Solution equilibria of mixed metal mixed ligand complexes of copper(II), nickel(II) and zinc(II) with glycylalanine and imidazole

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Solution equilibria of mixed metal mixed ligand complexes of CuII, NiII and ZnII with glycylalanine and imidazole investigated in aqueous solution by potentiometry, spectrophotometry and electron paramagnetic resonance spectroscopy. The stability constants of the binary, ternary and quaternary complexes are determined and it is obtained for the following species (A = glycylalaninate and B= imidazole): $M^{2+}, AH_2^+, AH^+, BH^+, B, M(OH)^+, M(OH)_2, M(A)H, M(A)(OH), M(B)^+, M(B)(OH)^+, M(A)(B)^-, M_2(A)(B)^2+, M_2(A)(B-H)^+, M_2M^+(A)(B)^2$ and $M_2^+(A)(B-H)^+, (N,H)$ deprotonation of bidentate coordinated bridging imidazole ligand in the binuclear complexes at $pH > 6.5$ is evident from spectral measurements. Stability constants of binary $M(A)^+, M(B)^2$ and ternary $M(A)(B)^+$ complexes follow Irving-Williams order.

Complexes of peptides and imidazoles with metal ions have aroused increasing interest in recent years because the imidazole group of histidine plays a fundamental role in several metal protein and metal enzyme reactions of living organisms1-3 where mixed metal mixed ligand equilibria are very common. Systematic studies with relatively simple molecules of known structure often yield valuable information, that gives idea to the roles of metal ions in biological systems4-8. A review of literature showed that very little equilibrium study on homo- and hetero binuclear imidazolate bridged complexes had been reported9-12. As a part of our programme on complex formation of transition metal ions with peptides and imidazoles, we describe here an equilibrium study on mixed ligand mixed metal complex formation of CuII, NiII and ZnII with glycylalanine (glyala) and imidazole (imH) in binary, ternary and quaternary systems at 25±1°C in aqueous solution at a constant ionic strength, $I = 0.1M$ (NaClO$_4$).

**Experimental**

All solutions were prepared by using glass double-distilled water. Metal(II) solutions were prepared from perchlorate salts and standardized by the usual procedure13. A CO$_2$ free NaOH solutions was prepared under an atmosphere of nitrogen. It was kept in polyethylene bottle with sodalime guard tubes. All solutions were made up to an ionic strength of 0.1 M NaClO$_4$.

Protonation constants of the ligands and stability constants of the copper(II) complexes were determined in a 0.1M NaClO$_4$ at 25°C using a Systronics pH-meter (335-pH meter). The total volume of the solution was 50 ml. The solution in the vessel was stirred continuously using magnetic stirrer. The temperature was maintained using a Yorke Thermostat. The general procedures for pH-metric titration was same as described in our earlier work10-12.

The copper binding sites in each of the quaternary complexes were determined spectrophotometrically on a Shimadzu UV-visible recording spectrophotometer UV-160 with 1 cm quartz cell in aqueous medium. Electronic spectra also afforded elucidation of the solution structures of the complexes. Similar sets of solutions to those in the potentiometric studies were carried out using similar concentration ranges of ligands and metal(II) and covering similar pH ranges.

The EPR spectra were recorded with a Varian E-line Century Series spectrometer, equipped with a dual cavity and operating at X-band 100 kHz modulation. TCNE was used as field marker. The $g ||$ and $A ||$ values were measured according to the standard procedure.

**Results and discussion**

**Solution equilibria**

Up to $pH$ 8.4, glycylalanine (AH$_2^+$) ($log \beta = 8.15$-11.34) in the absence of metal ions can release two hydrogen ions- one from the carboxylate and the other from the amino group. Imidazole possesses only one replaceable proton in the absence of metal ions and its protonated form considered as BH$^+$ ($log \beta = 7.1$). In the presence of metal ions, further deprotonation may occur from the peptide and pyrrole group. Complex formation equilibria have been derived on the basis of distribution curves of the complexes occurring at
different pH. The $\beta_{pkr}$ values were evaluated using SCOGS computer programme\textsuperscript{14}.

**Binary complexes**

The numerical refinement is consistent with the following species in calculating the binary stability constants, $\beta_{1000}$, $\beta_{0100}$, $\beta_{0010}$, and $\beta_{0001}$: 

- M$^{2+}$:A$^{-}$ system: \( \text{AH}^{2+}, \text{AH}, \text{A}^{-}, \text{M}^{2+}, \text{M(OH)}^{+}, \text{M(OH)}_2, \text{M(A)}^{+}, \text{M(A)(OH)} \)
- M$^{2+}$:B system: \( \text{BH}^{+}, \text{B}, \text{M}^{2+}, \text{M(OH)}^{+}, \text{M(OH)}_2, \text{M(B)}^{+}, \text{M(B)(OH)}^{+} \)

Here we have considered formation of hydroxo species, because the buffer regions corresponding to metal-ligand complex formation equilibria are found to be overlapping with the hydrolytic equilibria of the M$^{2+}$ (aq.). The distribution curve (Fig. 1) of the systems indicate the formation of \( \text{M(A)}^{+} \) (log$\beta$ = 4.1-5.81), \( \text{M(A)(OH)} \) (log$\beta$ = -3.0-0.80), \( \text{M(B)}^{+} \) (log$\beta$ = 2.53-3.65), complexes with all the three M$^{2+}$ (M = Cu, Ni and Zn) ions. Hydroxo species, Zn(OH)$^+$ and Zn(OH)$_2$ are however found to be higher in proportion.

**Ternary systems**

Formation of ternary species (log$\beta$ = 5.4-8.86) takes place in the pH range 4-7.0 according to the equilibria,

\begin{align*}
\text{M(A)}^{+} + \text{BH}^{+} & \rightleftharpoons \text{M(A)(B)}^{+} + \text{H}^+ \quad \ldots (1) \\
\text{M(A)(B)}^{+} + \text{H}_2\text{O} & \rightleftharpoons \text{M(A)(B)(OH)} + \text{H}^+ \quad \ldots (2)
\end{align*}

The homobinuclear ternary species, \( \text{M}_2(\text{AH}_2\text{B})^{2+} \) (log$\beta$ = 22.50) have been considered to exist in the complex equilibria of 2:2:1 M$^{2+}$:A$^{-}$:B systems. Distribution curves (Fig. 1) of Cu(A)$^{+}$, BH$^+$ and Cu$_2$(A)$_2$(B)$_2$$^{2+}$ in the pH range 3-7.5 suggest the formation of the binuclear Cu$_2$(A)$_2$(B)$_2$ complex according to equilibria,

\begin{align*}
2\text{Cu(A)}^{+} + \text{BH}^{+} & \rightleftharpoons \text{Cu}_2(\text{A})(\text{B})^{2+} + \text{H}^+ \quad \ldots (3)
\end{align*}

The corresponding binuclear complexes with Ni$^{II}$ (log$\beta$ = 19.00) and Zn$^{II}$ (log$\beta$ = 18.51) are however found to be formed according to equilibria,

\begin{align*}
\text{M}^{2+} + \text{AH} + \text{BH}^{+} & \rightleftharpoons \text{M(A)(B)}^{+} + 2\text{H}^+ \quad \ldots (4) \\
2\text{M}^{2+} + 2\text{AH} + \text{BH}^{+} & \rightleftharpoons \text{M}_2(\text{A})_2(\text{B})^{2+} + 3\text{H}^+ \quad \ldots (5)
\end{align*}

Simultaneous occurrence of the mononuclear ternary complexes, \( \text{M(A)(B)} \) and homobinuclear ternary complexes \( \text{M}_2(\text{A})_2(\text{B})^{2+} \) suggests \( (N_1, N_3) \) bridging bidentate coordination by imidazole ligand in these binuclear complexes.

**Quaternary complexes**

In addition to the mononuclear and homobinuclear complexes as discussed in above paragraphs, these systems are supported to involve the heterobinuclear complexes, \( \text{M}^{1}\text{M}^{2}(\text{A})_2(\text{B})^{2+} \). Stability constants fall in the range 13.00-22.10. The distribution curve indicates the formation of heterobinuclear complex by the following general equilibrium.

\begin{align*}
\text{M}^{1}(\text{A})^{+} + \text{BH}^{+} + \text{M}^{2}(\text{A})^{+} & \rightleftharpoons \text{M}^{1}\text{M}^{2}(\text{A})_2(\text{B})^{2+} + \text{H}^+ \ldots (6)
\end{align*}

**Deprotonated complexes**

The buffer region at pH$>$7 may be due to the metal induced deprotonation of the coordinated imidazole ligand. This leads to the formation of deprotonated species according to the equilibrium,

\begin{align*}
\text{M}^{1}\text{M}^{2}(\text{A})_2(\text{B})^{2+} & \rightleftharpoons \text{M}^{1}\text{M}^{2}(\text{A})_2(\text{B-H})^{+} + \text{H}^+ \ldots (7)
\end{align*}

The deprotonated imidazole, (B-H)$^-$ in the binuclear complexes provides \( (N_1, N_3) \) bridging bidentate coordination.

Deprotonated imidazole residue of histidine functions as bridging \( N_1, N_3 \) bidentate ligand in superoxide dismutase enzyme\textsuperscript{15}. Complexation
behaviour of imidazole residue of histidine was also earlier studied by Daniele et al.\textsuperscript{7}.

The \( N_1-H \) deprotonation constants for homobinuclear \( M_2(A)_2B^{2+} \) and heterobinuclear \( M'M^2(A)_2(B)^{2+} \) complexes may be given by equations,

\[
K_{20210}^H = \frac{[M_2(A)_2(B-H)]}{[M_2(A)_2(B)]} ... (8)
\]

\[
K_{11210}^H = \frac{[M'M^2(A)_2(B-H)]}{[M'M^2(A)_2(B)]} ... (9)
\]

Hence, \( pK_{20210}^H \) and \( pK_{11210}^H \) values may be evaluated with the help of equations,

\[
pK_{20210}^H = \log \beta_{20210} - \log \beta_{20211} \quad ... (10)
\]

\[
pK_{11210}^H = \log \beta_{11210} - \log \beta_{11211} \quad ... (11)
\]

**Stability constants with respect to metal ions**

The stability constants with respect to metal ions fall in the Irving-Williams order\textsuperscript{16,17}: \( Ni^{II}<Cu^{II}<Zn^{II} \). Overall stability constants, \( \beta_{20210} \) and \( \beta_{20211} \) of the binuclear complexes form a composite Irving-Williams order and this trend found to be as: \( Cu-Cu>Ni-Ni>Zn-Ni>Ni-Ni>Zn-Zn \). The stability constants of Ni-Ni complexes are found to be less than Ni-Zn complexes. The single \( Ni(n)\rightarrow B(n) \) back-bonding in \( ZnNi(A)_2(B)^{2+} \) complex adds to the stability of this complex, where as two opposing \( Ni(n)\rightarrow B(n) \) bonding in \( Ni_2(A)_2(B)^{2+} \) complex probably weaken each other by increasing the electron density on the B ligand.

In order to get more complete information about the bonding modes in \( Cu(II)-glyala \) (1:1), Copper(II)-glyala-imH (1:1:1) and homo- and heterometallic \( Cu_2(glyala)_{2}ImH \) (2:2:1), \( Cu-Zn(glyala)_{2}ImH \) (1:1:2:1), CuNi(glyala)_{2}ImH (1:1:2:1) copper(II) complexes, EPR measurements were also performed at different pH values. Selected spectra for the copper(II) systems are shown in Fig. 2. The conclusion that can be drawn from the EPR results are substantially in agreement with those derived from the equilibrium studies but interesting new information’s have been got also in homo- and heterometallic systems.

The details for the individual systems are described below:

**Cu(II)-glyala-ImH(1:1:0/1) systems**

The EPR spectra of frozen solutions of these binary and ternary complexes was recorded at 77K. The EPR spectra for these two binary and ternary systems exhibit usual line shape with \( g_\perp=g_{\perp} \geq 2.03 \), indicating an axial symmetry. The proposed donor atoms are OONN and ONNN in binary and ternary complexes respectively. The value of \( A_l \) in case of ternary complexes is found to be smaller (\( A_l = 170 \) G for binary and 150 G for ternary), this may be possible due to greater stronger field exerted by the ONNN groups.

\( (Cu(glyala)_{2}ImH/CuNi/Zn(glyala)_{2}ImH \) (2:2:1 or 1:1:2:1) systems

EPR spectra of frozen solutions these systems were also recorded at 77K. (Cu)_{2}(glyala)_{2}imH systems shows signals due to dimeric species present. The two broad signals at -3170 G and -3240 G were assigned to the split \( g_\perp \) signals, allowing the calculation of \( D \). A \( g \)-factor of 2.07 is measured at the middle point between these two resonances. However \( \Delta m=2 \) transition was not detected of this system. Similar spectral features were not observed in any binary, ternary and heterometallic copper(II) systems, therefore, it is likely that the dimer is the imidazolate
bridged complex. The most likely hypothesis is that the dimer is formed by bridging of 2Cu(glyala) by imidazole bridging ligand. On raising the pH only $A_{ii}$ value decrease which is obviously may be due to the deprotonation of imidazole ligand. The deprotonation takes place at the higher pH value and the deprotonated imidazole exerts the stronger ligand field that the neutral bridged imidazole. Hence due to stronger ligand field the value of $A_{ii}$ possibly decreases. The spectra of heterometallic CuNi/CuZn imidazole dimer show the usual line shape for the mononuclear copper(II) complexes with $g_{ll}>g_{ii}>2.03$, indicating an axial symmetry. The $g_{ll}(A_{ll})$ =157 cm$^{-1}$, indicating a rather pyramidal geometry with a $d_{x^2-y^2}$ ground state. On raising the pH again the value of $A_{ii}$ decreases which is owing to the stronger ligand field exerted by the imidazole ion. The possible donor atoms in this dimer are ONNN.

The EPR behaviour of the copper(II) centre in all these imidazole bridged binuclear systems is compared by means of Symon’s plot$^{18}$. This is in agreement with the geometry of copper(II) in Cu-Zn SOD model obtained for X-ray crystal structure$^{19,20}$ of the active site of the proteins.

The visible absorption spectra of various binary, ternary and binuclear liquid mixtures at pH values corresponding to the concentration maxima of the species were recorded. The $\lambda_{max}$ value along the $e_{max}$ are in the ranges 615-774 nm and $e_{max}$ 17-157 dm$^3$mol$^{-1}$cm$^{-1}$. The experimental conditions are same as those for pH-metric titrations. The present spectrophotometric data are in accordance with the above conclusions. In each case a single absorption band is seen which can be assigned to the spin-allowed Laporte-forbidden $d-d$ transition $2E_g \rightarrow 2T_{2g}$. Since this is a $d^9$ system, the crystal-field splitting is in turn affected by the coordination sphere of the metal ion and hence $\lambda_{max}$ yields a measure of the solution structure of the complex. As expected, the $\lambda_{max}$ value of the ternary species Cu(A)(B)$^+$ is found to be higher than $\lambda_{max}$ values of the two binary species Cu(A)$^+$ (650 nm) and Cu(B)$^+$ (760 nm). The binuclear complex, Cu$_2$(A)$_2$(B)$^+$ shows higher values of $\lambda_{max}$ (635 nm) and $e_{max}$ (110 dm$^3$mol$^{-1}$cm$^{-1}$) than the corresponding mononuclear complex, Cu(A)(B)$^+$. Both these effects are in accordance with addition of second copper(II) ion to an imidazole nitrogen. Thus suggesting a copper-glycylalaninate and copper-imidazole nitrogen bond for the two copper(II) ions in the complex, Cu$_2$(A)$_2$(B)$^+$. The extinction coefficient values ($75 \pm 1$ dm$^3$mol$^{-1}$cm$^{-1}$) for heterobinuclear species are roughly half than those of binuclear, species Cu$_2$(A)$_2$(B)$^+$ and Cu$_2$(A)$_2$(B-H)$^+$. This is indicative of that the one metal centre is different than copper(II) centre. The N$_1$-H deprotonation of the bridging imidazole ligand in homo- and heterobinuclear complex puts an additional electron on the bridging imidazole. The resulting anion, (B-H)$^-$ obviously exerts a stronger ligand field than the neutral B ligand as a result, the deprotonated homom and heterobinuclear species, Cu$_2$(A)$_2$(B-H)$^+$, Cu-Zn(A)$_2$(B-H)$^+$ and Cu-Ni(A)$_2$(B-H)$^+$ undergo blue shifts in their absorption maxima by 15-20 nm on raising the pH of the solution above 7.0. Also, the $e_{max}$ values are higher by 18-47 dm$^3$mol$^{-1}$cm$^{-1}$ for the different deprotonated species. Furthermore, only one absorption maxima is obtained in the electronic spectra of the Cu$_2$(A)$_2$(B)$^+$ and Cu$_2$(A)$_2$(B-H)$^+$, indicating a similar environment for both the copper(II) ions. For these data structure and is proposed for these species. Aiba et al.$^{21}$ have also collected the similar spectrophotometric data for some Cu$^{II}$ binuclear complexes. Thus, our findings are in good accordance with these reported values of binuclear complexes.

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References

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