Proc. Indian Acad. Sci. (Chem. Sci.), Vol. 95, Nos 5 & 6, December 1985, pp. 547–555.

Stability of the ternary complexes of inosine in solution

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MS received 10 April 1985; revised 5 July 1985

Abstract. The interaction of Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Mg(II) and Ca(II) with inosine and secondary ligands histidine and glycine in a 1:1:1 ratio was determined by potentiometric equilibrium measurements at 35°C and 0·10 M (KNO_3) ionic strength. The stability constants of the ternary complexes of xanthosine with histidine and glycine are also included for effective comparison. The ternary complexes were found to be stabler than the corresponding binary complexes. This higher stability of the ternary complexes is measured in terms of Δ log K which is the difference between the overall 1:1:1 stability constant and the corresponding 1:1 constant. Histidine forms stabler ternary complexes with inosine than does glycine. This is attributed to the π-accepting capacity of the imidazole ring of histidine and also to the stacking interaction between the purine part of inosine and the imidazole moiety of histidine.

Keywords. Inosine; histidine; glycine; ternary complexes; stacking interactions.

1. Introduction

During recent years considerable attention has been focussed on the ternary complexes of nucleosides and nucleotides in solution, due to their importance in many biological processes (Taqui Khan and Rabindra Reddy 1972, 1973; Rabindra Reddy *et al* 1976, 1978, 1979; Fischer and Sigel 1980; Scheller *et al* 1981; Arena *et al* 1983; Sigel *et al* 1983). Under biological conditions many potential ligands are likely to compete for metal ions and, as such, multiligand equilibria exist in solution and hence mixed ligand or higher order complexes are formed. Consequently, the determination of the stability of the ternary complexes will help in understanding the specific and selective interactions that occur in many biochemical reactions. Although many published data are available on the metal complexes of purine and pyrimidine bases and nucleotides (Izatt *et al* 1971; Taqui Khan and Rabindra Reddy 1972, 1973; Taqui Khan and Krishnamoorthy 1974; Taqui Khan and Rabindra Reddy 1975, 1976; Sigel 1975; Fischer and Sigel 1980; Arena *et al* 1983; Sigel *et al* 1983; Lippert 1983; Rabindra Reddy *et al* 1983a, b), very little is known about nucleoside complexes in solution. Nucleosides provide a link between bases and nucleotides, and hence any information on complexation properties of nucleosides will be worthwhile. In view of this, we systematically investigated the interaction of metal ions with nucleosides in solution, in the hope of evolving a better understanding of the base versus phosphate binding.

Earlier work on nucleosides is mainly restricted to xanthosine (Rabindra Reddy *et al* 1976, 1978, 1979, 1984; Rabindra Reddy and Harilatha Reddy 1983, 1985; Rabindra

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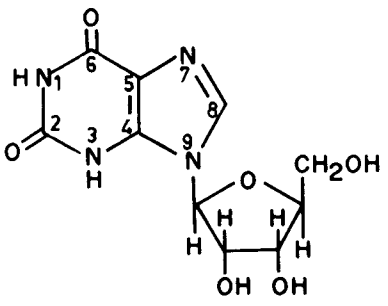
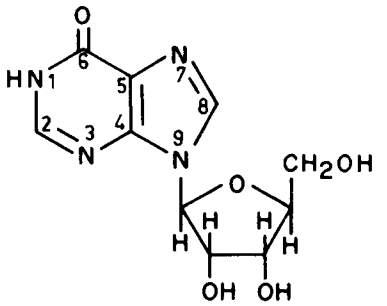


XANTHOSINE



INOSINE

Reddy and Venugopal Reddy 1983) and this study has provided useful information on the nature of metal nucleic acid interactions. However, the work on inosine was confined only to dissociation constants and the determination of the binary stability constants (Rabindra Reddy *et al* 1983b; Albert 1953; Tu and Friederich 1968). Here we report the data obtained from a detailed physico-chemical investigation of Cu(II), Ni(II), Co(II), Zn(II), Mn(II), Mg(II) and Ca(II) with inosine and biologically important secondary ligands, histidine and glycine, in a 1 : 1 : 1 ratio at 35°C and 0·10 M (KNO_3) ionic strength.

Inosine resembles xanthosine very closely (structures given above). Earlier investigations have shown that xanthosine binds to the metal through O(6) and N(7) (Rabindra Reddy *et al* 1978). No doubt, inosine is also expected to bind through the same sites, but the extent of interaction may vary due to the presence of an additional donor group O(2) in xanthosine. Therefore, any comparison of the stability data for the two systems will be highly informative. Such interactions and comparisons will hopefully pave the way for understanding the metal coordination in nucleosides and thus may help in the correct assignment of the binding sites of nucleic acid components.

2. Experimental

Inosine, histidine and glycine were obtained from the Sigma Chemical Company (USA). Transition and alkaline earth metal ions were of Analar grade and were standardised volumetrically by titration with the disodium salt of EDTA in the presence of a suitable indicator as outlined by Schwarzenbach (1957).

The experimental method employed consisted of a potentiometric titration of metal and inosine with a secondary ligand histidine or glycine, in a 1 : 1 : 1 ratio at 35° ± 0·1°C and 0·10 M ionic strength, with standard NaOH solution. The experimental conditions maintained were similar to those described earlier (Rabindra Reddy *et al* 1984).

3. Calculations

The acid dissociation constants of inosine and diprotonated secondary ligands histidine and glycine were calculated by the usual algebraic method (Rabindra Reddy *et al* 1983b; Rabindra Reddy and Harilatha Reddy 1983, 1985).

To calculate the stability constants of the ternary complexes of Cu(II), Ni(II), Co(II), Zn(II) and Mn(II) with inosine and histidine in a 1:1:1 ratio the following equations were used (excluding the charges):

$$M + HL + H_2A \rightleftharpoons MHLA + 2H^+ \tag{1}$$

together with the related equilibria:

$$M + L + HA \rightleftharpoons MHLA \tag{2}$$

$$K^M_{MHLA} = \frac{T_M - [M]}{[M][L][HA]} \tag{3}$$

where:

$$[M] = a'A \text{ or } b'L$$

$$L = \frac{a'\alpha}{a'b + b'a}; \quad HA = \frac{[H^+]}{K_{2aA}} \cdot A; \; A = \frac{b'\alpha}{a'b + b'a}$$

$$\alpha = (2 - m)T_L - H^+ + OH^-; \; a = \frac{2[H^+]^2}{K_{aA} \cdot K_{2aA}} + \frac{[H^+]}{K_{2aA}};$$

$$a' = \frac{[H^+]^2}{K_{aA} \cdot K_{2aA}} + \frac{[H^+]}{K_{2aA}} + 1; \; b = \frac{2[H^+]}{K_{aL}}; \; b' = \frac{[H^+]}{K_{aL}} + 1$$

T_M = total concentration of the metal ion species in solution;
T_L = total concentration of the ligand species in solution;
$[M]$ = concentration of the unbound metal ion;
m = moles of base added per mole of metal ion;
L = monoanion of inosine;
HA = neutral histidine;

where the subscripts A and L represent histidine/glycine and inosine, respectively. This equation was also used for the ternary complexes of Cu(II) with inosine and glycine.

However, for the ternary complexes of Mg(II) and Ca(II) with inosine and histidine in a 1:1:1 ratio the following equations were used (deleting charges);

$$M + HL + HA \rightleftharpoons MLA + 2H^+ \tag{4}$$

together with related equilibria

$$M + L + A \rightleftharpoons MLA \tag{5}$$

$$K^{MHA}_{MLA} = \frac{T_M - [M]}{[M][L][A]} \tag{6}$$

$$L = \frac{a'\alpha}{a'b + b'a}; \quad A = \frac{b'\alpha}{a'b + b'a}; \quad [M] = a'A \text{ or } b'L$$

$$\alpha = (2 - m)T_L - H^+ + OH^-; \; a = \frac{2[H^+]}{K_{2aA}} + 1; \quad a' = \frac{[H^+]}{K_{2aA}} + 1$$

$$b = \frac{2[H^+]}{K_{aL}} + 1; \quad b' = \frac{[H^+]}{K_{aL}} + 1$$

The above equations were also used for the calculation of the stability constants of

Ni(II), Co(II), Zn(II), Mn(II), Mg(II) and Ca(II) with inosine and glycine in a 1:1:1 ratio.

4. Results and discussion

4.1 *Metal-inosine-histidine system*

The mixed ligand titration curve of Cu(II)-inosine-histidine in a 1:1:1 ratio given in figure 1C shows an inflection at $m = 2$ indicating the simultaneous formation of a 1:1:1 mixed ligand complex in the buffer region between $m = 0$ and 2 (m = moles of base added per mole of the metal ion). The constant K^{M}_{MHLA} was calculated using (3) and presented in table 1. Similar trends were observed for all the metal ion studied except for Mg(II) and Ca(II) in which [figure 1B for Mg(II)] an inflection was obtained at $m = 1$. Accordingly, it was assumed that a ternary complex was formed from the monoprotonated binary (1:1) histidine complex ($m = 0$ and 1) and the constant K^{MHA}_{MLA} was calculated in the buffer region between $m = 1$ and 3, using (6). The constants so calculated are listed in table 1.

4.2 *Metal-inosine-glycine system*

The titration curve of Cu(II) with inosine and glycine in a 1:1:1 ratio (figure 2P) resulted in an inflection at $m = 2$ indicating the simultaneous formation of the mixed

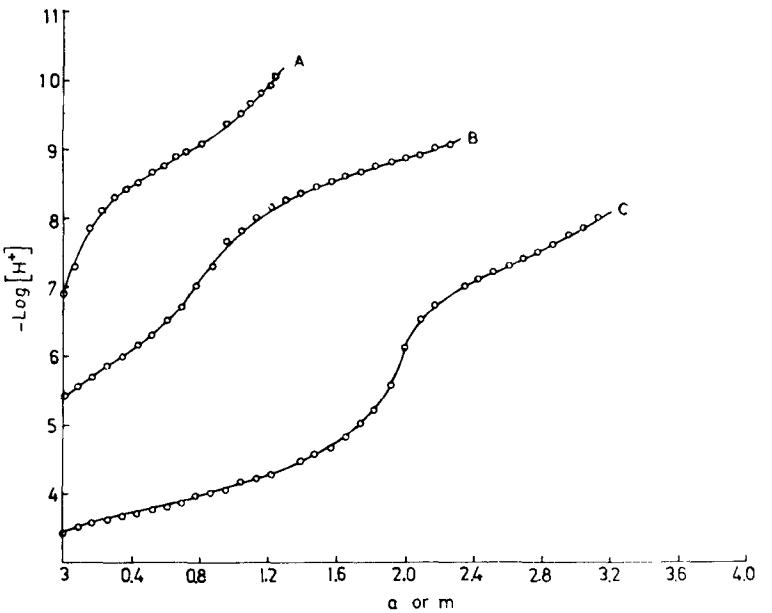


Figure 1. Mixed ligand titration curves for Cu(II)-inosine-histidine and Mg(II)-inosine-histidine in a 1:1:1 ratio at 35°C and $\mu = 0{\cdot}10$ M (KNO_3) [A = Inosine free ligand; B = Mg(II)-inosine-histidine; C = Cu(II)-inosine-histidine; a = moles of base added per mole of ligand (for curve A); m = moles of base added per mole of metal ion (for curves B and C)].

Table 1. Stability constants of the ternary complexes of inosine and xanthosine with histidine and glycine.

	M:inosine:glycine (1:1:1)		*M:xanthosine:glycine (1:1:1)		M:inosine:histidine (1:1:1)		**M:xanthosine:histidine (1:1:1)		$\Delta \log K$	*$\Delta \log K$
M(II)	K^{M}_{MHLA}	K^{MHA}_{MLA}	K^{M}_{MHLA}	K^{MA}_{MHLA}	K^{M}_{MHLA}	K^{MHA}_{MLA}	K^{M}_{MHLA}	K^{MA}_{MHLA}	M:Ino:Gly (1:1:1)	M:Xan:Gly (1:1:1)
Cu	12·57	—	12.8	—	14·55	—	13·19	—	—	1·13
Ni	—	11·23	10·9	—	13·18	—	11·84	—	2·13	2·06
Co	—	10·56	—	3·0	11·60	—	10·11	—	2·22	0·49
Zn	—	10·89	—	2·8	11·47	—	9·05	—	2·60	0·59
Mn	—	10·72	—	2·8	11·68	—	—	2·9	—	0·23
Mg	—	8·52	—	2·5	—	9·13	—	2·79	—	0·27
Ca	—	8·23	—	2·6	—	8·88	—	2·76	—	0·39

μ = 0·10 M (KNO_3); temperature = 35°C; K values are log K; deviations are omitted for clarity.

* Rabindra Reddy and Harilatha Reddy 1983

** Rabindra Reddy and Harilatha Reddy 1985

complex in the buffer region between $m = 0$ and 2 and the monoprotonated constant was calculated using (3); values thus determined are presented in table 1.

In figure 2 Q is the mixed ligand titration curve of Mg(II)-inosine-glycine in a 1:1:1 ratio. This curve indicated an inflection at $m = 1$. Accordingly, it was assumed that a ternary complex was formed from the monoprotonated (1:1) glycine ($m = 0$ and 1) complex, and the constant was calculated in the buffer region between $m = 1$ and 3 with the help of (6). The constants thus calculated are recorded in table 1. Similar results were also obtained for all the other metal ions studied.

The stability constants presented in table 1 show that the ternary complexes are more stable than their corresponding binary complexes, a trend which is not expected based only on the statistical considerations. This is because of the destabilization caused by the ligand repulsion being lower in the ternary complexes than in the binary complexes. This extra stability of the ternary complexes is usually measured in terms of $\Delta \log K$. $\Delta \log K$ is defined as the difference in the overall 1:1:1 stability constants and the corresponding constants of the 1:1 complexes. So the positive values of $\Delta \log K$ show that the ternary complexes are more stable than the binary complexes. The negative values of $\Delta \log K$ generally result from the absence of electronic effects or specific interactions between the two ligands in the ternary system. However, it should be noted that the negative values of $\Delta \log K$ do not preclude the formation of the ternary complexes in solution.

The $\Delta \log K$ values for Ni(II)-inosine-glycine, Zn(II)-inosine-glycine and Co(II)-inosine-glycine systems are reported in table 1. It can be seen from the table that the $\Delta \log K$ values for Zn(II) are more positive indicating the higher stability of Zn(II), as compared to the Ni(II) and Co(II) systems. This higher stability of Zn(II) exemplifies its

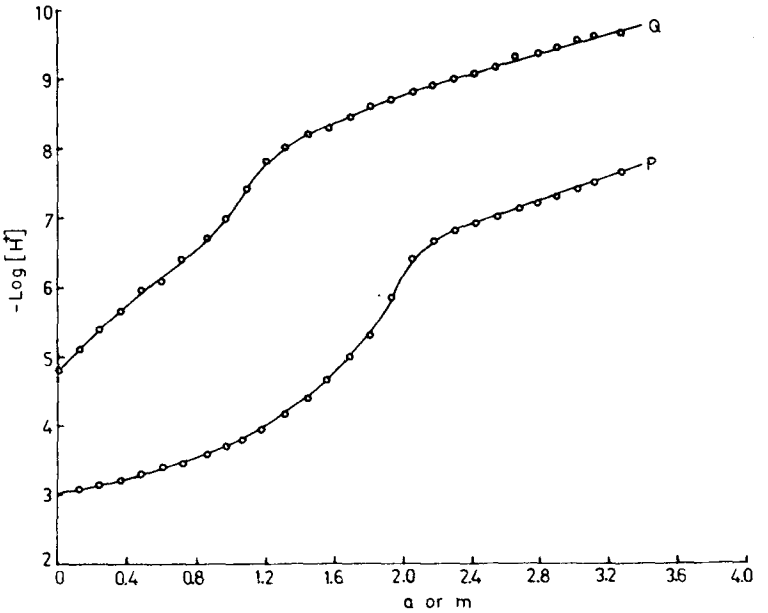


Figure 2. Mixed ligand titration curves of Cu(II)-inosine-glycine and Mg(II)-inosine-glycine in a 1:1:1 ratio at 35°C and $\mu = 0{\cdot}10$ M (KNO_3) [P = Cu(II)-inosine-glycine; Q = Mg(II)-inosine-glycine; m = moles of base added per mole of metal ion].

importance in many biochemical reactions. The $\Delta \log K$ values for the other systems under the present investigation could not be computed owing to the formation of different types of complexes in the ternary and binary systems. Meaningful information can, however, be obtained by comparing the absolute values of the stability constants of the ternary and binary systems. For e.g., the data in table 1 indicate the higher stability of the ternary complexes as compared to their binary counterparts. This enhanced stability of ternary complexes is due to various factors like charge neutralization, electronic or cooperative effects (formation of π bonds), and the stacking interaction (a phenomenon expected to occur between the aromatic moieties of the two ligands).

Also, it was shown in our earlier investigation that in mixed ligand complexes the reaction between the metal ion and the secondary ligands containing heteroaromatic nitrogen and oxygen donor atoms result in the formation of stabler ternary complexes than with the ligands containing pure nitrogen and oxygen donor atoms (Rabindra Reddy and Harilatha Reddy 1983). Although the secondary ligands in the ternary complexes (1:1:1) of both metal-inosine-histidine and metal-inosine-glycine have mixed N/O donor groups, the stabilities of histidine complexes are found to be higher than of the glycine complexes. This is due to (a) the stacking interaction, (b) the π accepting capacity of the imidazole ring of histidine, and (c) involvement of all the available donor sites in metal coordination. Stacking interaction occurs between the purine part of inosine and the imidazole moiety of histidine. This type of interaction was also observed earlier (Scheller *et al* 1981; Rabindra Reddy and Venugopal Reddy 1983; Rabindra Reddy *et al* 1984). The π accepting capacity of the imidazole ring in histidine will influence the transfer of electrons from the metal ion to the imidazole ring and this results in an increase in the positive charge on the metal ion than in the binary complex, and thereby increases the metal interaction in the ternary system. Thus, in the metal-inosine-histidine system a cooperative effect exists between the two aromatic rings resulting in the formation of stabler ternary complexes. Glycine, being an aliphatic ligand, can not participate in stacking interaction and also does not exert any cooperative effect on the aromatic ring of the inosine. This accounts for its lower stability. Also, the difference in the stability constants for the 1:1 Cu-glycine and 1:1 Cu-histidine systems is about 1.4 log K units. The same order of magnitude is observed in the $\Delta \log K$ values of ternary complexes of glycine and histidine with inosine. This clearly shows that histidine does not act like glycine but as a terdentate ligand involving all its available donor atoms in metal binding, otherwise the difference between them would not have been of this order of magnitude (Rabindra Reddy and Harilatha Reddy 1985).

It is of interest here to compare the $\Delta \log K$ values of the metal-inosine-glycine system with those of the metal-xanthosine-glycine system. The $\Delta \log K$ values for the inosine system are more positive than the corresponding complexes of xanthosine (Rabindra Reddy and Harilatha Reddy 1983). This shows that the ternary complexes of inosine are more favoured than the ternary complexes of xanthosine. The same conclusions can also be drawn from the absolute values of their stability constants. This may be due to the presence of an additional donor group O(2) in xanthosine which may compete for metal-coordination along with the O(6) and N(7) positions. Although, the O(2) participation in ternary complexes is not favoured sterically, it may exert an influence on the O(6) and N(7) binding positions thus making the metal interaction less effective and resulting in lower stability of the ternary complexes of xanthosine in solution.

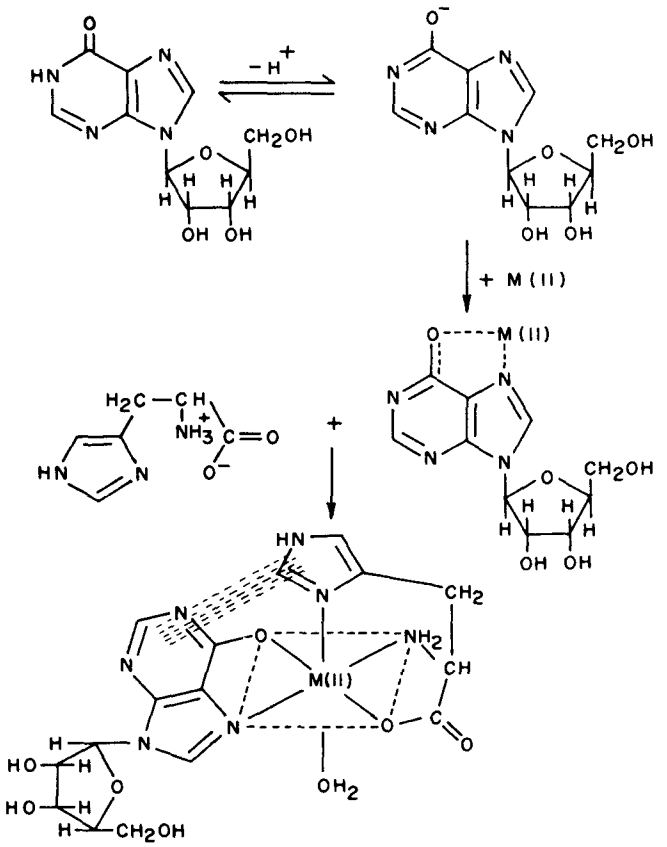


Figure 3. Tentative structure showing the proton dissociation and metal association reactions of inosine (binary and ternary) (R = Ribose).

It is important here to briefly mention the stabilities of xanthosine and inosine in their binary systems. Xanthosine (Rabindra Reddy *et al* 1976) forms stabler complexes as compared to inosine (Rabindra Reddy *et al* 1983b) with all the metal ions studied. This is in accord with the basicities of the ligands concerned. The higher stability of binary xanthosine complexes may also be partly responsible for the lower stability in ternary complexes.

A tentative structure showing the proton dissociation and metal association reactions of inosine (binary and ternary) given in figure 3 depicts the differences in the solvation energies of the above systems. Here, the entropy values rather than the enthalpy values may be responsible for the control of stabilities in solution.

Acknowledgement

One of the authors (MHR) is grateful to CSIR, New Delhi for a fellowship.

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