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Catalytic and Selective Conversion of Glycine into Serine by the Reaction with Formaldehyde in a Neutral Aqueous Solution

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Di- or trinuclear Cu(II) complexes efficiently catalyzed the condensation of glycine with formaldehyde to yield serine at pH 7.3, 50 °C.

The catalytic role of a multinuclear metal center is of current interest in fields related to bio-inorganic chemistry and catalytic chemistry. The α-CH_2 or α-CHR location of an α-amino acid is activated upon chelating with a metal ion, causing a racemization[1] and/or a C–C bond formation[2–7] under basic conditions. For such activation of α-amino acids, a multinuclear metal ion center is expected to be superior to a mononuclear metal ion center.

Pioneering works by Akabori et al. showed that the condensation of glycine, coordinated to Cu(II), with formaldehyde proceeded under basic conditions to yield serine selectively.[3] The condensation is known to occur similarly when a dipeptide[4] or a tripeptide[5] of glycine is used instead of glycine, and both reactions result in the selective modification of the *N*-terminal glycine residue. Because the *N*-terminal amino acid residue of peptides and proteins often plays important roles in determining their properties,[8] the chemical method for the conversion of an *N*-terminal amino acid residue into another one is potentially fascinating. For the practical applications, however, the reaction should be conducted under neutral conditions, in order to avoid undesirable side reactions of peptides.[6,7] This paper deals with the effect of multinuclear Cu(II) complexes for the activation of glycine to promote the condensation with formaldehyde under neutral conditions.

Ligands, **2**,[9] **3**, and **4**,[10] which have two or three bis(2-pyridylmethyl)amine (DPA, **1**) moieties, were used to generate di- or trinuclear Cu(II) complexes (Fig. 1), because DPA is known to bind Cu(II) with a sufficient stability (log K 9.31).[11] The Cu(II) complexes of **1**, **2**, **3**, and **4** were prepared by simply mixing $CuCl_2 \cdot 2H_2O$ and the ligand in an appropriate molar ratio in an aqueous solution just before the reaction.

The results for the reactions of glycine with formaldehyde by using the Cu(II) complexes are summarized in Table 1. Glycine was converted to serine at pH 7.3 and 50 °C in the presence of a catalytic amount of the Cu(II) complex, **2**–$(CuCl_2)_2$, **3**–$(CuCl_2)_2$, or **4**–$(CuCl_2)_3$. The reaction yielded serine as a main product, and a small amount of 2-(hydroxymethyl)serine[4] was formed. In contrast, the yield of serine was low when $CuCl_2$ or **1**–$CuCl_2$ was used.

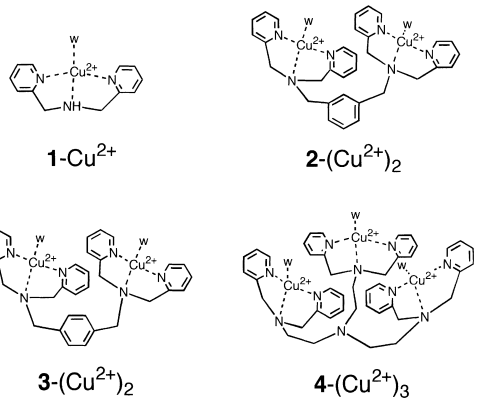

Fig. 1. Structures of the ligands used in this study and proposed coordination modes of the Cu(II) complexes. w denotes an appropriate ligand, typically water.

Table 1. The Condensation of Glycine with Formaldehyde Catalyzed by Cu(II) Complexes at pH 7.3 (200 mmol dm^{-3} HEPES buffer) and 50 °C for 2 h[a)]

Entry	Catalyst	Yield of serine/%	Turnover[c)]
1	$CuCl_2$	1	0.2
2	**1**–$CuCl_2$	1	0.2
3	**2**–$(CuCl_2)_2$	47	9.4
4[b)]	**2**–$(CuCl_2)_2$	19	3.8
5	**3**–$(CuCl_2)_2$	24	4.8
6	**4**–$(CuCl_2)_3$	33	6.6

a) $[glycine]_0$ = 20 mmol dm^{-3}, $[HCHO]_0$ = 3 mol dm^{-3}, [complex] = 1 mmol dm^{-3}. b) $[HCHO]_0$ = 0.3 mol dm^{-3}. c) Based on the copper(II) complex used.

Figure 2 shows α-amino acid distributions depending on the reaction time catalyzed by **2**–$(CuCl_2)_2$. Up to ca. 2 h, the amount of serine increased with the decrease of that of glycine, indicating the selective conversion of glycine into serine. Even

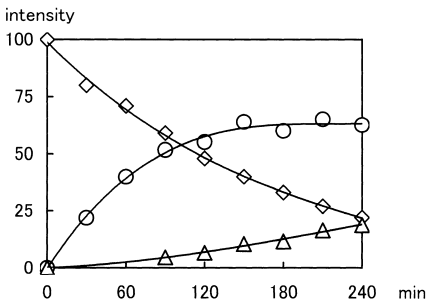

Fig. 2. Effect of reaction time for the condensation of glycine with formaldehyde catalyzed by **2**–$(CuCl_2)_2$. ◇: glycine, ○: serine, △: 2-(hydroxymethyl)serine. $[glycine]_0$ = 20 mmol dm^{-3}, $[HCHO]_0$ = 3 mol dm^{-3}, [**2**–$(CuCl_2)_2$] = 1 mmol dm^{-3}, pH 7.3 (200 mmol dm^{-3} HEPES buffer), 50 °C.

if one prolonged the reaction period, however, the amount of serine did not increase remarkably in spite of the decrease of glycine. Furthermore, the total amount of the starting and resulting α-amino acids decreased, indicating that side reactions besides the condensation occurred by prolonged treatment. Therefore, under the conditions employed, the best result for the selective conversion of glycine into serine was achieved with a short reaction time (ca. 2 h) by using **2**–$(CuCl_2)_2$.

When glycylglycine was used instead of glycine, the reaction with formaldehyde at pH 7.3 and 50 °C yielded serylglycine (20% by **2**–$(CuCl_2)_2$ and 30% by **4**–$(CuCl_2)_3$), but glycylserine was not formed at all. The selective formation of serylglycine indicates that the condensation proceeds via a Schiff base intermediate of glycylglycine and formaldehyde, i.e. *N*-methylideneglycylglycine. Such a mechanism has been proposed for the $CuSO_4$-assisted condensation of glycylglycine with formaldehyde in 0.2 M Na_2CO_3 at 100 °C, which also resulted in the selective formation of serylglycine.[4] It is noteworthy that the present reaction yielded only trace amounts of fragmentary amino acids as byproducts.[4] Because the present reaction was conducted under neutral conditions, the hydrolysis of the peptide bond proceeded only marginally.

The present results explicitly indicate the effectiveness of the multinuclear Cu(II) complexes in the condensation of glycine and formaldehyde under neutral conditions. The Schiff base intermediate, formed from glycine and formaldehyde, is in equilibrium between a major aminomethanol form and a minor imine form in aqueous solutions, and the condensation proceeds via the imine intermediate.[6b] Accordingly, possible roles of a multinuclear Cu(II) center in the present reaction are (1) to bind *N*-methylideneglycine more strongly than a mononuclear Cu(II) and (2) to shift the equilibrium between *N*-(hydroxymethyl)glycine and *N*-methylideneglycine to the latter. Hence, the dissociation of the α-methylene proton of the glycine moiety readily occurs, resulting in the smooth condensation even under neutral conditions.

In conclusion, although further effort to design a suitable catalyst is needed in order to achieve the conversion of glycine into serine more completely, a multiple Cu(II) center was found to be efficient as a catalyst for the condensation of glycine with formaldehyde under neutral conditions.

Experimental

Preparation of 2. **2** was prepared from 1,3-bis(bromomethyl)benzene and bis(2-pyridylmethyl)amine by procedures similar to those reported previously.[9] The product was purified by silica-gel chromatography eluted with chloroform/methanol saturated with ammonia (9:1 v/v), and was isolated as a perchlorate salt. Found: C, 42.94; H, 4.03; N, 9.18%. Calcd for $C_{32}H_{32}N_6 \cdot 4(HClO_4)$: C, 42.69; H, 4.02; N, 9.31%. ^{1}H NMR (D_2O, 300 MHz) δ 8.58 (d, 4H, J = 6Hz), 8.30 (t, 4H, J = 6Hz), 7.84 (d, 4H, J = 7Hz), 7.76 (t, 4H, J = 6Hz), 7.10 (s, 3H), 7.03 (s, 1H), 4.28 (s, 8H), 3.77 (s, 4H).

Preparation of 3. **3** was prepared from 1,4-bis(bromomethyl)benzene and bis(2-pyridylmethyl)amine similarly and was isolated as a chloride salt. Found: C, 58.08; H, 5.83; N, 12.65%. Calcd for $C_{32}H_{36}Cl_4N_6 \cdot H_2O$: C, 57.84; H, 5.76; N, 12.65%. ^{1}H NMR (D_2O, 300 MHz) δ 8.62 (d, 4H, J = 6Hz), 8.38 (t, 4H, J = 8Hz), 7.93 (d, 4H, J = 8Hz), 7.81 (t, 4H, J = 7Hz), 7.08 (s, 4H), 4.27 (s, 8H), 3.74 (s, 4H).

Condensation of Glycine with Formaldehyde Catalyzed by the Cu(II) Complexes. In a typical experiment, to a 9.0-mL portion of an aqueous solution of $CuCl_2 \cdot 2H_2O$ (0.020 mmol), **2**·4($HClO_4$) (0.010 mmol), and glycine (0.20 mmol) in buffer (200 mmol dm^{-3} HEPES, pH 7.5) was added 1.0 mL of aqueous formaldehyde solution (35%). The pH of the solution was adjusted to 7.30, and the resulting solution was heated at 50 °C. After an appropriate reaction period, the reaction was quenched by adding 0.20 mL of concentrated HCl to a 1.0-mL aliquot of the reaction mixture. The resulting solution was analyzed by HPLC: Column, TOSOH TSKgel Aminopak; eluent, citrate buffer (67 mM, pH 3.41); flow rate, 0.6 mL min^{-1}; fluorometrically detected (ex 345 nm, em 455 nm) after the post column reaction with *o*-phthalaldehyde at 60 °C. Condensations catalyzed by $CuCl_2$, **1**–$CuCl_2$, **3**–$(CuCl_2)_2$, or **4**–$(CuCl_2)_3$ were conducted similarly.

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