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Decomposition of O-Phosphoserine Accelerated by Copper(II) Complexes

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The decomposition of O-phosphoserine accelerated by the formation of ternary copper(II) complexes with other bidentate ligands has been kinetically investigated in aqueous solutions. The reaction was assigned to a β-elimination and the observed rate constant was expressed as a linear function of $[OH^-]$.

For the hydrolysis of O-phosphoserine(serine-3-phosphate; SP), there are the following two types of reactions[1)]

phosphoserine + H_2O → serine + orthophosphate (1)
phosphoserine + H_2O → pyruvate + ammonia + orthophosphate (2)

The former is the simple hydrolysis of the phosphate ester bond, while the latter contains the elimination of the phosphate group from the β-position and the sequent decomposition of the serine residue into pyruvate and ammonia. Both reactions closely relate to the enzyme-catalyzed metabolism of amino acids. Therefore, a number of nonenzymatic model reactions have been investigated for a better understanding of the enzyme mechanism. In particular, the β-elimination of electronegative substituents such as a phosphate group is remarkably catalyzed in the presence of pyridoxal and metal ions.[2)] This catalytic effect is attributed to the formation of Schiff base and the coordination to a metal ion which activate highly the α-proton of the amino acid.[3,4)]

This work is concerned with the hydrolysis of O-phosphoserine incorporated in ternary copper(II) complexes CuLSP, where L represents other bidentate ligands; the ternary complexes can be regarded as mimicking a metalloenzyme-substrate complex. The purpose of this paper is to decide which of the two reactions described above occurs in such ternary complexes and to estimate the effect of the partner ligand L on the reaction rate. The ligands used as partners of SP are ethylenediamine(en), N,N'-dimethylethylenediamine(N,N'-dmen), N,N-dimethylethylenediamine(N,N-dmen), histamine(hista), 2,2'-bipyridyl(bpy), 8-hydroxyquinoline-5-sulfonic acid(H_2SHQ), and iminodiacetic acid(H_2ida).

Kinetic runs for the hydrolysis of SP were carried out in aqueous solutions at 60 °C. The initial concentrations of sample solutions, in which copper(II) ion, L, and SP were mixed in a 1:1:1 molar ratio, were 2×10^{-3} M(1 M=1 mol dm^{-3}) and the ionic strength was 0.1 M($NaClO_4$). The pH was set in the range from 9 to 11 so that SP was able to chelate with the carboxylate and amino groups,[5,6)] and maintained constant during each run by use of a pH stat. In this pH range, the phosphate group seems to have no interaction with copper(II) ion,[6)] and hence the structure of the ternary complexes may be illustrated as I in Reaction Scheme. The amounts of orthophosphate and ammonia liberated in the course of reaction were determined spectrophotometrically: phosphate by the molybdate-acetone method[7] (350 nm) or the molybdate-amidol method[8] (800 nm) and ammonia by nesslerization[2] (390 nm).

Figure 1 shows the time-course of the conversion of SP at 60 °C and pH 10.5, estimated from the amount of liberated phosphate. From the diagrams, it turns out that the decomposition of chelated SP is much faster than that of noncoordinated SP and that the promotion effect is not the same for the different partner ligands. By Fig. 2 which shows the comparison of the liberation rates of phosphate and ammonia, it is also proved that the β-elimination is the main hydrolysis course in chelated SP.

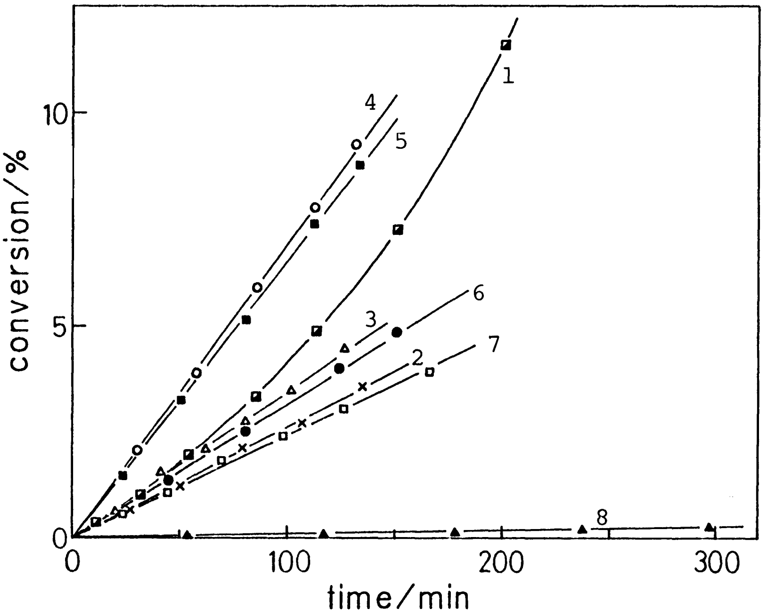


Fig. 1. Time-course of the decomposition of SP in the ternary complexes(CuLSP) at 60 °C and pH 10.5. Numbers in the figure represent L; 1:en, 2:N,N'-dmen, 3:N,N-dmen, 4:hista, 5:bpy 6:SHQ, 7:ida. 8 corresponds to the decomposition of free SP.

The first-order rate constant k_{obsd} in the initial stage can be obtained from the straight lines in Fig. 1,[9] except the en complex. This reaction is also accelerated with the increase of pH. The plots of k_{obsd} against the hydroxide ion concentrations, calculated from the value of Kw at 60 °C(9.614×10^{-14}), gave good linear relations as shown in Fig. 3. Therefore, the rate constant k_{obsd} can be expressed by Eq. 3, as a linear function of the hydroxide ion concentration

$$k_{obsd} = k_0 + k_{OH^-}[OH^-] \qquad (3)$$

This suggests that the decomposition of SP is composed of two reaction paths. The constants, k_0 and k_{OH^-}, are calculated from the lines in Fig. 3 and shown in Table 1. The decomposition rate of free SP is almost constant throughout the pH range used, which agrees with the result reported previously.[1]

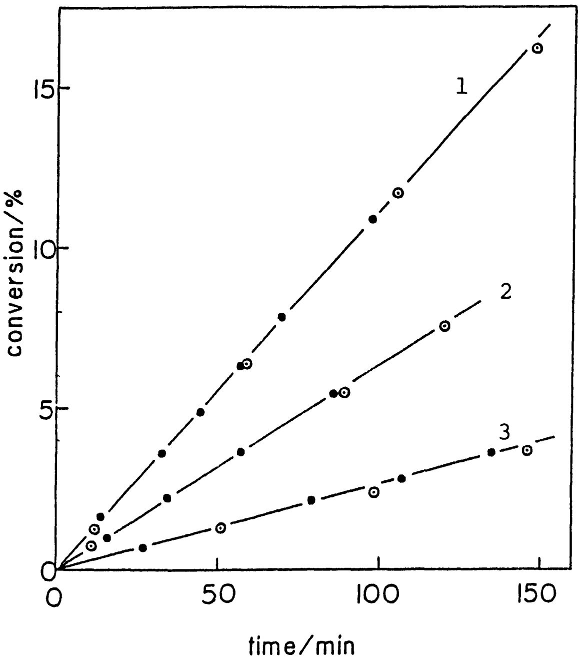


Fig. 2. Comparison of the liberation rates of phosphate(—●—) and ammonia(⊙). 1:hista(pH10.7), 2:bpy (pH10.5), 3:ida(pH10.5)

Since the reaction corresponding to k_{OH^-} shows a first-order dependence on $[OH^-]$, a reaction scheme is proposed, in which the OH^- attack on the α-proton of the serine residue and the formation of a carbanion intermediate II is a rate-determining step. This scheme is essentially identical with that proposed for the pyridoxal-copper(II) ion-catalyzed β-eliminations,[3)] except the absence of Schiff base formation with pyridoxal.

The dissociation of the α-proton is activated mainly by the electronwithdrawing character of copper(II) ion. However, the properties of partner ligands also have some influence on that activation, since a significant partner-dependence is observed for the values of k_{OH^-}: the values in the case of bpy and hista are considerably larger than those for the other partners (Table 1).

The ligands containing heteroaromatic N donors a strong ability to accept π-electrons and this property has been shown to increase the stability of the mixed-ligand complexes with charged ligands such as pyrocatecholate, oxalate, and amino acidate.[10,11)] Bpy and hista have such a π-accepting property and hence probably stabilize the ternary system, particularly the carbanion intermediate, more than the other aliphatic diamines. SHQ is also a heterocyclic N base but chelates with negative charges on itself. Therefore, the π-accepting property of SHQ may be small. With terdentate ida as a partner, SP may form a weak chelate ring with an apical coordination, so that the effect of copper(II) ion would be small.

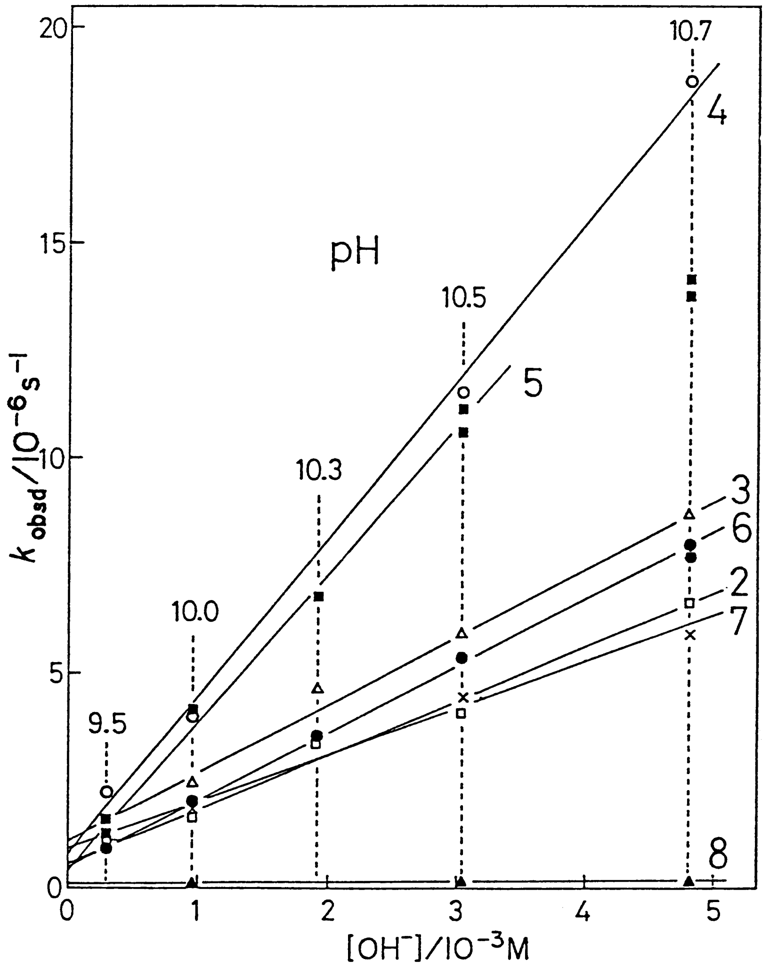


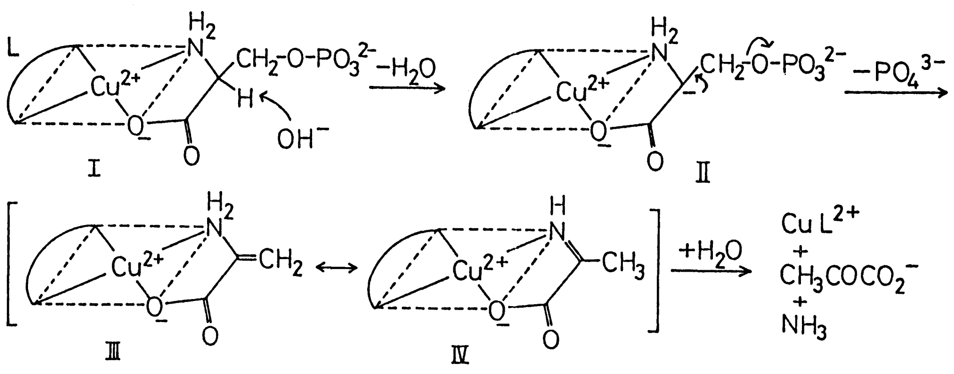
Fig. 3. Relationship between the observed rate constant k_{obsd} and the hydroxide ion concentration calculated from Kw at 60 °C. Numbers in the figure represent L(see Fig. 1).

Table 1. Rate constants for the decomposition of chelated SP obtained from Fig. 3

L	$\frac{k_0}{10^{-6}s^{-1}}$	$\frac{k_{OH^-}}{10^{-3}M^{-1}s^{-1}}$
bpy	0.4	3.5
hista	0.8	3.7
N,N'-dmen	0.6	1.3
N,N-dmen	1.1	1.6
SHQ	0.5	1.6
ida	0.9	1.1
none[a)]	0.1	<0.2

a) Decomposition of free SP.

Proposed Reaction Scheme



In Fig. 3, the data at pH 10.7 for Cu(bpy)SP are not on the line. This probably results from a destruction of the ternary complex which can be seen in the reduced absorvance of its d-d band at pH 10.7.[12)] In the case of Cu(hista)SP, however, the visible spectrum is almost unchanged in the pH range used.[12)] This indicates that, in a high pH region, Cu(hista)SP is rather stable, although the stability constant of Cu(hista)SP is a little smaller than that of Cu(bpy)SP.[6)] Therefore, the further detailed analysis of the ternary species is required to establish the relationship between the decomposition rate and the character of the partner ligands.

The value of k_0 is small for all partners and this reaction path is minor in the pH range used. Two reactions are possible for k_0: one is the water-catalyzed β-elimination[1)] and another the hydrolysis of the phosphate ester bond on which little effect of the partner ligands reaches. In the case of the en complex, the decomposition of SP does not follow the first-order kinetic law(Fig. 1). The origin responsible for this abnormal reaction is not clear at this stage.

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