Discriminating tendencies of cytidine and uridine towards stacking interactions – A phenomenon of biological significance

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Interaction of bivalent (Cu, Ni, Zn, Co, Mg and Ca) and trivalent (La, Pr, Nd, Gd, Dy and Y) metal ions with pyrimidine nucleosides (cytidine and uridine) in the presence of amino acids (alanine, phenylalanine and tryptophan) has been investigated by potentiometric pH measurements at 35°C and 0.10 mol dm^{-3} (KNO$_3$) ionic strength. Influence of charge on and the discriminating tendencies of these nucleosides towards stacking interactions have been discussed. Stabilization and destabilization of these ternary complexes are quantified in terms of Δlog K values. No interaction is observed with Mg(II) and Ca(II).

In recent years interest has been growing in the study of metal ion interactions with nucleic acids[1-3] since they lead to physiological effects which can be either beneficial[4] or harmful[5]. Further, nucleic acids and proteins recognise each other by very specific and selective biological interactions through amino acid side chains and nucleic acid constituents[6,7]. Most of these reactions are mediated through a metal ion resulting in the formation of ternary complexes[8,9]. Study of these complexes in solution has become very important as they act as models for many metalloenzyme reactions.

In the ternary systems there exist two types of complexes: (i) complexes in which interligand interactions occur and (ii) complexes in which there is no such interaction. The interligand interactions are effective in the formation and stabilization of ternary complexes in solution[10-15] and become much more important when the ligands participate in stacking interactions – a phenomenon of biological significance. The stacking interactions are neither automatic nor universal, but vary from metal ion to metal ion and ligand to ligand emphasizing the specificity and selectivity of these interactions. Therefore, it is important to gather more information on the ternary systems with a variety of metal ions and ligands (primary and secondary) to gain an insight into the nature of such interactions *in vivo*.

In the present paper a detailed investigation has been carried out on the interaction of some bivalent (Cu, Ni, Zn, Co, Mg and Ca) and trivalent (La, Pr, Nd, Gd, Dy and Y) metal ions with pyrimi-dine nucleosides (cytidine and uridine) and amino acids (alanine, phenylalanine and tryptophan) to assess the influence of donor atoms and size/ charge effects on the stacking interactions which eventually affect the structure and stability of the ternary complexes in solution.

Materials and Methods

Cytidine, uridine, α-alanine, α-phenylalanine and α-tryptophan were obtained from Sigma Chemical Company (USA). For every titration, fresh solid ligand was weighed out into the reaction cell to avoid possible concentration effects. Transition metal ions were of AR grade and all rare earth oxides were of Johnson Mathey's spectral grade. All these metal ions were standardized with disodium salt of EDTA[16]. Carbonate free sodium hydroxide was prepared and standardized by titration with potassium acid phthalate[17].

The experimental method consisted of potentiometric titration of the free ligands and of solutions containing metal ions nucleosides (cytidine and uridine) and amino acids (alanine, phenylalanine and tryptophan) in a 1:1:1 ratio at 35 ± 0.1°C with standard sodium hydroxide solution. For binary systems, a 1:1 ratio was maintained. The experimental conditions maintained were the same as those described earlier[15].

Calculations

The ionization constants of various ligands were calculated using the computer program PKAS[18]. All the formation constants were subject-

ed to refinement using the computer program BEST[19]. BEST was also used to generate the complete species distribution curves at various *p*H values. The refinement of the stability constants of ternary systems was done by considering all possible species present in the solution, i.e. HL^+, HA, L^-, L, A^-, ML, ML_2, MA, MA_2 and MAL. The refined values for these complexes thus obtained are given in Tables 1-4. The error limits in these constants were minimised (sigma fit = 0.001).

Results and Discussion

*Metal (II)-cytidine-amino acid (1:1:1) systems.*The interaction of metal ions [Cu(II), Ni(II), Zn(II) and Co(II)] with cytidine and amino acids (alanine, phenylalanine and tryptophan) resulted in the simultaneous formation of mixed ligand complexes in solution. This was further confirmed by the species distribution curves (Fig. 1). All the constants so calculated are listed in Table 2.

Metal (II)-uridine-amino acid (1:1:1) systems. Formation of ternary complexes in these ternary systems occurred in a step-wise manner. This is also supported by species distribution curves. The stability constants for these systems are presented in Table 3.

In Table 1 are given the ionization constants of ligands along with stability constants of the binary complexes (1:1) with bivalent and trivalent metal ions. Though the binary data is available in literature, we have determined the values under the ex-

perimental conditions used for the measurement of stability constants of the ternary complexes because even slight variations in binary data can have a notable effect on the $\Delta \log K$ values ($\Delta \log K$ is the difference between the overall stability constants of 1:1:1 ternary complexes and the corresponding constants for 1:1 binary complexes). However, there was an excellent agreement with the literature data[20-23].

Table 2 – Stability constants of M(II)-cytidine-amino acid (1:1:1) systems

[Temp. = 35°C; μ = 0.10 M(KNO$_3$)]

Metal ion (II) (M)	M-cyt-ala (1:1:1) K_{MLA}^M	M-cyt-phe (1:1:1) K_{MLA}^M	M-cyt-tryp (1:1:1) K_{MLA}^M
Cu	9.89	9.92	10.24
Ni	6.04	6.18	6.53
Zn	5.06	5.31	5.57
Co	5.22	5.05	5.62

Table 3 – Stability constants of M(II)-uridine-amino acid (1:1:1) systems

[Temp. = 35°C; μ = 0.10 M(KNO$_3$)]

Metal ion (II) (M)	M-urd-ala (1:1:1) K_{MAL}^{MA}	M-urd-phe (1:1:1) K_{MAL}^{MA}	M-urd-tryp (1:1:1) K_{MAL}^{MA}
Cu	3.73	3.71	3.70
Ni	2.51	2.49	2.48
Zn	2.08	2.05	2.04
Co	1.87	1.84	1.83

Table 1 – Formation constants* of various me .al ion-ligand (1:1) systems

[Temp. 35°C; μ = 0.10 M(KNO$_3$)]

Metal ion (M)	M-cytidine (*pKa* = 4.15 ± 0.03) $\log K_{ML}^M$	M-uridine (*pKa* = 9.01 ± 0.03) $\log K_{ML}^M$	M-alanine (*pKa* = 9.37 ± 0.03) $\log K_{MA}^M$	M-phenylalanine (*pKa* = 8.59 ± 0.03) $\log K_{MA}^M$	M-tryptophan (*pKa* = 9.13 ± 0.03) $\log K_{MA}^M$
Cu(II)	2.06	4.00	8.13	7.82	7.97
Ni(II)	0.97	2.77	5.32	5.10	5.37
Zn(II)	0.76	2.35	4.50	4.42	4.60
Co(II)	0.88	2.13	4.59	4.06	4.55
La(III)	2.30	4.10	4.93	4.71	5.02
Pr(III)	2.36	4.15	5.05	4.80	5.10
Nd(III)	2.43	4.21	5.11	4.89	5.18
Gd(III)	2.50	4.26	5.19	4.98	5.25
Dy(III)	2.57	4.32	5.30	5.09	5.33
Y(III)	2.70	4.49	5.42	5.27	5.48

* constants are accurate upto ± 0.03 log *K* units

It may be seen from the Tables 2-4 that the trivalent metal ions form more stable complexes (both binary and ternary systems) compared to the bivalent metal ions. These differences may be explained on the basis of the charge/size ratios of the respective metal ions. Lanthanides being smaller in size will permit a closer approach of the ligands resulting in the formation of more stable complexes.
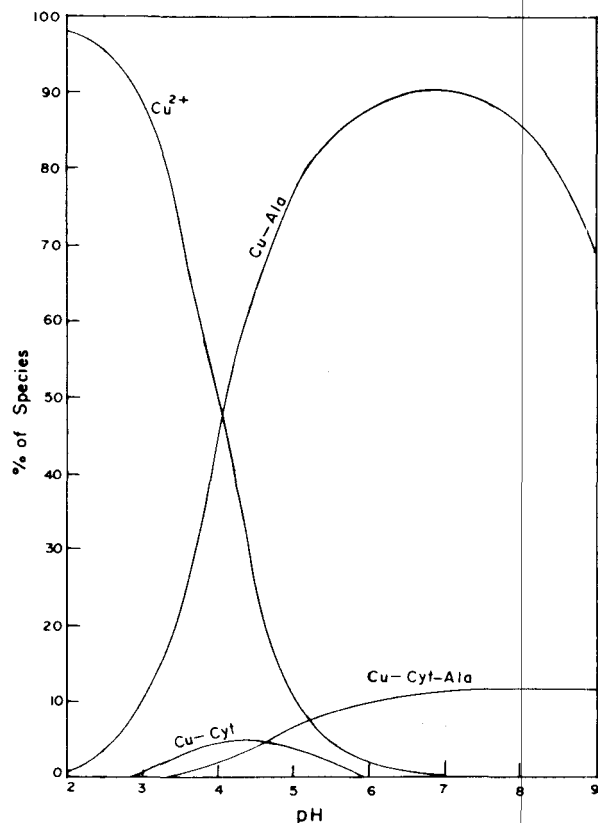

Fig. 1 – Species distribution curves of 1:1:1 Cu(II)-cytidine-alanine system

Table 5 lists the $\Delta \log K$ values of various systems under investigation. The $\Delta \log K$ values provide an insight into the various factors responsible for the formation and stabilization of ternary complexes in solution. Positive and negative $\Delta \log K$ values symbolize the stabilization and destabilization effects respectively. However, it should be noted here that the destabilization effects do not preclude the formation of ternary complexes. It can be seen from Table 5 that the $\Delta \log K$ values of metal-cytidine or -uridine complexes with amino acids increase in the order: alanine < phenylalanine < tryptophan indicating the dependance of the stabilities on the aromatic ring size. This is in agreement with the recent conclusions reached by Leporti and Odani and Yamauchi[24,25]. The $\Delta \log K$ values for the metal-cytidine-alanine systems are negative whereas they are positive for phenylalanine and tryptophan systems.

It is reasonably well established[12-14,26] that apart from the various factors like nature of metal ion and the ligand, geometry of the metal complex etc. affecting the stability of complexes, the stacking interaction (wherever possible) seem to be most effective in stabilizing the ternary complexes in solution. Thus, alanine being an aliphatic ligand cannot participate in stacking interactions resulting in the negative $\Delta \log K$ values. However, in case of phenylalanine and tryptophan systems, the aromatic moieties of these ligands are oriented in such a way that they are exactly parallel to cytidine ring which is perpendicular to metal plane. This results in a favourable stacking interaction (Structure I) and hence positive $\Delta \log K$ values for these systems.

The $\Delta \log K$ values of the uridine complexes are negative for all the systems studied. Uridine is unique among the nucleosides in the sense that its

Table 4 – Stability constants of the M(III)-cytidine/uridine-amino acids in 1:1:1 ratio

[Temp. = 35°C; $\mu = 0.10\ M(KNO_3)$]

Metal ion (III) (M)	M-cyt-ala (1:1:1) $\log K_{MLA}^{ML}$	M-cyt-phe (1:1:1) $\log K_{MLA}^{ML}$	M-cyt-tryp (1:1:1) $\log K_{MLA}^{ML}$	M-urd-ala (1:1:1) $\log K_{MAL}^{M}$	M-urd-phe (1:1:1) $\log K_{MAL}^{M}$	M-urd-tryp (1:1:1) $\log K_{MAL}^{M}$
La	4.73	4.77	5.11	8.73	8.48	8.77
Pr	4.85	4.87	5.19	8.90	8.62	8.89
Nd	4.91	4.96	5.28	9.01	8.77	9.03
Gd	4.99	5.05	5.35	9.14	8.90	9.15
Dy	5.09	5.16	5.43	9.31	9.07	9.29
Y	5.20	5.35	5.59	9.59	9.41	9.60

Table 5 − Δ log K values of various metal-ligand systems in solution
[Temp. = 35°C; μ = 0.10 M (KNO$_3$)]

Metal ion (M)	M-cyt-ala	M-cyt-phe	M-cyt-tryp	M-urd-ala	M-urd-phe	M-urd-tryp
Cu(II)	− 0.30	+ 0.10	+ 0.18	− 0.27	− 0.29	− 0.30
Ni(II)	− 0.25	+ 0.11	+ 0.19	− 0.26	− 0.28	− 0.29
Zn(II)	− 0.20	+ 0.13	+ 0.21	− 0.27	− 0.30	− 0.31
Co(II)	− 0.25	+ 0.11	+ 0.19	− 0.26	− 0.29	− 0.30
La(III)	− 0.20	+ 0.06	+ 0.09	− 0.30	− 0.33	− 0.35
Pr(III)	− 0.20	+ 0.07	+ 0.09	− 0.30	− 0.33	− 0.36
Nd(III)	− 0.20	+ 0.07	+ 0.10	− 0.31	− 0.33	− 0.36
Gd(III)	− 0.20	+ 0.07	+ 0.10	− 0.31	− 0.34	− 0.36
Dy(III)	− 0.21	+ 0.07	+ 0.10	− 0.31	− 0.34	− 0.36
Y(III)	− 0.22	+ 0.08	+ 0.64	− 0.32	− 0.35	− 0.37
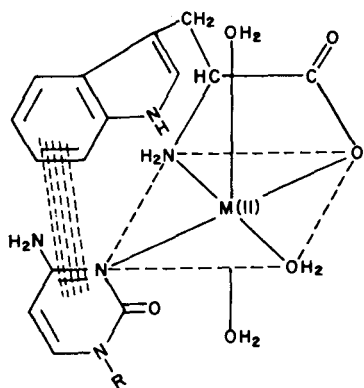
Structure (I) − Tentative structure of M(II)-cytidine-tryptophan, showing stacking interaction, (R = ribose).

metal binding capacity depends on the pH of the reacting medium. In acidic medium, it coordinates with metals through 0(4)[27] and in neutral or slightly basic medium through N(3),[28] since there is little competition from protons. In neutral medium the concentration of negative charge on the oxygen atoms approximately equals that on the ring nitrogen atom[29]. However, on coordination to metal the charge distribution pattern is altered. In the case of Cu(II)-uracilate system[30] it was concluded on the basis of the extended Huckel M.O. calculations, that Cu(II) alters the charge distribution pattern of practically all the atoms of uracil. The main effect seems to be the withdrawal of electron density from the negatively charged nitrogens and positively charged carbons with exocyclic oxygens remaining relatively unaltered[31]. This results in Cu(II) lying in the plane of (not perpendicular) the uracilate ring. In uridine,

though an additional ribose moiety is present similar situation is expected in the two systems as the metal coordinates through N(3). Thus, the uridine ring, being non-perpendicular to the metal plane, cannot take part in stacking interactions with aromatic moieties of phenylalanine and tryptophan. This may be the reason for the negative Δ log K values of these systems.

The Δ log K values of rare earth metal complexes are less positive than those of the transition metal complexes. This shows that the ternary complexes of trivalent metal ions are less stabilized as compared to those of the bivalent metal ions. The relatively lower stability of ternary complexes of trivalent metal ions may be due to the (i) formation of more stable binary complexes and (ii) expansion in coordination number of these metal ions which may affect the proximity of the ligands influencing the stacking interactions.

The above results emphasize the importance of stacking interactions and show how the ligands with slight variations may have discriminating tendencies towards it.

Acknowledgement

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