Aminoacidate Dechelation upon Imidazole Proton Ionization in a Mixed Ligand Metal Chelate, *N*-Carboxymethyl-L-histidinatovalinatocopper(II)

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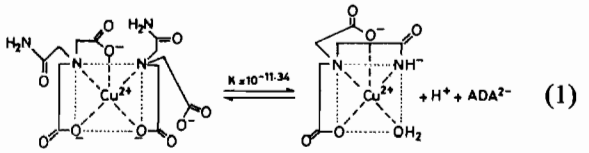
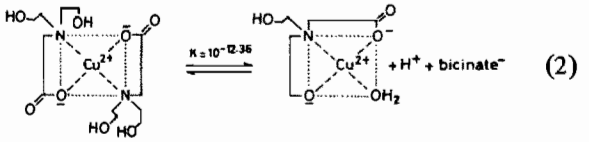
The removal of substrates from metalloenzyme centers is a neglected area of research. Several years ago, a program was initiated using deprotonation reactions of groups found at the active sites of metalloenzymes as potential models for substrate removal. Aminoacidate dechelation upon amide (asparagine, glutamine) deprotonation [1] was observed in the bis(*N*-acetamidoiminodiacetato)-copper(II) chelate, $[Cu(ADA)_2^{2-}]$ (eqn. (1)).



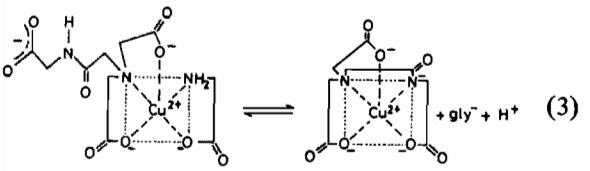
(1)

Hydroxyl group ionization (serine, threonine) was shown to lead to aminoacidate dechelation [2] in the bis(*N,N*-bis(2-hydroxyethyl)glycinato)copper(II) chelate $[Cu(bicine)_2]$ (eqn. (2)).



(2)

Also, aminoacidate dechelation was initiated upon peptide proton ionization [3] (protein backbone) in the mixed ligand chelate, (*N,N*-bis(carboxy-methyl)glycylglycinato)glycinatocopper(II), $[Cu(DGDA)gly^-]$ (eqn. (3)).



(3)

However, dechelation upon deprotonation of an imidazole group, which is the most ubiquitous functional moiety at the active site of metallo-enzymes, has proved elusive until now.

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Potentiometric formation curves and visible spectra* (not shown) of 1:1 Cu(II) to *N*-carboxy-methyl-L-histidine (CmHt), consist of three buffer zones with inflections at $a = 2.0$ and 3.0, moles of OH^- to moles of Cu(II). The reaction in the low pH ($a = 0$ to 2.0) buffer zone is the formation of $[Cu(CmHt)H_2O]$ (eqn. (4)); $\lambda_{max} = 660$ nm and

$$Cu^{2+} + H_2CmHt \rightleftharpoons [Cu(CmHt)H_2O] + 2H^+ \quad (4)$$

$\epsilon_{max} = 42\ M^{-1}\ cm^{-1}$. The reaction in the second buffer zone was found to be the ionization of the imidazole proton to yield $[Cu(H_{-1}CmHt)H_2O^-]$ (eqn. (5)).

$$[Cu(CmHt)H_2O] \rightleftharpoons [Cu(H_{-1}CmHt)H_2O^-] + H^+ \quad (5)$$

Visible spectra at $a = 3.0$ clearly show a large blue shift to 628 nm and an increase in ϵ_{max} to 62 M^{-1} cm^{-1}, which cannot indicate ionization of metal-bound H_2O. The formation of hydroxo complexes in metal chelates has been shown [4] to result in only small changes if any in λ_{max} and ϵ_{max}. Therefore, the only other available acidic proton would be that of the imidazole group. The ionization of an imidazole group should result in a much stronger σ-donor than imidazole itself and a large blue shift would be expected. The third buffer zone (high pH) involved the formation of the hydroxo complex, $[Cu(H_{-1}CmHt)OH^{2-}]$ (eqn. (6)).

$$[Cu(H_{-1}CmHt)H_2O^-] \rightleftharpoons [Cu(H_{-1}CmHt)OH^{2-}] + H^+ \quad (6)$$

Visible spectra at $a = 4.0$ show a λ_{max} of 630 nm and an ϵ_{max} of 66 M^{-1} cm^{-1}. As argued above, hydroxo complex formation should result in small changes in λ_{max} (in this case, 2 nm) and ϵ_{max} (4 M^{-1} cm^{-1}).

Potentiometric formation curves of 1:1:1 Cu(II) to *N*-carboxymethyl-L-histidine to valine show three buffer zones with inflections at $a = 2.0$ and 3.0. Visible data at $a = 2.0$ ($\lambda_{max} = 661$ nm, $\epsilon_{max} = 43$ M^{-1} cm^{-1}) are the same within experimental error as that of the 1:1 Cu(II) to H_2CmHt system at $a = 2.0$, indicating the formation of $[Cu(CmHt)H_2O]$. From $a = 2.0$ to $a = 3.0$, the formation of $[Cu(CmHt)val^-]$ was observed (eqn. (7))**.

$$[Cu(CmHt)H_2O] + val^- \rightleftharpoons [Cu(CmHt)val^-] \quad (7)$$

Visible spectral data confirm the formation of mixed ligand complexes. Although the λ_{max} values of

*Potentiometric and visible data obtained as in ref. 1.

**Inclusion of $[Cu(H_{-1}CmHt)H_2O]$ is necessary in order that iterative programs converge ($a = 2.0$ to 3.0).

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TABLE I. Formation Constants and Visible Spectra for Various Mixed Ligand Metal Chelates

Metal chelate	Ancillary ligand	log K_2 (λ_{max})[a]	log K_{1a}	K_d (λ_{max}, ϵ_{max})[b]
[Cu(ADA)]	β-alanine$^-$	5.51 ± 0.01 (710)	−10.04 ± 0.01	−13.60 ± 0.01 (747, 67)
[Cu(ADA)][c]	ADA^{2-}	3.12 (669)		−11.34 (745, 66)
[Cu(bicine)$^+$][d]	bicine$^-$	5.40 (610)		−12.36 (724, 65)
[Cu(DGDA)$^-$][e]	glycine$^-$	5.95 (665)		−11.68 (670, 120)
[Cu(CmHt)]	L-valine$^-$	3.15 ± 0.02 (660, 41)	−11.20 ± 0.02	−13.95 ± 0.02 (625, 64)[f]

[a]λ_{max} in nm of mixed ligand chelates, [CuLL'$^{x+}$]; ϵ_{max} in M^{-1} cm^{-1}, see eqn. (7). [b]λ_{max} in nm, ϵ_{max} in M^{-1} cm^{-1} of [Cu(H$_{-1}$L)OH$^{(x-2)+}$] species except [Cu(H$_{-1}$DGDA)$^{2-}$], see eqn. (8). [c]Ref. 1. [d]Ref. 2, bicine = $(HOCH_2CH_2)_2NCH_2COO^-$. [e]Ref. 3, DGDA = $(-OOCCH_2)_2NCH_2C(O)NHCH_2COO^-$. [f][Cu(H$_{-1}$CmHt)OH^{2-}] ($\lambda_{max}$ = 630 nm, ϵ_{max} = 66 M^{-1} cm^{-1}).

[Cu(H$_{-1}$CmHt)H_2O^-] and [Cu(CmHt)val$^-$] are similar (628 and 623 mm, respectively), the ϵ_{max} values (62 and 75 M^{-1} cm^{-1}, respectively), are sufficient to differentiate the two metal complexes.

Visible spectra of the above solution at several values from $a = 3.0$ to $a = 5.6$ show a monotonic increase in λ_{max} and decrease in ϵ_{max} confirming the potentiometric data that dechelation of valine and hydroxo complex formation occurs in the high pH buffer region (eqns. (8) and (6)).

$$[Cu(CmHt)val^-] \rightleftharpoons [Cu(H_{-1}CmHt)H_2O^-] + val^- + H^+ \quad (8)$$

Using iterative programs [5] it was found that convergence of the potentiometric data did not occur unless the species, [Cu(H$_{-1}$CmHt)val^{2-}] (eqn. (9)) was included above $a = 3.0$. Similar behavior, the

$$[Cu(CmHt)AmAc^-] \rightleftharpoons [Cu(H_{-1}CmHt)AmAc^-] + H^+ \quad (9)$$

formation of a deprotonated mixed ligand metal chelate, has been reported in the 1:1:1 Cu(II) to ADA to β-alanine system at high pH [6]. The dechelation of β-alanine with concomitant hydroxo complex formation upon amide proton ionization (eqn. (10)) is strikingly similar to the system reported here.

$$[Cu(ADA)\beta\text{-ala}^-] \rightleftharpoons [Cu(H_{-1}ADA)\beta\text{-ala}^{2-}] \rightleftharpoons [Cu(H_{-1}ADA)OH^{2-}] + \beta\text{-ala}^- \quad (10)$$

The formation and ionization constants of [Cu(CmHt)val$^-$], [Cu(H$_{-1}$CmHt)H_2O^-], [Cu(H$_{-1}$CmHt)val^{2-}] and [Cu(H$_{-1}$CmHt)OH^{2-}] are listed in Table I as are the corresponding λ_{max} and ϵ_{max} values. The initial goal of this program was to show that the ionization of protons from groups found at the active sites of metalloenzymes (amide, alcohol, peptide, and now imidazole) can help to initiate dechelation reactions of strongly bound groups such as aminoacidates. The basis for this work was the S_N1CB mechanism as formulated by Basolo and Pearson [7]. If the ionization of a coordinated ammine group could lead to substitution reactions in inert Co(III) complexes, the ionization of more acidic groups should lead to substitution reactions in mixed ligand chelates of labile metal ions. It is important to note, however, that no such reactions have been shown to occur at metalloenzyme active sites. Also, very little is known about substrate removal from active sites since that step is fast and usually preceded by the slow step. The above reactions may be models for substrate removal in that functional groups are thought to aid in substrate binding; it would be appealing if they also aided in substrate removal. These reactions occur just within the physiological pH range; if such reactions do occur in proteins, facile exchange may render them difficult to retain and detect.

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