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Stereochemistry of Hydroxymethylations of *N*-Salicylideneglycyl Dipeptide Copper(II) Complexes. A Kinetically and Also Thermodynamically Controlled Reaction

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(*N*-Salicylideneglycyl-L-valinato)copper(II) or (*N*-salicylideneglycyl-L-isoleucinato)copper(II) was treated with formaldehyde in an aqueous solution under relatively mild conditions. It was found that the resulting seryl residue was D-configuration in the early stage of the reaction. However, the configuration was inverted from D- to L-form after prolonged reaction. The epimerization reactions of authentic (*N*-salicylideneglycyl-D- or L-seryl-L-isoleucinato)copper(II) were carried out under similar conditions. These results indicate that the reaction as a whole is controlled kinetically as well as thermodynamically.

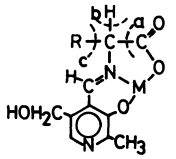
Nonenzymatic pyridoxal-catalyzed reactions of amino acids have been studied.[1-7] These are transamination, decarboxylation, racemization, and α–β bond cleavage (or formation) of α-amino acids. In these reactions, the metal complexes of the Schiff bases of amino acids with pyridoxal have been considered to be the key compounds. The presence of metal ion contributes to the formation of the Schiff base, and the resulting metal complex which has a planar geometry, facilitates the various reactions in the molecule. Because of the electron-attracting property of the metal ion in the complex, the chemical bonds around the α-carbon atom of the amino acid are activated and therefore show various interesting chemical properties in the nonenzymatic pyridoxal-catalyzed reactions of amino acids (Scheme 1).

Several studies on the activation of the α-carbon of amino acids or peptides by the formation of metal complexes have been reported. The reaction of acetaldehyde with glycine copper(II) complex in alkaline aqueous solution resulted in the formation of threonine and allothreonine.[8-11] The reaction of formaldehyde with glycylglycine copper(II) complex gave serylglycine.[12] The reaction of acetaldehyde with triglycine copper(II) complex also occurred to form threonylglycylglycine and allothreonylglycylglycine.[13]

It has been known that the reactions of aldehydes with the copper(II) complex of the Schiff base of glycine with salicyl aldehyde [aqua(*N*-salicylideneglycinato)copper(II)] proceeded smoothly to form hydroxy amino acids.[14] Alkyl halides[15] and acrylonitrile[16] react with the complex to form α-amino acids. These results indicate that the methylene group adjacent to the azomethine group in the copper complex is activated as in the pyridoxal-catalyzed reactions.[17]

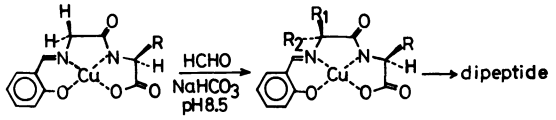
In this study employing the chemical properties of *N*-salicylidene amino acid copper(II) complex, an asymmetric synthesis of a seryl residue was carried out by the hydroxymethylation of (*N*-salicylideneglycyl-L-valinato)copper(II) complex (complex **1**) under relatively mild aqueous conditions (pH 8.5, 45—80 °C) (Scheme 2). The time courses of the reaction were studied. A part of the work was already published as a short communication.[18]

When glycyl-L-valine was used, the formation of D-seryl-L-valine was larger than the corresponding diastereomer in the beginning of the reaction. Prolonged reaction, however, resulted in the formation of L-seryl-L-valine in the reaction mixture. In the case of *N*-salicylideneglycyl-L-isoleucine copper(II) complex (complex **2**), D-seryl-L-isoleucine was formed in the initial stage of the reaction, but the amount of L-seryl-L-isoleucine increased with the reaction time. The formation of D-seryl-L-valine or D-seryl-L-isoleucine in the beginning of the reaction could be explained as a sterically controlled asymmetric Knoevenagel type reaction to the glycine residue. Formaldehyde attacked the methylene group of the copper(II) complex from the less bulky side of the



a: Decarboxylation
b: Racemization, transamination
c: α,β Bond cleavage (or formation)

Scheme 1.



Complex 1
Cu(sal=Gly-L-Val),
R: $-CH(CH_3)_2$
Complex 2
Cu(sal=Gly-L-Ileu),
R: $-CH(CH_3)C_2H_5$

$R_1=H$, $R_2=CH_2OH$
Cu(sal=D-Ser-L-Val)
Cu(sal=D-Ser-L-Ileu)
$R_1=CH_2OH$, $R_2=H$
Cu(sal=L-Ser-L-Val)
Cu(sal=L-Ser-L-Ileu)

Scheme 2.

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Table 1. Time Course of the Reaction of Complex **1** with Formaldehyde

Temp °C	Time h	Ser-L-Val/% D-L	Ser-L-Val/% L-L	Total yield/%	Optical purity of Ser[a]/%	HMSV[b] %	Recovery of Gly-L-Val/%
45	12	8.9	7.2	16.1	11 (D-L)	—	70.8
	25	17.7	14.4	32.1	10 (D-L)	—	26.4
	50	28.8	25.3	54.1	6.5 (D-L)	2	18.5
	75	29.3	26.3	55.6	5.4 (D-L)	2	10.3
	100	35.6	32.2	67.8	5.0 (D-L)	4	8.1
80	1	15.5	13.2	28.7	8.0 (D-L)	—	72.0
	2	24.6	22.7	47.3	4.0 (D-L)	0.7	51.8
	4	32.7	32.1	64.8	0.9 (D-L)	2	23.5
	6	39.1	39.2	78.3	0.1 (L-L)	4	13.7
	12	32.0	38.4	70.4	9.1 (L-L)	10	1.8
	25	16.0	22.4	38.4	16.7 (L-L)	20	Trace
	50	1.9	2.9	4.8	21 (L-L)	25	Trace

a) Optical purity $= |(\text{D-L}-\text{L-L})/(\text{D-L}+\text{L-L})| \times 100\%$. b) α-(Hydroxymethyl)seryl-L-valine.

planar molecule (from back side of the paper in Scheme 2).

Small amounts of the reaction mixture of Cu(sal=Gly-L-Val) and formaldehyde were withdrawn during the reaction, being treated with acid to decompose the copper complex. The resulting two diastereomers, D-seryl-L-valine and L-seryl-L-valine, and unchanged glycyl-L-valine were detected by an amino acid analyzer. The results are summarized in Table 1. The yields of seryl-L-valine increased up to 68% under the reaction conditions at 45 °C for 100 h, but the optical purities of D-serine residue decreased from 11 to 5%. At 80 °C, total yields of newly formed seryl-L-valine increase up to 78% after 6 h and then decrease gradually. In the early stage of the reaction, excess of D-seryl-L-valine was found in the reaction mixture. After 6 h, however, the content of L-seryl-L-valine increased. The increase of L-seryl-L-valine could be explained by the epimerization of the seryl residue in the dipeptide complex. The recovery of unchanged glycyl-L-valine decreased gradually and became trace amount after prolonged reaction at 80°C.

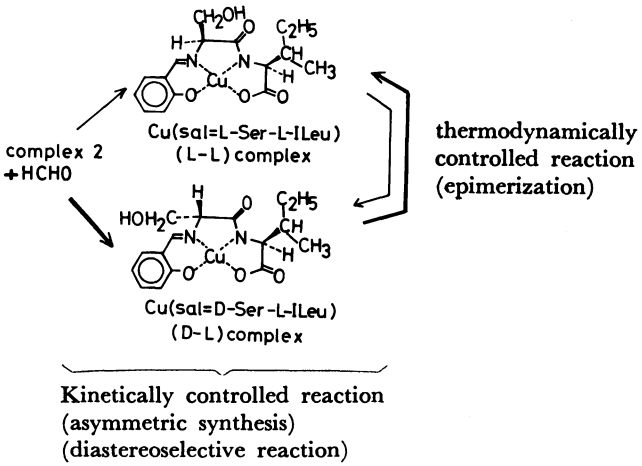
Table 2 shows the time course of the reaction of (*N*-salicylideneglycyl-L-isoleucinato)copper(II) (complex **2**) with formaldehyde at 60, 70, and 80 °C. At 60 °C, the yield of seryl-L-isoleucine reaches 63% after 15 h and the configuration of the newly formed seryl residue is D-form in the early stage of the reaction. Inversion of the configuration from D to L, however, takes place after 18 h as in the reaction of the glycyl-L-valine complex and the ratio of L-L-dipeptide to D-L-dipeptide increases gradually after longer reaction time. Similar behaviors were observed in the reactions at 70 and 80 °C. An unknown peak was detected in the reaction mixture on prolonged reaction by an amino acid analyzer. The hydrolysis of the reaction mixture showed the increase of the peak of α-(hydroxymethyl)serine. Therefore, the peak which is eluted more rapidly than the peaks of glycyl-amino acids and seryl-amino acids, was assumed to be due to α-(hydroxymethyl)seryl-L-valine (HMSV) and α-(hydroxymethyl)seryl-L-isoleucine (HMSI). Tables 1 and 2 show that the yields of HMSV and HMSI increase steadily depending on the reaction time, and the yield reaches about 20% at 80 °C. Small amounts of free amino acids such as serine, glycine, and valine or isoleucine were also found in the reaction mixtures. These amino acids formed by the hydrolysis of dipeptides during the reaction increased slowly with the reaction time. Thus the decrease of the total yield of seryl dipeptides could be caused by 1) hydrolysis of dipeptides, 2) formation of α-(hydroxymethyl)seryl dipeptides, and 3) decarboxylation, deamination, and other degradation of dipeptides.

After the reaction of complex **1** or **2** with formaldehyde, the reaction mixture was decomposed with acid and the resulting dipeptides were hydrolyzed. The amino acid mixtures were treated with 2,4-dinitroflouorobenzene and the resulting 2,4-dinitrophenyl derivatives of valine and isoleucine were separated by Celite column chromatography. The specific rotations showed that those derivatives were almost optically pure. The amino acid analysis of the hydrolyzed isoleucine exhibited no peak of alloisoleucine which would be generated by epimerization. These findings indicate that the epimerization of the dipeptide takes place only at the N-terminal amino acid and does not take place at the C-terminal of the dipeptide complex. The (D-L)-complex which is the major product of the asymmetric Knoevenagel type reaction in the early stage of the reaction, is epimerized to the (L-L)-complex under the conditions employed in the reaction, as shown in Scheme 3.

Table 2. Time Course of the Reaction of Complex **2** with Formaldehyde

Temp °C	Time h	Ser-L-Ileu/% D-L	Ser-L-Ileu/% L-L	Total yield/%	Optical purity of Ser[a]/%	HMSI[b] %	Recovery of Gly-L-Ileu/%
60	1	5.6	5.2	10.8	3.7 (D-L)	—	89.1
	4	17.1	14.6	31.7	7.9 (D-L)	—	65.4
	8	26.0	24.2	50.2	3.6 (D-L)	0.7	43.4
	12	30.5	29.0	59.5	2.5 (D-L)	1.2	26.4
	15	31.5	31.1	62.6	<1 (D-L)	1.6	22.0
	18	29.4	30.2	59.6	1.3 (L-L)	1.9	17.2
	24	25.5	26.9	52.4	2.9 (L-L)	2.9	9.1
	48	21.8	25.3	47.1	8.4 (L-L)	4.1	Trace
	72	12.1	17.3	29.4	17.5 (L-L)	4.3	Trace
70	1	12.4	11.0	23.4	6.0 (D-L)	—	73.6
	2	21.6	18.5	40.1	7.7 (D-L)	—	52.9
	3	25.8	22.9	48.7	6.0 (D-L)	1.4	37.5
	6	30.7	29.0	59.7	2.8 (D-L)	2.9	15.2
	8	31.7	31.6	63.3	<1 (D-L)	3.1	8.8
	10	31.2	33.0	64.2	2.8 (L-L)	3.5	5.4
	12	27.4	33.0	60.4	9.3 (L-L)	4.3	2.9
	24	15.5	22.4	37.9	18.2 (L-L)	6.7	Trace
80	1	18.3	15.7	34.0	7.7 (D-L)	—	63.3
	2	35.1	31.2	66.3	5.9 (D-L)	1.0	25.8
	3	37.0	36.9	73.9	<1 (D-L)	1.5	16.4
	6	30.4	34.2	64.6	5.9 (L-L)	4.2	4.2
	12	14.3	19.5	33.8	15.4 (L-L)	14.7	Trace
	24	10.6	15.5	26.1	18.8 (L-L)	20.7	Trace

a) Optical purity = $|(\text{D-L}-\text{L-L})/(\text{D-L}+\text{L-L})| \times 100\%$. b) α-(Hydroxymethyl)seryl-L-isoleucine.



Scheme 3.

When the authentic *N*-salicylidene complexes composed of L-seryl-L-isoleucine and D-seryl-L-isoleucine were synthesized and treated at 80 °C in aqueous solution in the presence of sodium hydrogencarbonate, epimerization took place simultaneously. The time course of the reaction is shown in Table 3. Starting from (L-L)- or (D-L)-complex, epimerization reached an equilibrium after 24 h and the ratio of (L-L):(D-L) was about 6 : 4. Therefore it could be considered that the epimerization is a typical thermodynamically controlled reaction, and the first-order asymmetric transformation takes place between the two diastereomeric complexes in the reaction mixture.

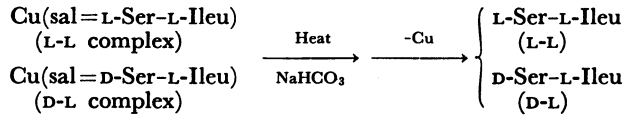
The reason for the enrichment of (L-L)-complex in the equilibrium is unknown at the present stage, although the (L-L)-complex is usually considered to be less stable thermodynamically than the (D-L)-complex because of the steric repulsion between the two side chains of serine and isoleucine.

The asymmetric Knoevenagel type reaction of complex **1** or **2** with formaldehyde could be regarded as a kinetically controlled reaction. Therefore, the whole reaction is controlled by two different mechanisms, i.e. kinetically controlled reaction in the early stage of the reaction, which causes the formation of D-seryl dipeptides, and also thermodynamically controlled reaction which leads to the formation of L-seryl dipeptides in the later stage of the reaction.

Experimental

Preparation of Authentic Dipeptides. Glycyl-L-valine and glycyl-L-isoleucine were purchased from Sigma Chemical Company, St. Louis Mo. U.S.A. Authentic

Table 3. Epimerization of Diastereomeric Complexes



Temp/°C	Time/h	Cu(sal=L-Ser-L-Ileu)		Cu(sal=D-Ser-L-Ileu)	
		L-L/%	D-L/%	L-L/%	D-L/%
80	0.5	83.4	16.4	32.0	68.0
	1	81.0	19.0	44.5	55.5
	2	75.6	24.4	52.7	47.3
	3	72.7	27.3	53.5	46.5
	6	66.3	33.7	56.0	44.0
	12	58.0	42.0	57.9	42.1
	24	57.8	42.2	58.1	41.9

Table 4. Characterization of Dipeptides

Dipeptide	Mp(decomp) θ_m/°C	$[\alpha]_D$	Elemental analysis/%			
				C	H	N
L-Ser-L-Val	224—226	−1.4	Found	46.83	7.82	13.50
		(*c* 2.0, 1 M[a] HCl)	Calcd	47.04	7.89	13.71
D-Ser-L-Val	214—216	−28.3	Found	47.33	7.95	13.87
		(*c* 2.0, 1 M HCl)	Calcd	47.04	7.89	13.71
L-Ser-L-Ileu	202—203	−4.9	Found	49.82	8.19	12.88
		(*c* 1.0, Water)	Calcd	49.52	8.31	12.83
D-Ser-L-Ileu	212—213	−25.2	Found	49.74	8.16	12.83
		(*c* 1.0, Water)	Calcd	49.52	8.31	12.83

a) 1 M = 1 mol dm^{-3}.

seryl dipeptides were synthesized by coupling reactions of *N*-benzyloxycarbonyl-L- or D-serine and L-valine or L-isoleucine benzyl ester using dicyclohexylcarbodiimide. Coupled products were recrystallized from ethyl acetate and petroleum ether. The yields of the products are in a range of 75—87%. The *N*-benzyloxycarbonyl dipeptide ester (1.0 g) was dissolved in a mixture of *t*-butyl alcohol and water (7:3), and the solution was applied to catalytic hydrogenolysis with 5% palladium on charcoal (1.0 g) under 1 atmosphere of hydrogen at room temperature for 6 h. After the catalyst was removed by filtration, the filtrate was evaporated to dryness under reduced pressure, and the residue was recrystallized from water and ethanol. The properties of the synthesized dipeptides are listed in Table 4. These dipeptides are chromatographically pure and the retention times of glycyl and seryl dipeptides observed by an amino acid analyzer are summarized in Table 5.

Preparations of (*N*-Salicylideneglycyl-L-isoleucinato)copper(II) and (*N*-Salicylideneglycyl-L-valinato)copper(II). Glycyl-L-isoleucine (2.5 mmol) was dissolved in a mixture of water and ethanol (2:1). To this solution, freshly distilled salicylaldehyde (2.5 mmol) and copper(II) chloride (2.5 mmol) were added. Aqueous potassium hydroxide was added to the solution, and the pH was adjusted to about 10. The solution was stirred for 15 min at room temperature. After filtration, the filtrate was evaporated to dryness under reduced pressure and purplish needles were obtained. The product was recrystallized from hot water–ethanol. molecular formula: KCu(sal=Gly-L-Ileu)· $1.5H_2O$; λ_{max} 558 nm(ε=185 M^{-1} cm^{-1}); Found: C, 42.66; H, 4.30; N, 6.77%; Calcd for $C_{15}H_{20}O_{5.5}N_2CuK$ C, 42.99; H, 4.82; N, 6.69%.

(*N*-Salicylideneglycyl-L-valinato)copper(II) was synthesized from glycyl-L-valine and bis(salicylaldehydato)copper(II) in a water–ethanol (2:1) mixture by adjusting the pH value to about 9, being isolated as barium salt. Found: C, 35.94; H, 4.52; N, 6.30%; Calcd for $C_{14}H_{22}O_{7.5}N_2CuBa_{0.5}$ C, 35.73; H, 4.72; N, 5.95%.

Hydroxymethylation of (*N*-Salicylideneglycyl-L-isoleucinato)copper(II) (Complex 2) The complex 2 (7.5×10^{-5} mol) was dissolved in 45 ml of water containing 1.5 mmol of $NaHCO_3$ and the pH of the solution was about 8.5. To this solution, 35% formaldehyde (9 mmol, 0.75 ml) was added and the reaction mixture was kept at a constant temperature under nitrogen atmosphere. During the reaction, small amounts of the sample was withdrawn from the reaction mixture, and the complex was decomposed with diluted hydrochloric acid. The resulting solution was applied to a Dowex 50 column. After the column was washed with water, peptides and amino acids were eluted with aqueous ammonia (1 mol dm^{-3}). The eluate was evaporated to dryness under reduce pressure. The residue was dissolved in water and an appropriate amount of the solution was applied to an amino acid analyzer to determine the diastereomeric ratio of the newly formed dipeptide and the amount of unchanged dipeptide. The

Table 5. Chromatographic Properties of Glycyl and Seryl Dipeptides[a)]

Dipeptide	Retention time/min	c-Value[b)]
Gly-L-Val	98	108
D-Ser-L-Val	78	106
L-Ser-L-Val	92	105
Gly-L-Ileu	128	114
D-Ser-L-Ileu	93	116
L-Ser-L-Ileu	120	118

a) Yanagimoto LC-5S amino acid analyzer; column, 0.8×50 cm; resin, cation-exchang resin SCX1001; elution buffer, pH 4.25 citrate buffer; temperature 53 °C. b) Observed constants for the integrations of peaks obtained by applying 1 μmol of each dipepeid.

hydroxymethylation of complex 1 was also carried out in a similar way.

Epimerization between (*N*-Salicylidene-L-seryl-L-isoleucinato)copper(II) and (*N*-Salicylidene-D-seryl-L-isoleucinato)copper(II). *N*-Salicylidenedipeptide complexes were prepared by mixing of L- or D-seryl-L-isoleucine (7.5×10^{-5} mol), salicylaldehyde, and copper(II) chloride in an aqueous ethanol solvent with 45 ml of an aqueous solution of 1.5 mmol of $NaHCO_3$. The solution was kept at 80 °C for epimerization under nitrogen atmosphere. Small amounts of the reaction mixture were collected at varying times, being treated with a Dowex-50 column. The resulting peptides and amino acids were analyzed by an amino acid analyzer. The results are shown in Table 3.

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