Aminoacidate Dechelation upon Hydroxo Complex Formation in Mixed Ligand Metal Chelates

JOSEPH S. DEMBOWSKI, DOUGLAS C. KURTZ and ROBERT NAKON*

Department of Chemistry, West Virginia University, Morgantown, W.Va. 26506, U.S.A.

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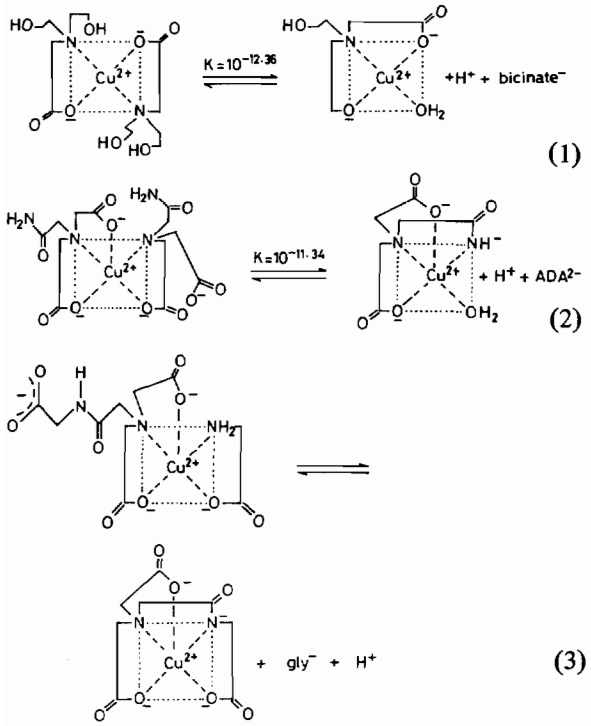
There are functional groups at the active sites of metalloenzymes for which there is no known purpose, *i.e.*, they are not involved in either the binding of substrate or the metal ion. Since the removal of these functional groups (via replacement of one amino acid by another) deactivates the enzyme, we thought the function of such groups is possibly to aid in substrate removal. Since it is well established that functional groups aid in substrate binding, it would be appealing that other such groups aid in substrate removal.

Of the various functional groups in proteins, the four strongest σ-donors to metal centers would be (1) an ionized amide group (asparagine or glutamine), (2) an ionized alcohol group (serine or threonine), (3) an ionized peptide group (protein backbone), and (4) an ionized imidazole group (histidine). We have successfully used these four groups to induce dechelation reactions of amino acids (eqns. (1)–(4)) [1–4].



*Author to whom correspondence should be addressed.

$$[Cu(CmHt)val^-] \rightleftharpoons [Cu(H_{-1}CmHt)H_2O^-] + val^- + H^+ \quad (4)$$

where **ADA** is *N*-acetamidoiminodiacetate, bicine is *N,N*-bis(2-hydroxyethyl)glycinate, gly is glycinate, CmHt is *N*-carboxymethyl-L-histidinate, and val is valinate.

Recent studies [5], however, showed the buildup and subsequent disappearance of a deprotonated mixed ligand chelate (eqn. (5)), [Cu(H$_{-1}$ADA)β-ala^{2-}], and indicated that the ultimate driving force in

$$[Cu(ADA)\beta\text{-ala}^-] \overset{-H^+}{\rightleftharpoons} [Cu(H_{-1}ADA)\beta\text{-ala}^{2-}] \overset{OH^-}{\rightleftharpoons} [Cu(H_{-1}ADA)OH^{2-}] + \beta\text{-ala}^- \quad (5)$$

the removal of β-alanine was hydroxo complex formation [5]. The above suggested that under appropriate conditions dechelation of amino acids in mixed ligand metal complexes could be effected by simply raising the pH of solutions even in the absence of a coordinating side chain or deprotonated ligand.

Table I contains the pK_a values of various amino acids, the mixed ligand formation constants of aminoacidates to nitrilotriacetatocopper(II), [Cu(NTA)$^-$], and the corresponding dechelation constants†. The potentiometric formation curves of 1:1:1 Cu(II) to H_3NTA to HAmAc where HAmAc is an amino acid consisted of three buffer zones with inflections at $a = 3.0$ and 4.0 (2.5 and 3.0 in the 2:1 H_3NTA to Cu(II) system), moles of OH$^-$ per mole of H_3NTA. In the low pH (2 to 4) region, the formation of [Cu(NTA)H_2O^-] was found to occur. The neutral buffer region (pH 6 to 8) entailed mixed ligand complex formation (eqn. (6)). At high pH (>9),

TABLE I. Mixed Ligand Formation Constants and Aminoacidate Dechelation Constants for [Cu(NTA)(H_2O)$^-$] at 25 °C and μ = 0.10 M (KNO_3)

Aminoacidate	pK_a	log K_2 [a]	log K_d [b]
β-Alanine	10.14 ± 0.01	4.48 ± 0.01	−13.91 ± 0.03
Proline	10.58 ± 0.01	6.23 ± 0.01	−15.86 ± 0.02
Norvaline	9.70 ± 0.01	5.18 ± 0.01	−14.78 ± 0.02
N-Methylleucine	9.74 ± 0.01	4.86 ± 0.01	−14.45 ± 0.01
Valine	9.54 ± 0.01	5.14 ± 0.01	−14.58 ± 0.02
α-Methylleucine	10.07 ± 0.01	5.28 ± 0.02	−14.77 ± 0.03

[a]Mixed ligand formation constant, see eqn. (6). [b]Dechelation constant, see eqn. (7).

†All constants were determined at 25.0 °C and an ionic strength of 0.10 M KNO_3. Procedures used are listed in detail in *Anal. Biochem., 133,* 492 (1983).

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TABLE II. Visible Spectra of 1:1 Cu(II) to NTA Complexes with Various Ancillary Ligands

a Values[a]	$[Cu(NTA)H_2O^-]$[b]	$[Cu(NTA)_2^{2-}]$	$[Cu(NTA)\beta\text{-ala}^{2-}]$	$[Cu(NTA)N\text{-}CH_3leu^{2-}]$
3.0	780(60)	710(33)	780(61)	780(60)
4.0	770(64)	750(54)	715(42)	650(30)
5.0	770(64)	780(61)[c]	760(55)	740(42)

[a] *a* is moles of OH^- per mole of NTA. [b] λ_{max} in nm (ϵ'_{max} in M^{-1} cm^{-1}). [c] 780(61) is for $a = 2.5$.

dechelation of the aminoacidate occurred concomitant with hydroxo complex formation (eqn. (7)).

$$[Cu(NTA)H_2O^-] + AmAc^- \rightleftharpoons [Cu(NTA)AmAc^-] \quad (6)$$

$$[Cu(NTA)AmAc^-] \rightleftharpoons [Cu(NTA)OH^{2-}] + AmAc^- + H^+ \quad (7)$$

Visible spectra[§] are tabulated in Table II and show that $[Cu(NTA)H_2O^-]$ was formed at $a = 3.0$ in all titrations except that of 2:1 H_3NTA to Cu(II) ($a = 2.5$). The large blue shifts observed from $a = 3.0$ to 4.0 ($a = 2.5$ to 3.0 for 2:1 H_3NTA to Cu(II)), indicate the coordination of a stronger σ-donor than H_2O, *i.e.*, aminoacidate binding (eqn. (6)). The hydroxo complex, $[Cu(NTA)OH^{2-}]$, absorbs at 770 nm, slightly lower than $[Cu(NTA)H_2O^-]$, but considerably higher than those observed, 650, 710, and 715 nm for the mixed ligand complexes (Table II). Above pH 9, the λ_{max} values and ϵ_{max} values both show a monotonic increase. This supports the potentiometric data in that the λ_{max} and ϵ_{max} values approach those of $[Cu(NTA)OH^{2-}]$ at high pH. However, the removal of the ancillary ligand is incomplete at $a = 5.0$, as indicated by the failure of each system to absorb at 770 nm. Using the K_d value in Table I, calculations indicate that for $[Cu(NTA)N\text{-}CH_3leu^{2-}]$, only 75% of the *N*-methylleucine is removed at pH 11.8. It is apparent that strong binders such as aminoacidates will only be totally removed when the concentration of OH^- is very high. However, weaker binding ligands should undergo dechelation at lower pH values.

Therefore, mixed ligand complex formation should not be assumed to be favorable at high pH. There is a specific pH region where the mixed ligand complex is predominant but hydroxide ion successfully competes with ancillary ligands at high pH. This may be put to use, however; the removal of materials from resins containing metal chelates may be accomplished in basic solutions rather than acidic ones or may obviate the need to use a strong complexing agent. For example Angelici *et al.* [6] were able to partially resolve D,L-mixtures of amino acids by using *N*-carboxymethyl-L-valinatocopper(II) which was bound to a sytrene polymer. Ethylenediamine was then used to replace the bound aminoacidates from the Cu(II)–chelate containing resin. One of the major problems encountered was that ethylenediamine, a strong complexing agent, caused leaching of Cu(II) from the resin. By choosing a high pH stable resin, removal of bound aminoacidates could be effected by raising the pH (as in eqn. (7)). Not only would the use of strong complexing such as ethylenediamine be avoided but there would also be little if any leaching of metal ions from the resins. The removal of reacted substrates from polymer-bound catalysts (batch catalysis) could also be effected in this manner.

[§] Visible spectra were obtained on a Cary 14R spectrometer; metal ion concentrations were approximately 5.0×10^{-3} M.

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