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ASYMMETRIC TRANSFORMATION OF AMINO ACIDS IN N-SALICYLIDENE AMINO ACYL-L-ISOLEUCINATOCOPPER(II)

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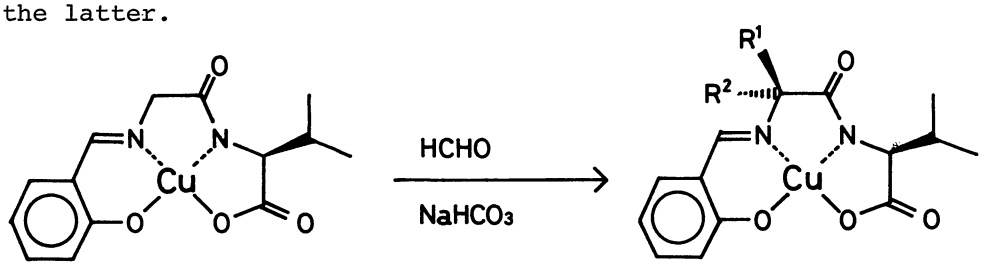
A new asymmetric transformation of amino acids was described. Thus, when N-salicylidene-D-alanyl-, D-phenylalanyl-, or D-phenylglycyl-L-isoleucinatocopper(II) was incubated at pH 8.5 and 80°C, the resulting mixture at equilibrium contained a complex of L-L-dipeptide in the contents of 63-76%. The epimerization of dipeptides is based on the enhanced activity of N-terminal amino acids through complex formation.

The reactivity of Gly[1] coordinated to copper(II) has been often utilized in reactions with aldehydes or alkyl halides to afford β-hydroxy or alkyl amino acids [2-8]. We previously reported a stereoselective reaction of formaldehyde with N-salicylideneglycyl-L-valinatocopper(II)[9] (Fig. 1). The predominant configuration of the N-terminal Ser produced from the Gly residue was D at the early stage of the reaction, whereas L-Ser subsequently exceeded the D-Ser residue. The overall reaction seemed to be kinetically as well as thermodynamically controlled. Thus, the addition of the methylene group in a Gly residue to formaldehyde from the less hindered side of the complex produces D-Ser-L-Val (D-L) complex, which was demonstrated to be in a thermodynamical equilibrium with an L-L complex resulting in an excess of the latter.

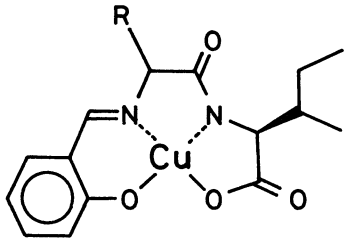


Cu^{II}(Sal=Gly-L-Val)

R^1=H, R^2=CH_2OH : Cu^{II}(Sal=D-Ser-L-Val)
R^1=CH_2OH, R^2=H : Cu^{II}(Sal=L-Ser-L-Val)

Fig. 1. Reaction of N-salicylideneglycyl-L-valinatocopper(II) with formaldehyde
Abbreviation; Sal:salicylidene

In the present communication we wish to report an asymmetric transformation of D- to L-amino acids (Ala, Phe, Phg) in dipeptides utilizing the above-mentioned equilibrium of N-salicylidene amino acyl-L-isoleucinatocopper(II) (Fig. 2). In this study L-Ile was employed as the C-terminal because of the facility in detection of D-alloIle produced by a possible undesirable racemization of the L-Ile residue.



R	N-terminal residue
CH_3	Ala
$CH_2-C_6H_5$	Phe
C_6H_5	Phg

Fig. 2. N-salicylidene amino acyl-L-isoleucinatocopper(II)

The peptides used here were prepared through coupling of benzyloxycarbonyl-D- or L-amino acid and L-Ile benzyl ester p-toluenesulfonate with dicyclohexylcarbodiimide followed by catalytic hydrogenation for deprotection in the total yields of 65-70% (Fig. 3). Each peptide was then subjected to the reaction with bis-salicylaldehydecopper(II) to provide the desired N-salicylidene amino acyl-L-isoleucinatocopper(II) as dark violet crystals in the yields of 46-65% (Fig. 3).

A typical procedure for epimerization of a complex is as follows. A complex (3.0 μmol) containing a peptide of L-L or D-L configuration was dissolved in 0.03M aqueous $NaHCO_3$ (2 ml), precipitated $BaCO_3$ filtered off and the pH of the solution adjusted to 8.5 with 0.1N HCl. The mixture was incubated at 80°C in a sealed tube. After a definite time of heating, the reaction was stopped by adjusting the pH of the solution to 3 with 2N HCl. The epimerized dipeptide was isolated by ion-exchange chromatography (Dowex 50, H form, 0.5^{ϕ} x 1.5 cm column, an eluting agent: 3N ammonia water). The ratio of two diastereomers (L-L and D-L form) of dipeptide was determined with an amino acid analyzer. Table 1 summarizes the analytical con-

$$\text{Z-AA-OH} + \text{H-Ile-OBzl}\cdot\text{Tos-OH} \xrightarrow[\text{HOBt}]{\text{DCC}} \text{Z-AA-Ile-OBzl} \xrightarrow{H_2 - Pd} \text{H-AA-Ile-OH}$$

$$\xrightarrow{Cu^{II}(Sal)_2} Cu^{II}(\text{Sal=AA-Ile})$$

Fig. 3. Synthesis of N-salicylidene dipeptide copper(II) complex
Abbreviations; Z:benzyloxycarbonyl, DCC:dicyclohexylcarbodiimide, HOBt:N-hydroxybenzotriazole, Sal: salicylidene or salicylaldehyde, AA:amino acid, Tos-OH:p-toluenesulfonic acid

Table 1. Analytical conditions and retention times of diastereomeric dipeptides

Dipeptide	Buffer (Citrate)	Retention time (min) D-L	L-L
Ala-Ile	pH 4.25 15% EtOH	84	96
Phe-Ile	pH 6.03 10% EtOH	50	30
Phg-Ile	pH 5.28	50	35

Yanagimoto LC5-S Amino Acid Analyzer

Column: Cation exchange resin SCX 1001, 0.8^{ϕ} x 30 cm

Temp.: 54°C

ditions and the retention times of the dipeptides.

The time-courses of the diastereomeric ratio were illustrated in Fig. 4 as to D- and L-Phe-L-Ile. As demonstrated in our previous study[10], the L-L and D-L isomers obviously coexist in equilibrium, since the curve obtained in the experiments using the L-L isomer coincides with that using the D-L isomer at reaction times longer than 100 hours.

In preliminary experiments the influences of pH and temperature on the epimerization were examined using Phe-Ile and Phg-Ile. Both pH (7.0, 8.5, 9.0, 10.0) and temperature (40, 60, 80°C) had almost no effect on the contents of isomers at equilibrium, but only affected the reaction rates. In addition, under these conditions, no decrease in the UV absorption of the reaction mixture was observed in the course of the reaction and no D-alloIle peptide was detected after the reaction, indicating that neither decomposition of the complexes nor racemization of the L-Ile residues took place.

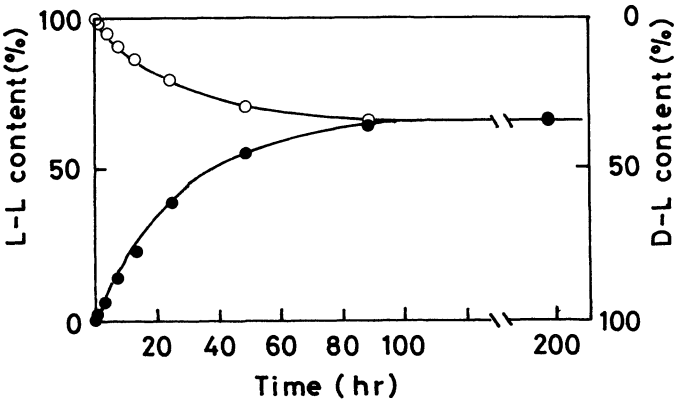


Fig. 4. Time-courses of the diastereomeric ratio in the epimerization of N-salicylidene-D- and L-phenylalanyl-L-isoleucinato-copper(II)

Reaction conditions; pH 8.5, 80°C

Asymmetric transformation of Ala, Phe, and Phg was successfully performed at pH 8.5 and 80°C according to the procedure stated above (Table 2). Especially with respect to D-Phg-L-Ile, the transformation

Table 2. Asymmetric transformation of N-terminal amino acids in dipeptide complexes

Dipeptide	k_1 (s^{-1})*	Content of L-L dipeptide(%)**
Ala-Ile	3.5×10^{-6}	63.0
Phe-Ile	8.2×10^{-6}	66.1
Phg-Ile***	1.3×10^{-4}	76.0

Reaction conditions: pH 8.5, 80°C

* First order reaction rate constant from L-L to D-L

** Content of L-L dipeptide at equilibrium

*** The data were obtained by experiments using L-L dipeptide.

proceeded so easily that on every attempt to prepare its copper complex only the complex containing L-Phg residue was obtained in the yield of 45%. This second-order asymmetric transformation of Phg will be reported elsewhere. Similar situation was experienced on preparation of the D-Phe-L-Ile complex, although in this case the desired complex could be obtained only when the work-up was carried out with great care.

In the present study, a new asymmetric transformation of amino acids in dipeptides was discovered by taking advantage of the lability of α-methine in N-terminal amino acids of N-salicylidene dipeptide copper(II) complex. It is extremely interesting that a complex containing an L-L dipeptide is enriched at equilibrium although it is considered to be thermodynamically unstable due to the steric repulsion of two side chains in the dipeptide. Further studies are now in progress in order to elucidate factors governing the stability of these complexes.

References and notes

1) Abbreviations according to IUPAC-IUB recommendation, J. Biol. Chem., 247, 977 (1972), are used. Phg: phenylglycine.

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10) Unpublished data, which will be soon reported elsewhere.

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