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Enzymatic Preparation of Optically Active Silicon-Containing Amino Acids and Their Application

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Abstract

Optically active 3-trimethylsilylalanine (TMS-Ala) was prepared by hydrolysis of *N*-acetyl-DL-TMS-Ala catalyzed by acylase I (aminoacylase; *N*-acylamino-acid amidohydrolase, EC 3.5.1.14). Acylase I from porcine kidney (PKA) was found to be more effective than that from *Aspergillus melleus* in the preparation of L-TMS-Ala. Under the optimized conditions, optically pure L-TMS-Ala (>99% enantiomeric excess, ee) was obtained with a 72% yield. Furthermore, a highly optically pure D-TMS-Ala (96% ee) could also be obtained with a 76% yield by chemical hydrolysis of the residual substrate. Enzymatic synthesis of peptides containing TMS-Ala was also attempted in ethyl acetate. Benzyloxycarbonyl (Z)-L-TMS-Ala served as the substrate for thermolysin, whereas L-TMS-Ala-OMe was inactive as the amino component. In the case of inhibitory activity of dipeptides toward thermolysin, L-Leu-(L-TMS-Ala) was found to be a more potent inhibitor than L-Leu-L-Leu, which is known to be one of the most effective inhibitors of thermolysin among the dipeptides consisting of natural amino acids.

Index Entries: Enzymatic preparation; silicon-containing amino acid; trimethylsilylalanine; acylase I; dipeptide; thermolysin; inhibitor.

Introduction

There have been only a few reports on enzymatic preparation of organosilicon compounds in spite of its usefulness in the field of synthetic organic chemistry. Organosilicon compounds are not present in nature, although silicon is the second most abundant element in the Earth's crust and belongs to the same group in the periodic table as carbon, which is one of the most important atoms for life. Organosilicon compounds show unique properties compared to conventional organic compounds owing to

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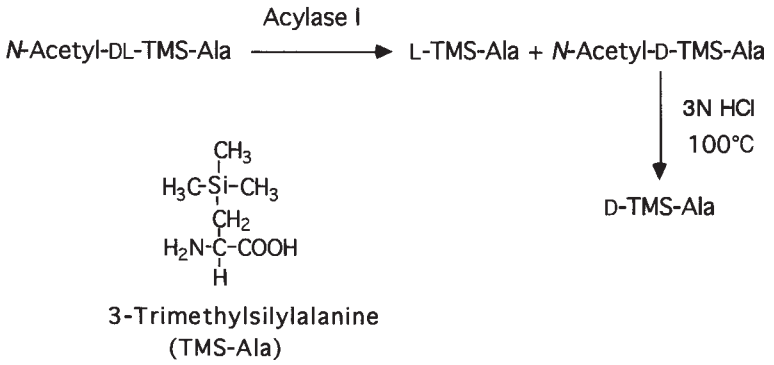

Fig. 1. Preparation of optically active TMS-Ala with acylase I.

the specific characteristics of the silicon atom, such as a larger atomic radius and smaller electronegativity, than those values for carbon atom *(1)*. We have already succeeded in the enzymatic preparation of optically active silicon-containing alcohols by hydrolases and dehydrogenase *(2,3)*. In this study, we selected optically active silicon-containing amino acids as the target because amino acids are very important compounds for organisms. Furthermore, optically active synthetic amino acids are very useful as precursors of pharmaceuticals, agricultural chemicals, food ingredients, and so on *(4–6)*, and novel synthetic amino acids, such as silicon-containing amino acids, and their derivatives can be expected to exhibit improved biological activities and new functions.

Preparation of Optically Active TMS-Ala with Acylase I

We constructed an efficient system for preparing an optically active TMS-Ala by enantioselective deacetylation of chemically synthesized racemic *N*-acetyl-TMS-Ala with acylase I (Fig. 1) *(7)*. TMS-Ala may be useful as a novel hydrophobic amino acid and especially as an analog of leucine because of the similarity in their structures.

Both acylases I from porcine kidney (PKA, grade I; Sigma, St. Louis, MO) and from *Aspergillus melleus* (AMA; Sigma) were examined in the deacetylation of *N*-acetyl-DL-TMS-Ala in 100 m*M* potassium phosphate buffer (pH 7.5) containing 40 m*M* KOH at 40°C, and were found to be able to catalyze the deacetylation (Fig. 2). As far as we know, this is the first report on the bioconversion of silicon-containing amino acid derivatives. It took 24 h to achieve a 50% conversion with 30 mg AMA used. On the other hand, it took only 4.5 h in the case of 0.3 mg of PKA. In both cases, the conversion did not exceed 50%, suggesting that the enantioselectivity should be high. Then we selected PKA for the further studies.

The effects of reaction conditions on the PKA-catalyzed deacetylation of *N*-acetyl-DL-TMS-Ala were investigated to optimize the reaction system. First, the pH dependence of PKA-catalyzed deacetylation was investigated in the presence or in the absence of 0.5 m*M* cobaltous ion, which is known

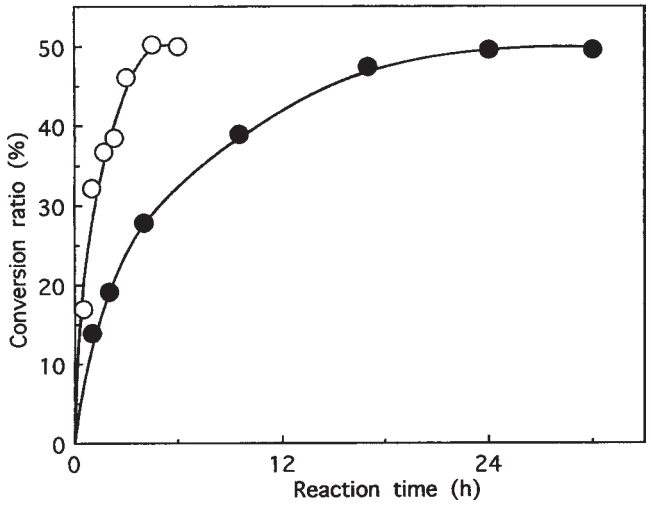

Fig. 2. Time-courses of deacetylation of *N*-acetyl-DL-TMS-Ala with acylase I. Reaction was carried out in 1.5 mL of 100 m*M* potassium phosphate buffer (pH 7.5) containing 40 m*M* of *N*-acetyl-DL-TMS-Ala, 40 m*M* of KOH, and 0.3 mg of PKA (○) or 30 mg of AMA (●).

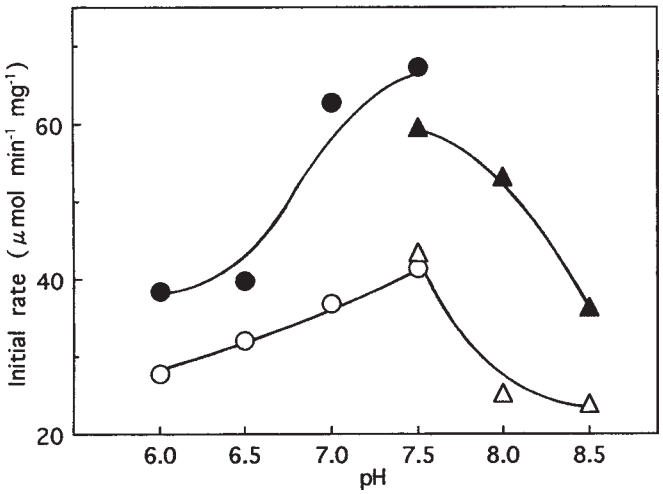

Fig. 3. Effects of pH and Co^{2+} on activity of PKA toward *N*-acetyl-DL-TMS-Ala. Deacetylation of *N*-acetyl-DL-TMS-Ala was carried out in 1 mL of 100 m*M* potassium phosphate buffer (○,●) or Tris-HCl buffer (△,▲) containing 40 m*M* of *N*-acetyl-DL-TMS-Ala and 40 m*M* of KOH, without (○,△) or with 0.5 m*M* of $CoCl_2$ (●,▲).

to affect the activity of PKA *(8)*. As shown in Fig. 3, the optimum pH of the reaction was 7.5 independent of cobaltous ion. This optimum pH is in the range of that of PKA toward the usual *N*-acylamino acids (pH 7.0–8.0) *(9,10)*. Thus, introduction of a silicon atom into the substrate did not change the optimum pH. Figure 3 also shows that the reaction was accelerated by

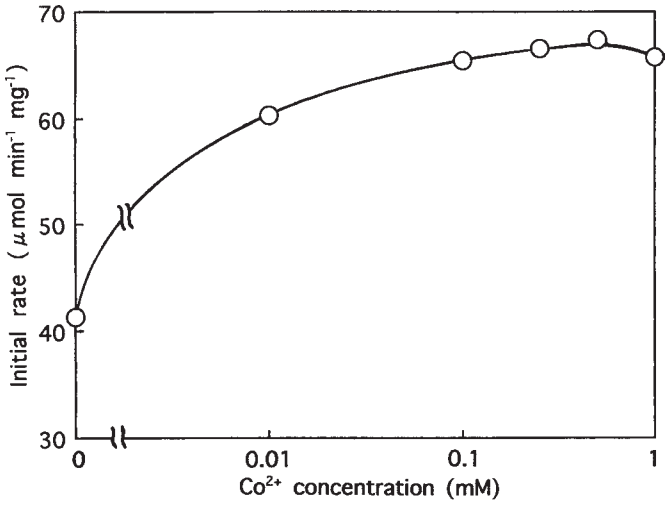

Fig. 4. Effect of Co^{2+} concentration on activity of PKA toward *N*-acetyl-DL-TMS-Ala. Deacetylation of *N*-acetyl-DL-TMS-Ala was carried out in 1 mL of 100 m*M* potassium phosphate buffer (pH 7.5) containing 40 m*M* of *N*-acetyl-DL-TMS-Ala, 40 m*M* of KOH, and 0–1 m*M* of $CoCl_2$.

the addition of 0.5 m*M* cobaltous ion. We then optimized the concentration of cobaltous ion and found that 0.5 m*M* cobaltous ion gave the highest reaction rate under the conditions used (Fig. 4).

Finally, enantioselective deacetylation of 2.436 g (12 mmol) *N*-acetyl-DL-TMS-Ala with 30 mg PKA was attempted under the optimized conditions to demonstrate the utility of this system for the preparation of optically active TMS-Ala. The conversion reached 50% after only 3.5 h of reaction. The yield of L-TMS-Ala was 700 mg (72%) and its optical purity was more than 99% ee (Table 1). A highly optically pure D-TMS-Ala (96% ee) was also obtained with a 76% yield (734 mg) after chemical deacetylation of the residual substrate (Table 1).

Enzymatic Synthesis of Dipeptides Containing TMS-ala

Thermolysin-catalyzed synthesis of dipeptides containing TMS-Ala was also attempted. Considering the structural similarity of TMS-Ala to Leu, we selected Leu-Leu as a target, which is known to inhibit several zinc proteases *(11)*, and effects of introduction of TMS-Ala instead of Leu were investigated.

Table 2 shows that thermolysin from *Bacillus thermoproteolyticus* Rokko (Daiwa Kasei, Osaka, Japan) could catalyze the synthesis of not only Z-L-Leu-L-Leu-OMe but also Z-(L-TMS-Ala)-L-Leu-OMe. In contrast, Z-L-Leu-(L-TMS-Ala)-OMe and Z-(L-TMS-Ala)-(L-TMS-Ala)-OMe were not synthesized with thermolysin even after 120 h when L-TMS-Ala-OMe was used as the amino component. Addition of L-TMS-Ala-OMe (40 m*M*) into the reaction mixture did not inhibit the synthesis of Z-L-Leu-L-Leu-OMe, suggesting

Table 1
Preparation of Optically Pure TMS-Ala[a]

	Yield	% ee
L-TMS-Ala	700 mg (72%)	>99
D-TMS-Ala	734 mg (76%)	96

[a]Enantioselective deacetylation of *N*-acetyl-DL-TMS-Ala was carried out with PKA for 3.5 h. Reaction mixture consisted of 60 mL of 100 m*M* potassium phosphate buffer (pH 7.5) containing 12 mmol of *N*-acetyl-DL-TMS-Ala, 12 mmol of KOH, 30 μmol of $CoCl_2$, and 30 mg of PKA. D-TMS-Ala was obtained by chemical deacetylation of the residual substrate.

Table 2
Thermolysin-Catalyzed Synthesis of Dipeptides Containing TMS-Ala[a]

Carboxyl component	Amino component	Conversion (%)[b]
Z-L-Leu	L-Leu-OMe	86
Z-L-Leu	L-TMS-Ala-OMe	ND[c]
Z-L-TMS-Ala	L-Leu-OMe	87
Z-L-TMS-Ala	L-TMS-Ala-OMe	ND[c]

[a]Reaction was carried out in 10 mL ethyl acetate saturated with 50 m*M* MES/NaOH buffer (pH 6.5) containing 5 m*M* $CaCl_2$ in the presence of 40 m*M* carboxyl component, 40 m*M* amino component, and thermolysin (54 mg) adsorbed on Celite at 30°C.

[b]Conversion after 120 h reaction.

[c]Product was not detected by HPLC.

that TMS-Ala-OMe could not be incorporated into thermolysin so that the corresponding dipeptides were not synthesized. The initial rate of synthesis of Z-(L-TMS-Ala)-L-Leu-OMe was about 1.3 times higher than that of Z-L-Leu-L-Leu-OMe. From the kinetic analysis, it was suggested that the acceleration of the initial rate resulted mainly from a smaller value of K_m in the case of Z-L-TMS-Ala than that in the case of Z-L-Leu. Z-L-TMS-Ala having a more hydrophobic trimethylsilyl group may interact with the S1 subsite of thermolysin more effectively in peptide synthesis than Z-L-Leu since the S1 subsite is known to be wide and hydrophobic *(12–14)*. On the other hand, the S1' subsite of thermolysin is relatively narrow *(14)*. Consequently, TMS-Ala-OMe cannot be incorporated into thermolysin because of the bulkiness of trimethylsilyl group.

Furthermore, the inhibitory activity of dipeptides containing L-TMS-Ala toward thermolysin was examined after the removal of protecting groups to investigate the effects of the introduction of TMS-Ala as mentioned above. Results are summarized in Table 3. L-Leu-(L-TMS-Ala), which was synthesized chemically, was a more potent inhibitor than L-Leu-L-Leu, whereas (L-TMS-Ala)-L-Leu and (L-TMS-Ala)-(L-TMS-Ala) showed weaker activity.

Table 3
Inhibition of Thermolysin-Catalyzed Hydrolysis of *N*-(3-[2-furyl]acryloyl)-Gly-Leu-NH_2 by Dipeptides

	Relative inhibitory activity[a]
L-Leu-L-Leu	100
(L-TMS-Ala)-L-Leu	8
L-Leu-(L-TMS-Ala)	144
(L-TMS-Ala)-(L-TMS-Ala)	24

[a]Inhibitory activity of L-Leu-L-Leu was expressed as 100.

These results demonstrate the possibility of silicon-containing amino acids as novel synthetic amino acids.

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