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Can Co(II) or Cd(II) substitute for Zn(II) in zinc fingers?

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Abstract. Zinc finger domains consist of sequences of amino acids containing cysteine and histidine residues tetrahedrally coordinated to a zinc ion. The role of zinc in a DNA binding finger was considered purely structural due to the absence of redox chemistry in zinc. However, whether other metals e.g. Co(II) or Cd(II) can substitute Zn(II) is not settled. For an answer the detailed interaction of Co(II) and Cd(II) with cysteine methylester and histidine methylester has been investigated as a model for the zinc core in zinc fingers. The study was extended to different temperatures to evaluate the thermodynamic parameters associated with these interactions. The results suggest that zinc has a unique role.

Keywords. Zinc finger; cobalt; cadmium; cysteine methylester; histidine methylester; stability constants; thermodynamic parameters.

1. Introduction

Zinc finger proteins are widespread in eukaryotes and the zinc finger motif is the most commonly used structural element for nucleic acid recognition in eukaryotic cells[1]. Within this large family of proteins, xenopus transcription factor III A (TF III A) is unique, in that the zinc finger motif was originally identified as a Zn(II)-stabilized structural mini domain in TF III A[2–11]. These domains consist of sequences of amino-acids containing cysteine and histidine residues tetrahedrally coordinated to a zinc ion. The role of zinc in the DNA-binding finger was considered purely structural[12] as it might have an advantage over a disulphide bridge in bringing together two parts of a protein because it cannot be reduced in the reducing environment that prevails in the cell due to the absence of redox chemistry in zinc. Whether other metals can substitute zinc is not yet clear.

Hence, the detailed interaction of Co(II) and Cd(II) with cysteine methylester (Cysme) and histidine methylester (Hisme) has been studied as a model for the zinc core. Stability and thermodynamic data have been evaluated in solution as a function of *p*H for clearer understanding of these systems in solution. The data is compared with that of the zinc system for a comprehensive understanding of the role of zinc.

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2. Experimental

2.1 *Materials and methods*

Cysteine methylester hydrochloride and histidine methylester dihydrochloride were obtained from the Sigma Chemical Company (USA). AnalaR grade zinc nitrate, cobalt chloride and cadmium chloride obtained from E Merck (Darmstadt, Germany) were used.

2.2 *pH-metry*

The study involved potentiometric titration of ligands in the absence and presence of metal ions under controlled experimental conditions. Stock solutions of analytically pure metal salts were prepared and their concentrations were determined by the usual volumetric methods[13]. Each titration was repeated at least twice under carefully controlled experimental conditions. Ionic strength was maintained at constant level by using 0·1 mol dm^{-3} KNO_3 as supporting electrolyte and relatively low concentrations of the ligand and metal (1×10^{-3} mol dm^{-3}). 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 *p*H-meter fitted with combined SCE–glass microelectrode was used to determine hydrogen ion concentration. The electrode system was calibrated by direct titration of acetic acid and an observed *p*H-meter reading was compared with the actual hydrogen ion concentration. *p*H regions below 3·5 and above 10·5 were calibrated by measurements in HCl and NaOH solutions respectively. Further details can be found elsewhere[14].

The protonation constants of ligands, cysteine methylester and histidine methylester were determined by computer program PKAS[15] using experimental data (table 1). Although complexes ML, MA, and MLA account for the metal-binding by cysteine methylester and histidine methylester, the formation of protonated, hydroxo and polynuclear complexes were considered for obtaining the stability constants listed in table 1. The equilibria are shown below (omitting the charges except for H^+).

$$\mathrm{M} + \mathrm{H_2L} \rightleftharpoons \mathrm{ML} + 2\mathrm{H}^+,$$

$$\mathrm{M} + \mathrm{L} \rightleftharpoons \mathrm{ML},\ K^{\mathrm{M}}_{\mathrm{ML}} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]}, \tag{1}$$

$$\mathrm{M} + \mathrm{H_2A} \rightleftharpoons \mathrm{MA} + 2\mathrm{H}^+,$$

$$\mathrm{M} + \mathrm{A} \rightleftharpoons \mathrm{MA},\quad K^{\mathrm{M}}_{\mathrm{MA}} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{A}]}, \tag{2}$$

where H_2L and H_2A are Cysme and Hisme respectively and M = Zn(II), Co(II) and Cd(II).

To determine the stability constants for the M(II)–cysteine methylester–histidine methylester ternary system in a 1:1:1 ratio in the buffer region between m = 0 and 4, the following equations were used (omitting the charges except for H^+),

$$\mathrm{M} + \mathrm{H_2\,L} + \mathrm{H_2\,A} \rightleftharpoons \mathrm{MLA} + 4\mathrm{H}^+,$$

Table 1. Stability constants* of M(II)–cysteine methylester and histidine methylester systems at different temperatures; μ = 0·1 mol dm^{-3} (KNO_3).

System	Composition of the complex	$\log b^{M}_{MLA}$ 25°C	35°C	45°C
Co(II)–Cysme	K^{M}_{ML} 1:1	–	–	–
Co(II)–Hisme	K^{M}_{ML} 1:1	4·76	4·65	4·17
Co(II)–Cysme–Hisme	b^{M}_{MLA} 1:1:1	16·08	15·99	14·63
$\Delta \log K = [\log b^{M}_{MLA} - (\log K^{M}_{ML} + \log K^{M}_{MA})]$				
Cd(II)–Cysme	K^{M}_{ML} 1:1	9·37	8·20	7·53
Cd(II)–Hisme	K^{M}_{ML} 1:1	4·63	4·31	3·96
Cd(II)–Cysme–Hisme	b^{M}_{MLA} 1:1:1	17·01	16·16	15·09
$\Delta \log K = [\log b^{M}_{MLA} - (\log K^{M}_{ML} + \log K^{M}_{MA})] = +3 \cdot 42$				
Zn(II)–Cysme–Hisme	b^{M}_{MLA} 1:1:1	17·09	16·30	15·15
$\Delta \log K = [\log b^{M}_{MLA} - (\log K^{M}_{ML} + \log K^{M}_{MA})] = +3 \cdot 68$				
pk values of:				
Cysteine methylester:	SH–6·16; $\overset{+}{N}H_3$ – 8·89			
Histidine methylester:	$N\overset{+}{H}$ –5·47; $\overset{+}{N}H_3$ – 7·17			

*The values are accurate to ± 0·02

$$M + L + A \rightleftharpoons MLA,\ b^{M}_{MLA} = \frac{[MLA]}{[M][L][A]}. \quad (3)$$

All the formation constants were subjected to refinement considering all possible species in the solution, i.e. H_2L^+, HL, L^-, H_2A^{2+}, HA^+, A, ML, ML_2, MA, MA_2, MLA using the computer program BEST[16]. 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 *p*H.

2.3 *Thermodynamic constants*

For this investigation as in a number of similar investigations reported in the literature, the ionic strength of the medium was held constant using 0·1 mol dm^{-3} (KNO_3). The activity coefficient of any species is held constant in a highly dilute solution of the species that permits use of concentrations in place of activities in the equilibrium expression to arrive at equilibrium constants corresponding to the ionic strength of the

medium. Thus for any species x, its activity a_x is related to its concentration $[x]$ and activity coefficient f_x as,

$$a_x = [x]\, f_x .$$

Values of ΔH_f° were obtained at each temperature interval by the relationship,

$$\Delta H_f^\circ = \frac{2 \cdot 303 R T_1 T_2 \log(K_2 / K_1)}{(T_2 - T_1)}. \tag{4}$$

ΔG_f° and ΔS_f° for the various reactions were calculated by the relationships,

$$\Delta G_f^\circ = - R\, T \ln K \tag{5}$$

and

$$\Delta S_f^\circ = \frac{\Delta H_f^\circ - \Delta G_f^\circ}{T}. \tag{6}$$

3. Results and discussion

Histidine methylester contains two nitrogen atoms in the aromatic imidazole ring, one of which protonates in the biologically relevant *p*H range of 5–6. This nitrogen atom can strongly coordinate to metal ions, especially when histidine residue is part of a protein chain. Cysteine methylester by virtue of its *b*-thiol group has a high affinity for soft and borderline metal ions. Thus Cysme coordinates to the metal through sulphur and nitrogen donor atoms whereas Hisme coordinates through the imidazole and amino nitrogens. The proton dissociation constants of Cysme and Hisme correspond to the dissociation of protons from SH, $\overset{+}{\mathrm{N}}\mathrm{H}_3$ and $\overset{+}{\mathrm{N}}\mathrm{H}$, $\overset{+}{\mathrm{N}}\mathrm{H}_3$ in the two cases respectively. Although the proton dissociation constants of these protonated ligands and formation constants of the binary complexes of these ligands with the metal ions are available[17], these were redetermined under conditions similar to those applied here for determining formation constants of ternary complexes and avoid possible errors in the evaluation of $\Delta \log K$, where

$$\Delta \log K = [\log\, b^{\mathrm{M}}_{\mathrm{MLA}} - (\log K^{\mathrm{M}}_{\mathrm{ML}} + \log K^{\mathrm{M}}_{\mathrm{MA}})].$$

The data presented in table 1 are in good agreement with the reported values considering the differences in the conditions applied for their evaluation.

The titration curves of Cysme and Hisme (figure 1a, b) showed an inflection at $A = 1$ and $A = 2$ (where A = moles of base added per mole of ligand) followed by a buffer region, indicating the stepwise and simultaneous dissociation of their protons. Titration curves of Cd(II) with Cysme and Co(II), Cd(II) with Hisme showed an inflection at $A = 2$ (figure 1c, d, e), followed by a buffer region. Accordingly, it was assumed that the complex is formed in the buffer region around $A = 0$–2.

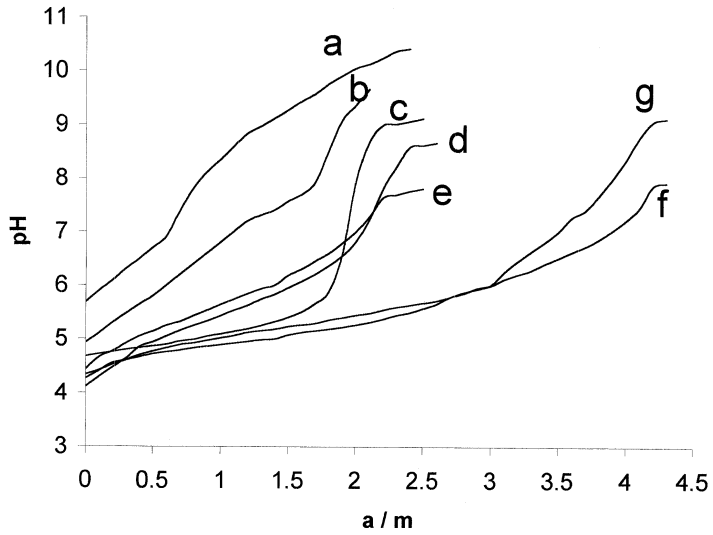

Figure 1. Potentiometric titration curves of (a) free Cysme, (b) free Hisme, (c) Cd(II)–Cysme (1:1 system), (d) Co(II)–Hisme (1:1 system), (e) Cd(II)–Hisme (1:1 system), (f) Co(II)–Cysme–Hisme (1:1:1 system), (g) Cd(II)–Cysme–Hisme (1:1:1 system), at T = 35°C and μ = 0·10 mol dm^{-3} (KNO_3).

Equilibrium constants K^{M}_{ML} and K^{M}_{MA}, for the binary complexes ML and MA, were determined using (1) and (2). Mixed ligand titration curves (figure 1f, g) of the Co(II), Cd(II)–cysteine methylester–histidine methylester system in 1:1:1 ratio at 35°C, showed an inflection at m = 4 (where m is moles of base added per mole of metal ion), followed by a buffer region. This indicates that the sulphur and amino nitrogen, and imidazole and amino nitrogens of Cysme and Hisme respectively are involved in metal ion coordination. Similar trends were observed for Zn(II) [18] and for all the metal ions at other temperatures also. Therefore it was assumed that 1:1:1 ternary complexes are formed in the buffer region between m = 0 and 4.

Values of the constant log b^{M}_{MLA} were calculated using (3) and are listed in table 1. These were subjected to refinement considering all possible species in solution. The 2:1 complexes with respect to ligand and metal are not taken into account as they are known to redistribute into ternary complexes,

$$ML_2 + MA_2 \rightleftharpoons 2MLA.$$

Stability of the ternary system as compared to the corresponding binary systems is measured in terms of $\Delta\log K$. 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, while if the values for $\Delta\log K$ are negative, the reverse holds good [19]. However, the negative values of $\Delta\log K$ do not preclude the formation of ternary complexes in solution. Thus the $\Delta\log K$ values clearly emphasize the amount of extra stabilization in these complexes. $\Delta\log K$ values for the Cd(II) system are given in table 1. $\Delta\log K$ values for the Co(II) system could not be compiled owing to the formation of different types of complexes in binary and ternary systems. The high positive values of these systems are significant

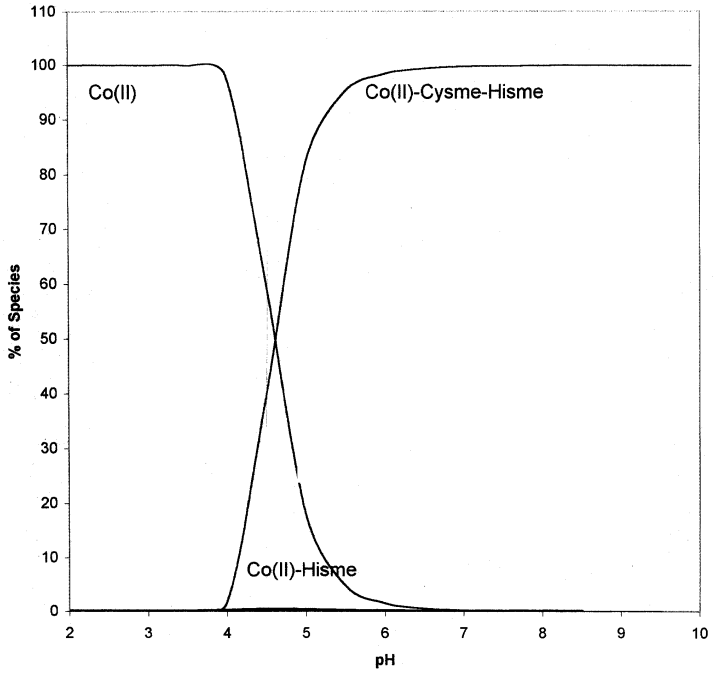

Figure 2. Species distribution curve for Co(II)–Cysme–Hisme (1:1:1 system).

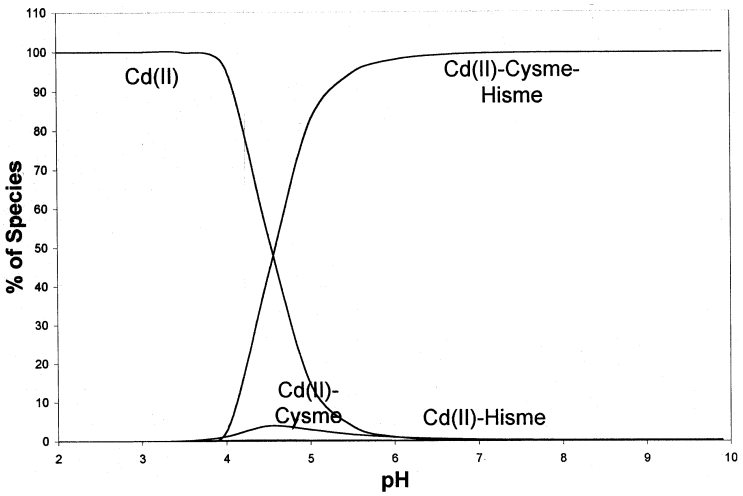

Figure 3. Species distribution curve for Cd(II)–Cysme–Hisme (1:1:1 system).

since the Δlog *K* values for ternary complexes of Zn(II) or Co(II) with cysteine and histidine range from the less positive to the negative [20]. This is also evident from the species distribution curves (figures 2 and 3) of cobalt and cadmium systems. It may be seen from figures 2 and 3 that the M(II)–Cysme – Hisme species reaches almost 100% at

biological *p*H, while 1:1 M(II)–Cysme and M(II)–Hisme species are almost negligible. It may also be seen from the table that zinc forms more stable complexes compared to cobalt and cadmium with Cysme and Hisme ligands, the order being Zn(II)>Cd(II)> Co(II). This may be explained on the basis of the changes in their ligand field stabilization energies (LFSE) which provide the rationale for the enhanced stability of the zinc system [21]. For example, metal ligand interactions are accompanied by a transition from an octahedral environment in the hexa aquo complex to a tetrahedral environment in the metal–ligand complex. Such a process involves changes in the LFSE, the energy associated with differential destabilization and occupation of *d* orbitals in a complex with a particular geometry [22]. For the d^7 Co^{2+} ion in a high spin octahedral complex, the LFSE is $\approx -4/5\ \Delta o$, whereas for a tetrahedral complex it is $\approx -6/5\ \Delta t$, where Δo and Δt are the splittings between the sets of *d* orbitals in octahedral and tetrahedral ligand fields respectively. For the corresponding d^{10} Zn^{2+} complexes the LFSE values are zero since this ion has a completely filled *d* shell. The same is the case with the Cd^{2+} ion. This might be the reason for the comparable stabilities of zinc and cadmium systems and the lower stability of the cobalt system. A tentative structure for the ternary complexes is depicted in figure 4.

A better picture emerges from the thermodynamic data (table 2) of the above systems. The enthalpy and entropy values for Zn(II), Cd(II) and Co(II) systems decrease in the order Zn(II)>Cd(II)>Co(II). The high negative enthalpy and entropy values are indicative of substantial contributions from the former towards stabilization. The entropy values indicate a high degree of solvation of the complex. These effects manifest in the

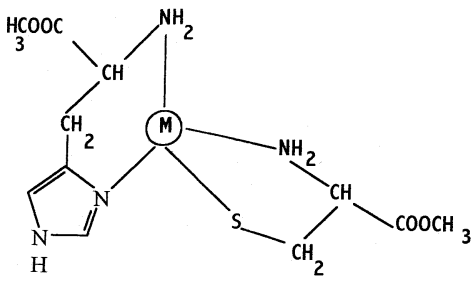

Figure 4. Coordination of ligands around M(II) in the model.

Table 2. Thermodynamic parameters* for the interaction of M(II)–cysteine methylester and histidine methylester systems at different temperatures; μ = 0·1 mol dm^{-3} (KNO_3).

System	$\Delta H_f°$ (kJ mol^{-1})	$\Delta G_f°$ (kJ mol^{-1})	$\Delta S_f°$ (J mol^{-1} deg^{-1})
Co(II)–Cysme	–	–	–
Co(II)–Hisme	–54·29	–27·16	–91·04
Co(II)–Cysme–Hisme	–134·14	–91·75	–142·23
Cd(II)–Cysme	–166·06	–53·46	–377·85
Cd(II)–Hisme	–60·88	–26·42	–115·66
Cd(II)–Cysme–Hisme	–174·74	–97·05	–260·69
Zn(II)–Cysme–Hisme	–176·83	–97.51	–266.18

*The values are accurate to ± 0.01

desolvation of the metal ion and its binding site, as the metal ion binds to the ligands, the entropy gain of solvent release is not significant since the complex is ionic. Thus the entropy loss from the metal-binding site organization is expected to surpass the entropy gain of metal desolvation. 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 [23–26]. Therefore, it is clear that the Zn(II)–Cysme–Hisme system which mimics the zinc core in TF III A is stabilized considerably compared to other systems. This indicates a functional role for zinc.

It is well-known that zinc enzymes lose their catalytic activities on removal of zinc metal. However, the activity is regained when Co(II) is added. But in transcription factor proteins it is clear that Zn(II) cannot be substituted by other metal ions, since it forms the most stable complexes as compared to other metal ions. This is evident from model studies [21,27]. In fact, nature seems to have followed the stability criteria in selecting zinc over other metal ions. Zinc may have been selected not because it is more abundant and readily available, but because of its basic ability or potential to stabilize the core for specific biological objectives.

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