

Inter-ligand stacking interactions in some *o*-phenanthroline amino acid biligand complexes

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Abstract. Biligand complexes of the type metal/*o*-phenanthroline/amino acid, where metal = Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} ; and amino acid = β -phenyl alanine or tryptophan have been found to show inter-ligand stacking interactions in aqueous solutions. This interaction has been evaluated quantitatively from a knowledge of the formation constants of these complexes (and that of the identical biligand complex with α -alanine) as determined by potentiometric pH titrations at 25°C and an ionic strength, $I = 0.2$ (mol dm^{-3} , KNO_3) by following the Irving-Rossotti approach. The sequence of interaction is tryptophan > β -phenyl alanine.

Keywords. Stacking interactions; biligand metal-phenanthroline-amino acid complexes.

1. Introduction

Stacking interactions in biligand complexes are included in general category of hydrophobic interactions (Sigel and Fischer 1980). The hydrophobic effect is the most important single factor which governs the organization of the constituent molecules of living matter into complex structural entities (Tanford 1973). These inter-ligand interactions enhance the stability of the biligand complexes. Since *o*-phenanthroline is structurally similar to porphyrins, its simultaneous bonding with biochemically important metal ions and amino acids make a useful study. This has prompted us to design the present ternary systems of the type [MAL], where $M = \text{Co}^{2+}$, Ni^{2+} , Cu^{2+} , Zn^{2+} or Cd^{2+} ; $A = o$ -phenanthroline (*phen*); $L = \alpha$ -alanine (α -*ala*), β -phenyl alanine (β -*phe*) or tryptophan (*try*). An attempt has been made to demonstrate quantitatively the extent upto which extrastabilization is caused due to stacking.

2. Experimental

All chemicals used were of standard purity (BDH analar/Merck GR). The solutions were prepared in double distilled water. The strength of the metal solutions was adjusted finally after their complexometric titrations with EDTA (West 1969). The metal and ligand solutions of 1.0×10^{-3} mol dm^{-3} (final)

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concentration and an NaOH solution of 0.2 mol dm^{-3} concentration were used. The formation constants were determined by means of potentiometric pH-titrations in aqueous medium at 25°C and an ionic strength, $I = 0.2$ (mol dm^{-3} , KNO_3) by following the Irving-Rossotti approach (Irving and Rossotti 1953, 1954; Bhattacharya and Chidambaram 1970). An Elico digital pH-meter (model LI-120) was used for pH measurements. The titrations were repeated to obtain reproducible pH data. The proton-ligand formation constants ($\log K_n^H$) were redetermined in order to get their values under experimental conditions. These were used in the evaluation of metal-ligand formation constants ($\log K_{\text{ML}}^M$ and $\log K_{\text{MAL}}^{\text{MA}}$). The formation constants were refined by the method of linear plots (Rossotti and Rossotti 1955) and also subjected to statistical refinement by the 'Q-test' (Pecsok *et al* 1976).

3. Results and discussion

The calculated values of formation constants and $\Delta \log K$ ($= \log K_{\text{MAL}}^{\text{MA}} - \log K_{\text{ML}}^M$) are presented in table 1. Figures in parentheses represent standard deviations. It may be seen that the $\Delta \log K$ values with α -ala are uniformly negative with all the metal ions, but with β -phe these values are less negative or even positive (with Cu^{2+}), and with *try* all the values are positive. Less negative or positive values of $\Delta \log K$ indicate greater stabilization of the biligand complexes. This is significant as normally one would expect the β -phe and *try* biligand complexes to be less stable ($\Delta \log K$ more negative) because of their bulkier side chains ('phenyl' with β -phe and 'indole' with *try*). Greater stabilization

Table 1. Formation constants of the binary ML and ternary MAL complexes. Temperature = 25°C Ionic strength, $I = 0.2$ (mol dm^{-3} , KNO_3)

Formation constant	Co^{2+}	Ni^{2+}	Cu^{2+}	Zn^{2+}	Cd^{2+}
<i>α-ala</i>					
$\log K_{\text{ML}}^M$	4.71 (± 0.03)	5.83 (± 0.02)	7.83 (± 0.03)	5.29 (± 0.02)	4.85 (± 0.02)
$\log K_{\text{MAL}}^{\text{MA}}$	4.34 (± 0.02)	5.28 (± 0.02)	7.17 (± 0.02)	4.94 (± 0.03)	4.58 (± 0.01)
$\Delta \log K$	-0.37	-0.55	-0.66	-0.35	-0.27
<i>β-phe</i>					
$\log K_{\text{ML}}^M$	3.89 (± 0.03)	4.83 (± 0.03)	7.34 (± 0.02)	4.57 (± 0.03)	4.21 (± 0.01)
$\log K_{\text{MAL}}^{\text{MA}}$	3.60 (± 0.01)	4.54 (± 0.01)	7.60 (± 0.03)	4.35 (± 0.02)	4.04 (± 0.03)
$\Delta \log K$	-0.29	-0.29	+0.26	-0.22	-0.17
<i>try</i>					
$\log K_{\text{ML}}^M$	4.10 (± 0.03)	5.50 (± 0.03)	8.08 (± 0.03)	4.79 (± 0.02)	4.33 (± 0.02)
$\log K_{\text{MAL}}^{\text{MA}}$	4.50 (± 0.02)	5.75 (± 0.01)	8.69 (± 0.02)	5.11 (± 0.02)	4.59 (± 0.03)
$\Delta \log K$	+0.40	+0.25	+0.61	+0.32	+0.26

Note: Values in parentheses are standard deviations.

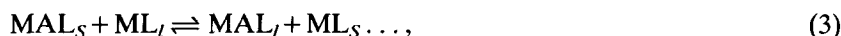
of these complexes is a consequence of inter-ligand stacking interaction in the β -*phe* and *try* biligand complexes. The extrastabilization caused due to this may be worked out from a knowledge of the experimental values of $\log K_{MAL}^{MA}$ and $\Delta \log K$. The quantity $\Delta \log K$ may be regarded as an equilibrium constant for the reaction



Another stability quantifying parameter $\Delta \Delta \log K$ may be defined as

$$\Delta \Delta \log K = \Delta \log K_1 - \Delta \log K_S, \quad (2)$$

where $\Delta \log K_1$ and $\Delta \log K_S$ stand for the experimental values of $\Delta \log K$ with the 'large-sized' (β -*phe*, and *try* in the present case) and 'small-sized' (here α -*ala*) ligands, respectively. The calculated values of $\Delta \Delta \log K$ are reported in table 2; positive and significant values qualitatively indicate extrastabilization due to stacking interaction between the primary and secondary ligands within the ternary system. $\Delta \Delta \log K$ may also be regarded as equilibrium constant for the reaction



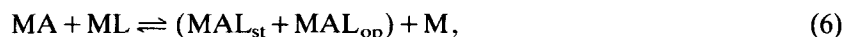
involving ligand selectivity. The subscripts 'I' and 'S' mean the same as defined above. We may conceive of the existence of an equilibrium of the type



(where MAL_{op} = 'open' and MAL_{st} = 'stacked' forms of MAL) in view of the fact that the $\Delta \Delta \log K$ values are quite small as compared to $\log K_{MAL}^{MA}$ suggesting that rather weak forces are involved in stacking. The equilibrium constant, K_I corresponding to this equilibrium will evidently be dimensionless and may be written as

$$K_I = [MAL_{st}]/[MAL_{op}], \quad (5)$$

Equation (1) can be written in two ways,



and



for the existence or absence of stacking interaction, respectively. These would in

Table 2. $\Delta \Delta \log K$ values, equilibrium constant (K_I), and percentage of stacked form (% MAL_{st}) in the ternary systems.

System	Property	Co ²⁺	Ni ²⁺	Cu ²⁺	Zn ²⁺	Cd ²⁺
<i>M. phen.</i> (β - <i>phe</i> / α - <i>ala</i>)	$\Delta \Delta \log K$	+0.08	+0.26	+0.92	+0.13	+0.10
	K_I	0.20	0.81	7.31	0.34	0.25
	(%) MAL_{st}	24.00	44.00	87.96	25.00	31.25
<i>M. phen.</i> (<i>try</i> / α - <i>ala</i>)	$\Delta \Delta \log K$	+0.77	+0.80	+1.27	+0.67	+0.53
	K_I	4.88	5.30	17.62	3.67	2.38
	(%) MAL_{st}	82.99	84.12	94.62	78.58	70.40

turn lead to two modified values of $\Delta \log K$ (corresponding to (6) and (7), respectively) as

$$\Delta \log K_{\text{exp}} = [\text{MAL}_{\text{st}} + \text{MAL}_{\text{op}}] [\text{M}]/[\text{MA}] [\text{ML}], \quad (8)$$

(subscript 'exp' stands for 'experimental') and

$$\Delta \log K_{\text{op}} = [\text{MAL}_{\text{op}}] [\text{M}]/[\text{MA}] [\text{ML}]. \quad (9)$$

Assuming the $\Delta \log K_{\text{op}}$ value as equal to the experimental $\Delta \log K$ value for the 'small-sized' ligand, combination of (2), (5), (8) and (9) and rearrangement of terms gives K_I in terms of $\Delta \Delta \log K$ as

$$K_I = 10^{\Delta \Delta \log K} - 1. \quad (10)$$

The percentage of stacked form (% MAL_{st}) may be expressed as

$$(\%) \text{MAL}_{\text{st}} = [K_I/(1 + K_I)] \times 100. \quad (11)$$

The values of K_I and (% MAL_{st}) for the β -*phe* and *try* biligand complexes (in comparison to the α -*ala* biligand complex regarding the $-\text{CH}_3$ side-chain in α -*ala* as negligible for the present purpose) have been calculated using (10) and (11) above and shown in table 2. These values show greater stacking interaction with *try* than with β -*phe*. It seems that the larger indole moiety in *try* as compared to a smaller phenyl moiety in β -*phe* is responsible for the sequence *try* > β -*phe*. For the same reason perhaps the variations in K_I and (% MAL_{st}) values are larger with β -*phe* than with *try*.

The data on energetics of the formation of biligand complexes and their extrastabilization have been recorded in table 3. The free-energy changes ΔG_1 and ΔG_2 correspond to $\log K_{\text{MAL}}^{\text{MA}}$ and $\Delta \Delta \log K$ values, respectively. The extent of extrastabilization may be expressed as

$$(\%) \text{stabilization} = (\Delta G_2/\Delta G_1) \times 100. \quad (12)$$

The biligand complexes with *try* and β -*phe* are extrastabilized by about 0.10 to 1.73 kcal mole⁻¹. This range agrees well with the theoretical calculations on inter-ligand (intramolecular) interactions (Scheraga 1979). The (%) stabilization values lie in the range 2.2 to 17.1%; a similar observation has been reported (Sigel

Table 3. Energetics of complexation : ΔG_1 ($= -2.303 RT \cdot \log K_{\text{MAL}}^{\text{MA}}$) and ΔG_2 ($= -2.303 RT \cdot \Delta \Delta \log K$) values-(in kcal mole⁻¹) and (%) stabilization of the ternary complexes.

System	Property	Co ²⁺	Ni ²⁺	Cu ²⁺	Zn ²⁺	Cd ²⁺
<i>M. phen.</i>	$-\Delta G_1$	4.90	6.18	10.35	5.92	5.50
(β - <i>phe</i> / α - <i>ala</i>)	$-\Delta G_2$	0.1090	0.3543	1.2539	0.1771	0.1363
<i>M. phen.</i> β - <i>phe</i>	(%) Stabilization	2.22	5.73	12.11	2.99	2.47
<i>M. phen.</i>	$-\Delta G_1$	6.13	7.83	11.84	6.96	6.25
(<i>try</i> / α - <i>ala</i>)	$-\Delta G_2$	1.0495	1.0904	1.7310	0.9132	0.7223
or <i>M. phen.</i> <i>try</i>	(%) Stabilization	17.10	13.92	14.61	13.12	11.55

and Fischer 1980) for the biligand *phen* complexes with aliphatic amino acids like leucine, valine, isoleucine and iso-valine. The (%) stabilization for Cu^{2+} shows a somewhat smaller value with the *try* systems, which may perhaps be due to its tendency to prefer a square planar configuration.

Another significant aspect of the interaction seems to be the nature of the metal ion which plays the role of a bridge in the stacked biligand complexes. The geometry of the complex and the mode of bonding of the amino acid (through the two equatorial or one equatorial and one axial positions) may also affect the extent of interaction. In the present case stacking interaction has been noticed with all the metal ions under study (Co^{2+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+}) but by and large greater interaction occurs with Cu^{2+} . A recent report (Sigel *et al* 1985) on such interactions with phenyl acetate (or propionate) also shows the sequence $\text{Cu}^{2+} > \text{Zn}^{2+}$

It may reasonably be concluded from the present studies that in the biosystems (biligand complexes of the type metal ion/enzyme/ATP, etc.) the properties of ternary complexes may be, to a good extent, the consequence of stacking interactions.

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