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Bis(L-serinato)copper(II) Exhibits Serine Aldolase Reactivity

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The enzymatic reactions of transformation of amino acids mediated by pyridoxal have received much attention by inorganic chemists because the related nonenzymatic reactions are usually strongly catalyzed by metal ions; many of the mechanisms proposed for pyridoxal catalysis are actually based on studies of such reaction systems [1]. Cleavage of α-amino-β-hydroxyacids in the presence of pyridoxal, or pyridoxal analogues, and metal ions has been extensively studied by Snell [2] and by other authors [3–6]. Several products are formed (Fig. 1) according to reaction pathways which may involve common intermediate species. However, detailed analysis of the species involved has generally proven prohibitively difficult for the occurrence of a large number of metal–ligand equilibria. Of the reactions indicated in Fig. 1 racemization [7–12] and oxidative deamination [13–15] of amino acids, including α-amino-β-hydroxyacids, are known to occur even in the absence of pyridoxal as the result of an aerobic degradation of the metal–amino acid system. We report here that, together with the above reactions, also serine dealdolization occurs by aerobic alkaline degradation of bis(L-serinato)copper(II).

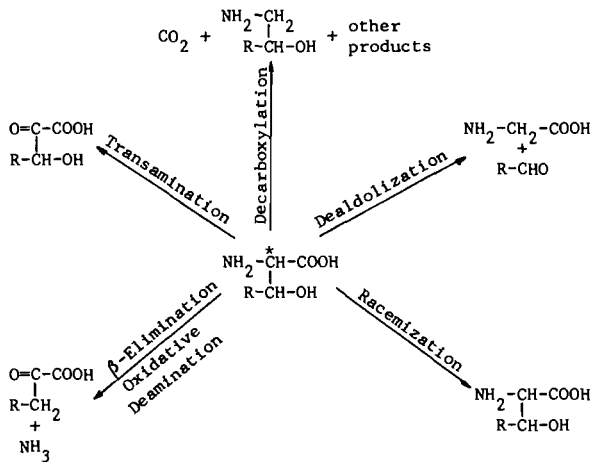

Fig. 1. Simplified scheme of pyridoxal mediated reactions of α-amino-β-hydroxyacids. Pyridoxamine is also formed by transamination. Hydrogen peroxide is apparently the other reaction product of oxidative deamination, but this has never been clearly established. Decarboxylation of α-amino-β-hydroxyacids gives rise to several products [25].

On heating for a few hours an aqueous alkaline solution (pH ~ 11) of Cu^{2+} and L-serine (1:2) a decrease in the optical activity of the solution is observed and precipitation of brown cupric hydroxide and red copper(I) oxide occurs. After filtration and acidification, treatment of the solution on a column of Dowex 50W × 8 (H^+) resin and elution with dilute ammonia enables the isolation of a mixture of serine and glycine.* Identification of the amino acids was made through ^1H and ^{13}C NMR spectroscopy [16] and by gas-chromatographic analysis of their N-trifluoroacetyl O-n-butyl esters [17]. Formation of ammonia and a large extent of racemization of the serine recovered show that the reaction of dealdolization to glycine is flanked by oxidative deamination and racemization (Fig. 1). The rate of serine dealdolization, like the other reactions, increases at high temperature and high pH, however the reaction takes also place at room temperature and pH slightly above 10, albeit very slowly. In previous studies it was reported [13] that alkaline degradation of the copper(II)–serine system leads to almost complete liberation of ammonia by oxidative deamination. It is likely that under the severe conditions employed there, complete degradation of the amino acid system occurred. By operating in milder conditions various routes accessible to serine degradation become observable.

The features of electronic and CD spectra of bis(L-serinato)copper(II) in aqueous solution undergo dramatic changes upon raising the pH from 8.5 to 11.1 (Fig. 2). These changes may indicate a change in the number of copper coordination sites. Indeed, recent spectroscopic [18] and thermodynamic [19] studies suggest that in alkaline solution (pH > 10) the hydroxy-group of the α-amino-β-hydroxyacid ligand can provide an effective third coordination site even in the secondary coordination sphere of copper(II), through strong hydrogen bonding to a hydroxide ion coordinated in apical position. This type of outer-sphere coordination is apparently favored with respect to a genuine tridentate binding mode because the latter would impose considerable steric strain. Interestingly, the positive CD band near 310 nm,

*Typically: copper(II) sulphate (5 mmol) and L-serine (10 mmol) in aqueous solution (100 ml) were kept at pH 11.1 and at 50 °C. Ammonia was collected by bubbling into a standard sulfuric acid solution. After a reaction time of 7 h the products recovered were: glycine 1.8 mmol; serine 5.2 mmol; ammonia 1.1 mmol. Only 34% of the serine recovered was optically active ($[\alpha]_D^{25}$ = +12.4°, (c = 3, 5 N HCl), for pure L-serine).

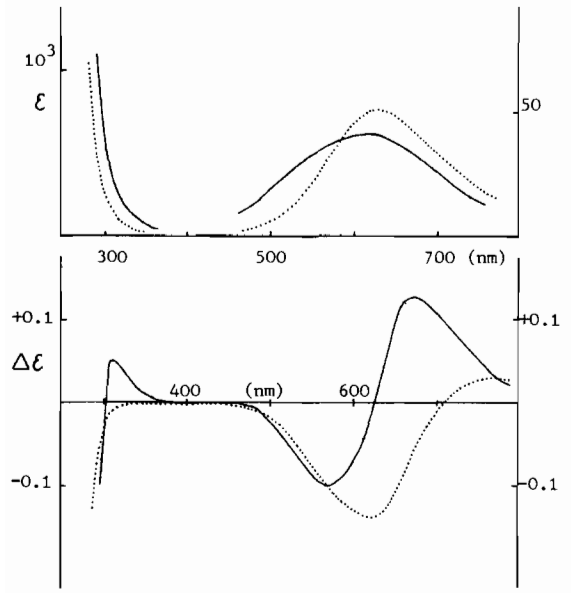

Fig. 2. Electronic and CD spectra of bis(L-serinato)copper(II) in aqueous solution at pH 8.5 (....) and at pH 11.1 (——).

largely buried under the stronger negative band at higher energy, has never been observed in the CD spectra of copper(II)–L-amino acid complexes [20], and has been considered as typical for copper(II)–peptide complexes [21]. Previous assignment of the 310 nm CD band to features of the peptide amide group [20] may therefore be incorrect.

The electronic and CD spectra of alkaline solutions of bis(L-serinato)copper(II) kept at pH 11.1 do not show detectable changes in the position or shape of the bands with time, but show only a decrease in their intensity as a result of the reactions undergone by the ligand and of the reduction in copper(II) content of the solution caused by precipitation of copper(I) oxide. The loss of optical activity at room temperature is rather slow and does not show the presence of either an induction period or an auto-

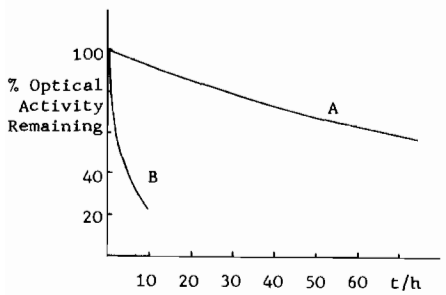

Fig. 3. Loss of optical activity of bis(L-serinato)copper(II) in aqueous 0.05 *M* solution at pH 11.1: (A) 25 °C; (B) 50 °C.

catalytic path (Fig. 3). At 50 °C the loss of optical activity is very fast, having a time for 50% reduction of approximately 4 h. Although there is no spectral evidence for species other than the starting copper–serine complex, it seems unreasonable that serine dealdolization can occur by direct α-carbon–β-carbon bond fission of the coordinated ligand. A different pathway for this reaction can be provided by the formation of a Schiff base between coordinated serine and some carbonyl compound formed by other degradation routes. Pyruvate is probably the catalytic species capable of activating serine dealdolization at the early stages of the reaction, since it is the only carbonyl compound which can be formed by direct oxidation of coordinated serine. In fact, catalysis by pyruvate formed by oxidation of alanine has been recently observed in the racemization of bis(L-alaninato)copper(II) in alkaline solution [12] and it is known that in the presence of pyruvate or other α-keto acids and Cu^{2+} transamination and dealdolization of α-amino-β-hydroxyacids take place easily [3]. The very low stability at high pH of copper(II) Schiff base complexes of N-pyruvylidene-amino acids [22, 23] would prevent the accumulation of such species in the conditions employed here. Indeed, addition of pyruvate to the Cu^{2+}: L-serine (1:2) system at pH 11, up to a 1:2 pyruvate:Cu^{2+} mol ratio, does not produce noticeable changes in the electronic and CD spectra but increases remarkably the rate of the loss of optical activity, which can be taken as an indication of how fast the reactions of serine degradation occur. Formation of pyruvate would give an easy access to all the reactions indicated in Fig. 1, and competing paths for serine degradation will then be provided by the products formed in the early reactions.

In conclusion, a rather complex pattern of reactions is undergone by the copper(II)–serine system in alkaline medium. There is evidence for the occurrence of three processes (oxidative deamination, dealdolization and racemization) which are usually related to catalysis by pyridoxal or its analogues, and other reactions are likely to occur since the products isolated do not reach the material balance of serine degradation. The presence of reduced copper suggests that some reacting species is involved in redox reactions, and we are currently trying to characterize all the products of serine degradation. The results reported here may provide some additional complication to the full understanding of pyridoxal model systems, since they show that alternative routes to pyridoxal catalysis are accessible to copper(II)–amino acid systems without need of intervention by external activating species. This could be of relevance considering a number of amine and amino acid oxidase enzymes requiring copper for activity, for which participation of pyridoxal is still disputed or has been disproved [24].

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