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Resolution of α-Formyl-ε-acyl-DL-lysines by Acylase

Yoshihiro MINEMATSU, Yasuyuki SHIMOHIGASHI, Michinori WAKI, and Nobuo IZUMIYA

Laboratory of Biochemistry, Faculty of Science 33, Kyushu University, Higashi-ku, Fukuoka 812

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Synopsis. Taka-acylase hydrolyzed an α-formyl derivative of ε-acyl-L-lysine faster than the corresponding α-acetyl derivative. Preparative resolution by crude Taka-acylase afforded ε-formyl or ε-benzyloxycarbonyl-L-lysine from the corresponding α-formyl-ε-acyl-DL-lysine. The action of *p*-toluenesulfonic acid on α-formyl-ε-benzyloxycarbonyl-D-lysine under mild conditions afforded pure ε-benzyloxycarbonyl-D-lysine.

Many ε-acyl-L-lysines have been prepared by enzymatic resolution. For example, Chibata *et al.* prepared ε-benzoyl-L-lysine by the resolution of α-Ac-ε-benzoyl-DL-lysine[1] with Taka-diastase,[2] and Izumiya *et al.* ε-benzoyl-L-lysine from ε-benzoyl-DL-lysinamide by hog kidney amidase.[3] From recent findings that For-L-phenylalanine is hydrolyzed most rapidly among series of acyl-L-phenylalanines by Taka-acylase, we have undertaken the resolution of α-For-ε-acyl-DL-lysines by crude Taka-acylase. α,ε-Diformyl-DL-lysine was prepared by the action of formic anhydride[4] on free DL-lysine, and α-For-ε-Z-DL-lysine by that on ε-Z-DL-lysine.

Prior to the preparative resolution of α-For-ε-acyl-DL-lysines, their optimum pH values were determined by using reference compounds such as α-Ac-ε-Z-DL-lysine and Ac-DL-phenylalanine (Table 1). The hydrolytic rates of α-formyl derivatives and reference compounds at various concentrations were determined at each optimum pH, the relative hydrolytic rates at 0.005 M concentration being given in Table 1. The results indicate that α-For-ε-Z-L-lysine is more susceptible than the corresponding α-acetyl derivative. Although α,ε-diformyl-L-lysine is less susceptible than α-For-ε-Z-L-lysine, the resolution of α,ε-diformyl-DL-lysine might be achieved with a slightly increased amount of crude Taka-acylase. Actually, ε-For(or Z)-L-lysine and α-For-ε-For(or Z)-D-lysine were isolated in good yields from the incubation mixture of α-For-ε-For(or Z)-DL-lysine (see Experimental).

We have attempted selective removal of formyl group by a solution-phase procedure. α-For-ε-Z-D-lysine dissolved in aqueous dioxane was treated with one equivalent of *p*-toluenesulfonic acid at 50 °C, the reaction mixture being examined by TLC at selected intervals. After *ca.* 6 h an α-formyl group in the compound was removed, the ε-benzyloxycarbonyl group remaining intact. Wünsch and Fürst reported that by heating a suspension of α-For-*O*-benzyl-D-serine in 1 M hydrobromic acid until dissolution, *O*-benzyl-D-serine was isolated.[5]

Table 1. Optimum pH and relative hydrolytic rate of α-acylamino acids by crude Taka-acylase

Substrate[a]	Optimum pH	Relative hydrolytic rate
Ac-DL-Phe-OH	7.5	1.0
For-DL-Lys(For)-OH	7.1	1.25
For-DL-Lys(Z)-OH	7.4	2.9
Ac-DL-Lys(Z)-OH	7.6	0.72

a) Substrate concentration in respect to L-form, 0.005 M.

Experimental

The amino acid derivative was determined with a Hitachi amino acid analyzer KLA-5 with spherical resin under the following conditions: flow rate, 30 ml/h; jacket temperature, 55 °C.

For-DL-Lys(For)-OH. DL-Lysine monohydrochloride (3.65 g, 0.02 mol) dissolved in water (15 ml) was put on a column (2×10 cm) of Dowex 50X8 (H^+ form). The column was washed with water and eluted with 2 M NH_4OH (80 ml). The eluate was evaporated *in vacuo*. Evaporation was repeated several times by addition of water, and the residue was dissolved in 99% formic acid (84 ml). Acetic anhydride (28 ml, 0.28 mol) was added to the solution at 0 °C under stirring . After being stirred at 0 °C for 2 h and at room temperature overnight, the solution was evaporated, and the residual solid was recrystallized from EtOH–acetone; yield, 2.71 g (67%); mp 143—144 °C.

Found: C, 47.27; H, 6.96; N, 13.54%. Calcd for $C_8H_{14}O_4N_2$: C, 47.52; H, 6.98; N, 13.86%.

For-DL-Lys(Z)-OH. H-DL-Lys(Z)-OH[6] (0.02 mol) in 99% formic acid (42 ml) was treated with acetic anhydride (0.14 mol) as described above. The residual solid was recrystallized from EtOH–water; yield, 88%; mp 116—117 °C.

Found: C, 58.33; H, 6.56; N, 9.00%. Calcd for $C_{15}H_{20}O_5N_2$: C, 58.43; H, 6.54; N, 9.09%.

Ac-DL-Lys(Z)-OH. This was prepared by the following procedure in a better yield than in the previous synthesis, in which H-DL-Lys(Z)-OH was treated with acetic anhydride and sodium hydroxide.[6] A suspension of H-DL-Lys(Z)-OH (0.01 mol) in AcOH (10 ml) and acetic anhydride (0.014 mol) was refluxed for 10 min. The resulting solution was evaporated, evaporation being repeated several times by addition of water. The residual solid was recrystallized from EtOH–water; yield, 89%; mp 115—116 °C. Reported values; yield, 66%; mp 114 °C.[6]

Crude Taka-acylase. This was prepared by a procedure almost the same as that of Shimohigashi *et al.*[7] Takadiastase powder (200 g) was suspended in water (1100 ml), the insoluble material being filtered off at 0 °C. The filtrate was treated with $(NH_4)_2SO_4$ (165 g, 0.26 saturation) and centrifuged at 0 °C. The supernatant phase was brought to 0.85 saturation with $(NH_4)_2SO_4$ (375 g) and centrifuged. The sediment was suspended in 30% acetone (400 ml) at −5 °C and the suspension was centrifuged. The sediment was dissolved in a small amount of water, dialyzed, and lyophilized; yield of crude Taka-acylase, 1.21 g.

Determination of Optimum pH. To a solution of acyl-DL-amino acid (0.02 mmol) and 0.1 M NaOH (0.2 ml) in a 2-ml flask were added 1/7 M sodium barbital buffer (1 ml) with a specified pH, 1/80 M $CoCl_2$ (0.2 ml), and an aqueous enzyme

solution (0.2 ml) containing a specified weight. The solution was made up to 2.0 ml with water. The reaction mixture was incubated at 38 °C, an aliquot being withdrawn after 1 h. Appearance of L-amino acid was determined with an amino acid analyzer: column, 0.6×10 cm; buffer, standard 0.2 M sodium citrate at pH 3.25 for H–L-Lys(For)–OH, pH 4.25 for H–L-Phe–OH, and pH 5.28 for H–L-Lys(Z)–OH. Calculation of each L-amino acid produced was made on the basis of the color value determined for the corresponding authentic amino acid with the analyzer.

Determination of Relative Hydrolytic Rate of α-Acylamino Acids by Taka-acylase. A mixture of an acyl-DL-amino acid (0.02 mmol), 0.1 M NaOH (0.2 ml), 1/7 M sodium barbital buffer (1 ml) at optimum pH, 1/80 M $CoCl_2$ (0.2 ml) and an aqueous enzyme solution (0.2 ml) was made up to 2.0 ml with water. The rate of hydrolysis at 38 °C was followed by the analyzer under the conditions described above. The hydrolysis of substrates followed zero-order kinetics within experimental error. The relative hydrolytic rate was calculated by taking the hydrolytic rate of Ac-L-phenylalanine as 1.0.

Resolution of For–DL-Lys(For)–OH. H–L-Lys(For)–OH: For–DL-Lys(For)–OH (2.02 g, 10 mmol) was dissolved in aqueous triethylamine, the pH being adjusted to 7.1. To the solution were added crude Taka-acylase (80 mg), 1/80 M $CoCl_2$ (100 ml), and water. The solution (1000 ml) was left to stand at 38 °C, the pH of the solution being adjusted occasionally to 7.1. After 3 days, pH of the solution was adjusted to 5 with formic acid. A small amount of Norit was added, and the mixture was maintained at 50—60 °C for 10 min. To the filtrate was added Dowex 50X8 (H^+ form) (20 ml) at 0 °C. After a few minutes, the resin was collected and washed with cold water (the combined filtrates were used for the preparation of For–D-Lys(For)–OH). The resin was suspended in 2 M NH_4OH (60 ml) and stirred at room temperature for a few minutes. The filtrate was evaporated, and the residual solid was recrystallized from water–EtOH; yield, 0.79 g (91%); $[\alpha]_D^{20}$ +13.9° (*c* 2, saturated $NaHCO_3$). Reported value; $[\alpha]_D^{26}$ +15.5° (saturated $NaHCO_3$).[8]

For–D-Lys(For)–OH: The combined filtrates obtained above were evaporated, and the residual solid was recrystallized from water–acetone; yield, 0.80 g (75%); mp 119—121 °C; $[\alpha]_D^{20}$ +3.3° (*c* 2, H_2O).

Found: C, 45.64; H, 6.85; N, 13.14%. Calcd for $C_8H_{14}O_4N_2 \cdot 1/2H_2O$: C, 45.49; H, 7.16; N, 13.27%.

H–L-Lys–OH·HCl: To a solution of H–L-Lys(For)–OH (1 mmol) in a mixture (5 ml) of dioxane–water (1: 1, v/v) was added TosOH·H_2O (1.1 mmol). After being left to stand at 50 °C for 10 h, the solution was treated with 2 ml of Dowex 50X8 (H^+ form). The resin was eluted with 2 M NH_4OH. The eluate was evaporated, and the residue was dissolved in water, the pH being adjusted to 7 with 1 M HCl. The solution was evaporated, and the residual solid was recrystallized from water–EtOH; yield, 94%; $[\alpha]_D^{20}$ +20.8° (*c* 2, 5 M HCl). Reported value, $[\alpha]_D^{15}$ +20.5° (5 M HCl).[3]

H–D-Lys–OH·HCl: This was prepared from For–D-Lys(For)–OH (0.5 mmol) with TosOH·H_2O (1.1 mmol) as described above; yield, 77%; $[\alpha]_D^{20}$ −20.1° (*c* 2, 5 M HCl).

Resolution of For–DL-Lys(Z)–OH. H–L-Lys(Z)–OH: For–DL-Lys(Z)–OH (0.77 g, 2.5 mmol) was dissolved in aqueous triethylamine, the pH being adjusted to 7.4. To the solution were added crude Taka-acylase (16 mg), 1/80 M $CoCl_2$ (25 ml), and water. After the solution (250 ml) had been left to stand at 38 °C for 3 days, the precipitate formed was collected and washed with water, and the combined filtrates were evaporated to a small volume. 6 M HCl was added to the solution until it reached a pH of 3. An oily product was extracted with EtOAc. The extract was used for the preparation of For–D-Lys(Z)–OH. The aqueous layer and the above precipitate dissolved in a small volume of 1 M HCl were put on a column (2.2×5 cm) of Dowex 50X8 (H^+ form). The column was eluted with 2 M NH_4OH (30 ml). The eluate was evaporated, and the residue was recrystallized from water–EtOH; yield; 0.28 g (80%); mp 233—235 °C (dec); $[\alpha]_D^{20}$ +16.7° (*c* 1, 5 M HCl). Reported value; $[\alpha]_D$ +17.3° (2 M HCl).[9]

For–D-Lys(Z)–OH: The extract obtained above was dried (Na_2SO_4) and evaporated. The residual solid was recrystallized from EtOH–water; yield, 0.31 g (79%); mp 93—95 °C; $[\alpha]_D^{20}$ −10.7° (*c* 2, EtOH). Reported values for the L-isomer; mp 74—78 °C; $[\alpha]_D^{24}$ +9.6° (EtOH).[8]

H–D-Lys(Z)–OH: To a solution of For–D-Lys(Z)–OH (0.3 mmol) in a mixture (10 ml) of dioxane–water (9: 1, v/v) was added TosOH·H_2O (0.33 mmol). After being left to stand at 50 °C for 6 h, aqueous triethylamine was added to the solution until its pH became 7, and the solution was evaporated. The residual solid was recrystallized from water–EtOH; yield, 75%; $[\alpha]_D^{20}$ −16.4° (*c* 1, 5 M HCl). Reported value for the L-isomer; $[\alpha]_D$ +17.3° (2 M HCl).[9]

References

1) Abbreviations used: Ac, acetyl; For, formyl; TosOH, *p*-toluenesulfonic acid; Z, benzyloxycarbonyl.

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