formation of protonated, hydroxo and polynuclear complexes were considered for obtaining stability constants listed in Table. The equilibria are described below:(omitting charges)

\[
M + H_2L \rightleftharpoons ML + 2H^+ \\
M + L \rightleftharpoons ML \\
K_{ML}^M = \frac{[ML]}{[M][L]} \quad \ldots (1)
\]

\[
M + H_2A \rightleftharpoons MA + 2H^+ \\
M + A \rightleftharpoons MA \\
K_{MA}^M = \frac{[MA]}{[M][A]} \quad \ldots (2)
\]

where H₂L and H₂A are Cysme and Hisme, respectively and M=Zn(II).

To determine the ternary stability constants for the Zn(II) - cysteinemethylester - histidinemethylester system in a 1:1:1 ratio in the buffer region between 1=0 and 4, the following equations were used: (Omitting charges)

\[
M + H_2L + H_2A \rightleftharpoons MLA + 4H^+ \\
M + L + A \rightleftharpoons MLA \\
\beta_{MLA}^M = \frac{[MLA]}{[M][L][A]} \quad \ldots (3)
\]

<table>
<thead>
<tr>
<th>System</th>
<th>Composition of the complex</th>
<th>25°C</th>
<th>35°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn(II) - Cysme</td>
<td>K_{ML}^M 1:1</td>
<td>8.28</td>
<td>7.83</td>
<td>7.23</td>
</tr>
<tr>
<td>Zn(II) - Hisme</td>
<td>K_{MA}^M 1:1</td>
<td>5.04</td>
<td>4.89</td>
<td>4.23</td>
</tr>
<tr>
<td>Zn(II) - Cysme-Hisme</td>
<td>\beta_{MLA}^M 1:1:1</td>
<td>17.0</td>
<td>16.30</td>
<td>15.15</td>
</tr>
</tbody>
</table>

Δ log K = [log \beta_{MLA}^M - (log K_{ML}^M + log K_{MA}^M)] = +3.68

Cysteinemethylester: SH - 6.16; \\ NH$_3$ - 8.89.

Histidinemethylester: \\ NH - 5.47; \\ NH$_3$ - 7.17.

**Thermodynamic Data**

<table>
<thead>
<tr>
<th>System</th>
<th>ΔH° (KJ mol$^{-1}$)</th>
<th>ΔG° (KJ mol$^{-1}$)</th>
<th>ΔS° (J mol$^{-1}$ deg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn(II) - Cysme - Hisme</td>
<td>-176.83</td>
<td>-97.51</td>
<td>-266.18</td>
</tr>
<tr>
<td>Zn(II) - Cys - His$^{**}$</td>
<td>-103.40</td>
<td>-110.29</td>
<td>+23.13</td>
</tr>
</tbody>
</table>

*The constants are accurate to $\pm$0.02
$^{**}$ The constants are accurate to $\pm$1.0.

Ref. 29
All the formation constants were subjected to refinement considering all possible species in the solution i.e. $H_3^+$, $HL$, $L$, $H_2A^{2+}$, $HA$, $A^{-}$, $ML$, $ML_2$, $MA$, $MA_2$, $MLA$ using computer program BEST. The error limits in these constants were minimized ($\Sigma$ fit=0.001 to 0.0001). BEST was also used to generate the complete species distribution curves as a function of pH.

Thermodynamic constants
For this investigation as well as for a large number of similar investigations in the literature, the standard state is defined as 0.1M(KNO$_3$) solution so that the activity of any species become unity when it is highly dilute in 0.10M(KNO$_3$) i.e. the activity of any reacting ionic or non-ionic species x becomes

$$\lim_{x \to 0} \left[ x \right] = 1$$

in the pure ionic medium. On this basis all the constants determined are very close to the thermodynamic constants$^{22}$. 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 $\Delta H^0_f$ are about the same for the two concentrations. The values of the enthalpy of complex formation $\Delta H^0_f$ were obtained at each temperature interval by the relationship,

$$\Delta H^0_f = \frac{2.303RT \log(K_2/K_1)}{(T_2 - T_1)} \quad \text{...(4)}$$

Equation(4) gave a better statistical variation of $\Delta H^0_f$ than those obtained as a plot of $\log K$ against $1/T$. The values of $\Delta G^0_f$ and $\Delta S^0_f$ for the various reactions involved were calculated by the relationships

$$\Delta G^0_f = -RT \ln K \quad \text{...(5)}$$

$$\Delta S^0_f = \frac{\Delta H^0_f - \Delta G^0_f}{T} \quad \text{...(6)}$$

where $\Delta H^0_f$, $\Delta G^0_f$ and $\Delta S^0_f$ are standard enthalpy, free energy and entropy changes, respectively and $R$ = gas constant, $T$ = absolute temperature and $K$ = equilibrium constant.

The $^1$H NMR spectra of individual ligands and ligands together in the absence and presence of zinc ion were recorded at room temperature (20-25°C) on a Varian Gemini 200MHz pulsed FT NMR spectrometer in D$_2$O. TMS was used as an internal standard.

![Fig 1](image1.png)

Fig 1—Potentiometric titration curves of (a) Free Cysme ; (b) Free Hisme ; (c) Zn(II) - Cysme (1:1 system) ; (d) Zn(II) - Hisme (1:1 system) ; (e) Zn(II) - Cysme - Hisme (1:1:1 system), at $T = 35^\circ C$ and $\mu = 0.10$ mol.dm$^{-3}$ (KNO$_3$).

![Fig 2](image2.png)

Fig 2—Species distribution curve for Zn(II) - Cysme - Hisme (1:1:1 system);

Results and Discussion
Histidinemethylster contains two nitrogen atoms in the aromatic imidazole ring, one of which protonates in the biologically relevant pH range of 5-6. This nitrogen atom can coordinate to metal ions, especially when histidine residue is part of a protein chain. Cysteinemethylster by virtue of its $\beta$ - thiol group has a high affinity for soft and boarder line metal ions. The Cysme coordinates to the metal through sulfur and nitrogen donor atoms whereas Hisme will coordinate through the imidazole and amino nitrogens. The protonation constants of Cysme and Hisme correspond to the dissociation of protons from SH, NH$_2$ and NH, NH$_2$ respectively. The dissociation and the binary constants of these ligands with zinc were redetermined under conditions similar to those applied here for determining ternary con-
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Fig 3—(a) 1H-NMR spectra of Cysme – Hisme system; (b) 1H-NMR spectra of 3a in presence of Zn(II) metal ion.

The stability of the ternary system compared to its corresponding binary systems is measured in terms of $\Delta \log K$. Thus the $\Delta \log K$ reflects the extent of stabilization in the ternary complex. The $\Delta \log K$ value is given in the table. It is to be noted here that if the $\Delta \log K$ values are positive the ternary complexes are more stable than the corresponding binary complexes and if the values for $\Delta \log K$ are negative the binary complexes are more stable than the ternary complexes. However, the negative values of $\Delta \log K$ does not preclude the formation of ternary complexes. Therefore, the high positive value of $\Delta \log K (3.68)$ for the Zn(II) – Cysme – Hisme complex indicates that the complex is stabilized more than 3000 times as compared to the statistical considerations. The extra stabilization of 1:1:1 ternary complex is also clearly evident from the species distribution curve (Fig.2). It may be seen from Fig.2, that the Zn(II) – Cysme – Hisme species reaches almost 100% at biological pH, while 1:1 Zn(II) – Cysme and Zn(II) – Hisme species are negligible. In order to determine the factors responsible for extra stabilization of Zn(II) – Cysme – Hisme system, the study was extended to different temperatures to evaluate thermodynamic parameters, as they provide a comprehensive understanding of these reactions in solution. The enthalpy and entropy values associated with the Zn(II) – Cysme – Hisme system are given in Table. The high negative enthalpy and negative entropy values are indicative of substantial contributions from the former quantity for the stabilization of the Zn(II) – Cysme – Hisme system.

The 1H NMR spectra were utilized to identify the binding sites in cysteinemethylester and histidinemethylester. The spectra of individual ligands and ligands together in the absence and presence of zinc were recorded (Fig.3) to identify the impact of the metal ion on the chemical shifts and the values are CH(α)– 4.30, 4.28; CH₂(β)– 4.04, 4.10; CH(δ)– 7.18, 7.40; CH(ε)– 8.05, 8.58; OCH₃– 3.82, 3.85; CH₂(β¹)– 3.28, 3.10; CH(α¹)– 3.10, 3.00; OCH₃¹– 3.38, 3.38 for the Cysme-Hisme system in absence and presence of zinc ion respectively.
All compounds which contain imidazole exhibit chemical shift due to CH(α) around δ 8.0 ppm in D$_2$O. The chemical shift of this proton would be expected to shift down field upon binding with a metal ion. This effect is observed for the proton in the 2-position of imidazole ring in histidinemethylester which shifts by ~ δ 0.6 ppm down field in the corresponding metal complex. Consistent with this the CH(δ) chemical shift was also shifted to down field on metallation. However, since Zn(II) would cause deprotonation of NH$_3$ proton on binding, an upfield shift of the CH(α) chemical shift was observed. In cysteinemethylester CH$_2$β) and CH(α') chemical shifts are shifted upfield in metal complex indicating the involvement of sulfur and nitrogen atoms in zinc coordination. Thus, the spectra suggests the bidentate coordination of these ligands resulting in a tetra coordination around zinc.

It is clear from the potentiometric and NMR studies that zinc coordinates to cysteinemethylester and histidinemethylester through sulfur, amino, imidazole and amino nitrogen, respectively resulting in a tetra coordination around it (Structure II). Since zinc is not subjected to ligand field stabilization effects (LFSE = 0), the change from an octahedral hexa aquo species M$^{2+}$(H$_2$O)$_6$ to the tetrahedral metal- ligand complex M$^{2+}$L$_4$ is energetically favorable$^{27,28}$. Thus a tetrahedral geometry is reflected in the large favorable enthalpy values, and is also evident from the entropy values indicating a high degree of solvation in the complex. These effects manifest in the desolvation of the metal ion and its binding site, as the zinc ion binds to the ligands, the entropy gain of solvent release is not substantial since the complex is ionic. Thus the entropy loss from zinc – binding site organization is expected to surpass the entropy gain of metal desolvation. It is important and of interest to compare the thermodynamic quantities$^{29}$ associated with the interaction of zinc with free histidine and cysteine (Table) with that of the Zn(II)-Cysme-Hisme system. The less negative enthalpy and positive entropy values of the former as compared to the latter are due to the differences in the charges of their respective ternary complexes.

In the metal – binding sites of proteins, metal ligands are engaged in hydrogen bond networks which minimize the conformational entropy gain conferred by metal binding$^{30-33}$. The present data, which clearly establishes the stability of the zinc core, may provide an opportunity in the design of proteins for specific biological objectives.

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References