Complexation behavior and stability of ternary complexes of glutathione and thymine involving Hg^{II}, Pb^{II}, Cd^{II}, Zn^{II}, Co^{II} and Ni^{II} metal ions

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Abstract : The overall stability constants of ternary complexes of Hg^{II}, Pb^{II}, Cd^{II}, Zn^{II}, Co^{II} and Ni^{II} with reduced glutathione and thymine have been determined in biologically relevant conditions and at an ionic strength of 0.1 M $NaNO_3$. The stability constants and complexation equilibria have been determined on the basis of speciation curves obtained through SCOGS. The stability sequence of 1 : 1 : 1 GSH(A) : M(II) : Thy(B) has been found as : GSH(A)-Hg^{II}-Thy(B) > GSH(A)-Pb^{II}-Thy(B) > GSH(A)-Cd^{II}-Thy(B) > GSH(A)-Zn^{II}-Thy(B) > GSH(A)-Co^{II}-Thy(B) > GSH(A)-Ni^{II}-Thy(B).

Keywords : Thymine, glutathione, ternary complex, metal ions, stability.

Introduction

Reduced glutathione (GSH) is a non-protein tripeptide of the sequence γ-Glu-Cys-Gly. The most important functions of Glutathione include detoxification of xenobiotics and heavy metals, reduction of oxidation-prone protein thiols, maintenance of cellular membranes and deactivation of free redicals[1]. Metal-thymine complexes have antitumor[2] and antimicrobial[3] activity. An understanding of the nature and extent of binding of glutathione to Hg^{II} is of importance in seeking explanations for the manifestation of the acute and chronic toxic effect of Hg^{II}. Glutathione has been used as an antidote for mercury poisoning, but without much success. Knowledge of the magnitude of the formation constants of the Hg^{II}-glutathione complexes is essential for the development of effective antidote for mercury poisoning. Zinc is involved, among others, in DNA transcription (enzymes, zinc fingers) and in intracellular signalling[4,5]. Nickel compounds are human carcinogens, while it is agreed that intracellular Ni^{II} is responsible for neoplastic transformation. Several divergent concepts in nickel carcinogenesis have been developed as reviewed recently[6].

We report herein a potentiometric study using Irving-Rossotti pH-titration technique[7] in biologically relevant conditions. The results presented provide a quantitative and structural description of ternary reduced glutathione-M(II)-thymine complexes. The solution structure for these complexes have been discussed following the complexation equilibria deduced, which in turn have been derived on the basis of speciation curves obtained through SCOGS[8].

Results and discussion

Glutathione (GSH) is a naturally occurring tripeptide with the sequence γ-L-glutamyl-L-cysteinyl-L-glycine. Its molecule possesses eight potential donors of electronic density towards metal ions. They can be grouped into three classes : the glutamic (amino acid like) set of amine and carboxylate donors, the thiol and the peptide bonds. Among these potential donors, the carboxyl, the amine and the thiol are protonated/deprotonated spontaneously in aqueous solution. GSH has a total of four such groups. At physiological conditions, GSH exists as H_2L^- ions, with deprotonated carboxylate groups and still protonated basic functions. The refined values of acid dissociation constants are listed in Table 1.

As is revealed from the speciation curves of (1 : 1 : 1) GSH(A)-Zn^{II}-Thy(B) (Fig. 1), which has been considered as the representative system, the binary species GSH(A)-Zn^{II} is the most prevalent species in the present

Table 1. Stability constants and other related constants of binary and ternary complexes of reduced glutathione (A) and thymine (B) with different metal ions in aqueous solution at 37 ± 1 °C, $I = 0.1$ M NaNO$_3$

(i) Metal-ligand constants (log β_{p0r00}/log β_{0qr00}/log β_{p00s0}/ log β_{0q0s0}) : Binary systems

	Hg	Pb	Cd	Zn	Co	Ni
MA	15.38	14.13	13.85	13.83	12.78	12.20
MB	13.45	13.33	11.33	7.06	6.34	7.20

(ii) Metal-ligand constants (log β_{p0rs0}/log β_{0qrs0}) : Ternary systems

	Hg	Pb	Cd	Zn	Co	Ni
MAB	21.47	19.31	19.23	17.87	16.15	15.15

centration of mixed ligand complex GSH(A)-Zn^{II}-Thy(B) is accompanied by a concomitant fall in the concentration of BH and binary complex GSH(A)-Zn^{II}, which suggests its stepwise formation.

All other systems of the present study have been found to follow a similar pattern of species distribution almost in the same pH range. The maximum abundance ($\sim 70\%$) of ternary complex with Zn^{II} may be observed at pH ~ 9.4. Observing the same nature of speciation the maxima of ternary complex with Hg^{II}, Pb^{II}, Cd^{II}, Co^{II} and Ni^{II} metal ions have been found 93, 92, 87, 67 and 45%
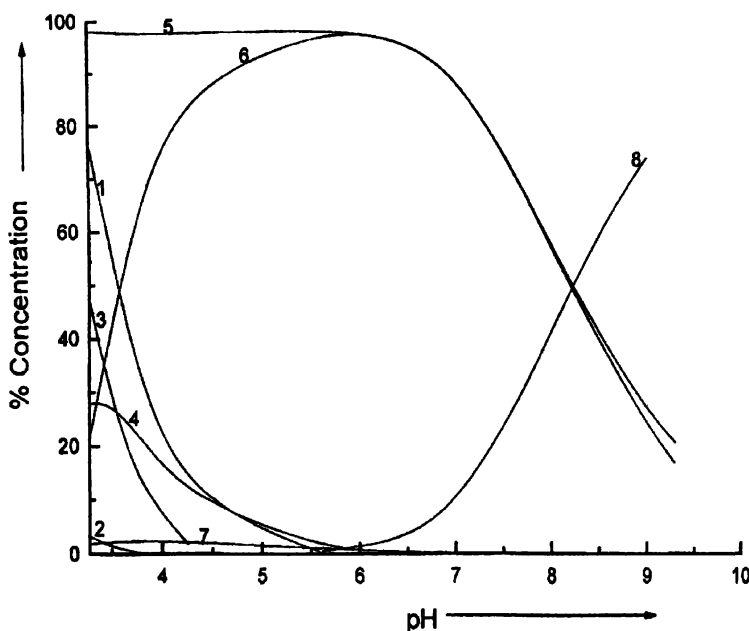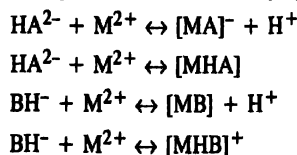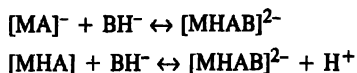

Fig. 1. Distribution curves of 1 : 1 : 1 reduced glutathione (A)-Zn^{II}-thymine (B) system : (1) Zn^{2+}, (2) AH$_4$, (3) AH$_3$, (4) AH$_2$, (5) BH, (6) A-Zn^{II}, (7) B-Zn^{II}, (8) A-Zn^{II}-B.

system, its maximum concentration being about 90% at pH ~ 6.0 beyond which it declines. However, the binary complex Thy(B)-Zn^{II} shows its appearance in the lower pH range, decreasing gradually with the gradual addition of alkali attaining minima at pH 6.5. Protonated ligand species of the secondary ligand i.e. BH at first increases and then decreases with the rise in pH. The gradual fall in its concentration beyond pH ~ 6.0 shows its further involvement in ternary complexation. Rise in the con-

respectively within the pH range 8.5-9.5.

On the basis of above description of the speciation curves obtained through SCOGS, following equilibria have been proposed for all the ternary systems :

$$HA^{2-} + M^{2+} \leftrightarrow [MA]^- + H^+$$
$$HA^{2-} + M^{2+} \leftrightarrow [MHA]$$
$$BH^- + M^{2+} \leftrightarrow [MB] + H^+$$
$$BH^- + M^{2+} \leftrightarrow [MHB]^+$$

$$[MA]^- + BH^- \leftrightarrow [MHAB]^{2-}$$

$$[MHA] + BH^- \leftrightarrow [MHAB]^{2-} + H^+$$

Numerous studies have shown that Hg^{II} binds strongly to ligand with free mercapto groups. A very high formation constant of Hg^{II}-glutathione complex results in very low free Hg^{2+} concentrations that cannot be measured experimentally. It has been proposed on the basis of ^{13}C chemical shifts that Hg^{II} forms a chelate with GSH by binding sulfhydryl group and a deprotonated peptide nitrogen in Hg^{II}-GSH complexes of 1 : 1 stoichiometry[9]. The binding of Pb^{II} by GSH is considerably more selective than is the binding of Zn^{II} or Cd^{II}, with Pb^{II} binding only to the glycine carboxylate group[9]. The formation constants for the Pb(HL) complex indicates the binding to the sulfhydryl group to be similar to that in the analogous Cd^{II} complex and slightly stronger than in the Zn^{II} complex (Table 1).

The Zn^{II} ion, a borderline Lewis acid, is suited for interactions with such a versatile ligand as GSH. The simultaneous presence of amino acid like and thiolate functions in its molecule results in the variety of Zn^{II} coordination modes, depending on pH and molar ratios. Studies of coordination equilibria performed by our predecessors, in particular those applying NMR techniques, demonstrated the involvement of the thiol function in Zn^{II} coordination in the entire pH range of the binding[10-13]. The sterical constraints due to the simultaneous coordination of the thiol and the glutamic acid donor, exclude the Gly carboxylate from the coordination to the same Zn^{II} ion. Ni^{II} exhibits little preference among sulphur, nitrogen and oxygen as potential donor atoms, similarly to Zn^{II}. This results in similarities in coordination modes for GSH between these two metal ions, limited however by differences in complex geometries, which are usually square planar for thiolate containing Ni^{II} complexes and octahedral for the Ni^{II} complexes without thiolate coordination[14]. The binding of Cd^{II} by GSH has also been studied earlier by pH titration methods[10,15]. The ^{13}C NMR results[9] indicate the complexation of Cd^{II} by GSH to be very similar to that of Zn^{II}. The Cd^{II} binds more strongly to the sulfhydryl group than does Zn^{II}. Evidence suggests that the predominant binding site in the Co^{II} complex of stoichiometry Co(HL) is the $^-O_2CCHNH_2$ group of the γ-glutamyl residue[9].

It seems quiet plausible that the coordination spheres

of these metal ion complexes[16,17] under study are not saturated with one molecule of GSH and hence these binary systems are prone to the formation of ternary complexes.

From the structural view point, following solution structure may be proposed for the mixed-ligand complex of the ternary 1 : 1 : 1 GSH(A)-M(II)-Thy(B) system.
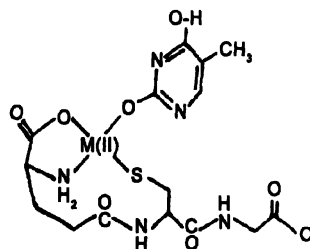
Scheme 1. A motif of M(II)-ternary complex with glutathione (A) and thymine (B)

The protonation constants of the ligands, binary and ternary constants for all the systems taken under study have been complied to present in Table 1 A comparison of log β values obtained through SCOGS shows following order of the ternary complexes : GSH(A)-Hg^{II}-Thy (B) > GSH(A)-Pb^{II}-Thy(B) > GSH(A)-Cd^{II}-Thy(B) > GSH(A)-Zn^{II}-Thy(B) > GSH(A)-Co^{II}-Thy(B) > GSH(A)-Ni^{II}-Thy(B).

Experimental

Stock solution (0.01 M) of reduced glutathione (GSH) was prepared in boiled double distilled water. Metal nitrate solutions were prepared and standardized by EDTA titrations[18]. A 0.01 M solution of thymine was prepared by dissolving it into one equivalent of alkali (NaOH) which itself was prepared in double distilled water and had been standardized against a standard oxalic acid solution. Carbonate free sodium hydroxide (E. Merck) and nitric acid (A.R.) were prepared in double distilled water and standardized as usual.

The other experimental details have been described elsewhere[19]. The ionic strength of all mixture solutions was kept 0.1 M NaNO$_3$. The free acid concentration was kept equal in each case i.e. 0.02 M. Preliminary estimates of binary and ternary constants obtained by least

square method[20] were used as input data for running the SCOGS computer program. The evaluation and refinement of stability constant of the complexes formed have been done and species distribution curves were obtained by plotting % concentration vs pH. Ionic product of water (K_w) and activity coefficient of hydrogen ion under the experimental conditions were obtained from the literature[21].

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