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ENZYMATIC FORMATION OF AN ISOPEPTIDE BOND INVOLVING THE ε-AMINO GROUP OF LYSINE

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Summary: Bacterial protease and lipase act as regioselective preparative catalysts of peptide bond synthesis in anhydrous organic solvents: when a derivative of lysine is used as the amino component, only its ε-NH_2 group reacts (to give an isopeptide linkage).

There is a substantial interest in peptides containing unnatural linkages, i.e., those involving functional groups located in the amino acid side chains, for such structurally unusual peptides may have a number of attractive properties.[1] In particular, the biological characteristics of ε-substituted lysine derivatives are quite distinct from those of the "normal" α-isomers.[2] Enzymatic peptide synthesis has a number of virtues in terms of mild reaction conditions, minimal protection and activation requirements, absence of racemization, and selectivity.[3] It has been invariably assumed that enzymes link the α-carboxyl group of one amino acid to the α-amino group of another; the possibility of the involvement of side chains is usually not even discussed.[4,5]

Kullmann[5] reported the use of α-chymotrypsin as a catalyst in the reaction between N-Boc-L-Tyr-OEt and L-Lys-N_2H_2Ph and implied that only the natural peptide bond (i.e., involving the α-NH_2 group of lysine) was formed. We investigated a similar reaction, between N-Ac-L-Phe-OEt and L-Lys-O-tert-Bu, under analogous conditions (4.7 mL of 0.14 M aqueous carbonate buffer (pH 10.0) containing 32% dimethylformamide, 0.5 and 1.0 mmol of the substrates, respectively, and 4 mg of chymotrypsin, 20°C) and found that in fact after a 1-hr incubation, when the former substrate was exhausted, the ratio of α to ε dipeptide was only 7:3, as determined by HPLC analysis (Waters μBondapak C_{18} column; 30:70 acetonitrile-aqueous buffer (phosphate-triethylamine, pH 3.6) as eluent, 1 mL/min; detection at 220 nm).

In contrast, another protease, that from Bacillus subtilis (subtilisin Carlsberg), afforded an overwhelming (>99%) selectivity toward the ε-NH_2 group in the aforementioned peptide synthesis under the same conditions. Unfortunately, the degree of conversion in this reaction never exceeded 30% because of the competing enzymatic hydrolysis. This problem, however, was eliminated when anhydrous tert-amyl alcohol was employed as the reaction medium for subtilisin-catalyzed peptide synthesis[6]: 2.5 mmol of N-Ac-L-Phe-OEtCl and 3.0 mmol of L-Lys-O-tert-Bu were dissolved in 50 mL of the solvent, followed by the addition of 170 mg of the enzyme (lyophilized from pH 7.8[7]), and the suspension was shaken at 45°C for 24 h. The product,[8] obtained with an 85% isolated yield, was pure by HPLC and ^{1}H NMR, and was found to be[9] exclusively the ε-isomer of N-Ac-L-Phe-L-Lys-O-tert-Bu.

Similar data were obtained when a non-protease, Pseudomonas sp. lipoprotein lipase, was used as a catalyst of peptide synthesis[10] in anhydrous toluene: 0.57 g of the pure (by HPLC and ^{1}H NMR) ε-isomer of the dipeptide was prepared with a 51% isolated yield.[11] It is worth noting that when the Phe and Lys amino acid derivatives (0.1 M each) were non-enzymatically reacted in tert-amyl alcohol at 70°C for 4 days, only a 28% conversion was achieved with the ratio of ε to α isomer of 5:1.

Therefore, subtilisin and lipase (both commercially available enzymes), when used in anhydrous organic solvents, are regioselective practical catalysts of peptide synthesis and with lysine as a nucleophile afford the unnatural, ε peptide linkage. Interestingly, this peptide bond is much more stable toward proteolysis than its α-isomer: after a 1-hr incubation with chymotrypsin (0.2 mg/mL) in aqueous solution (pH 7.6, 10% CH_3CN) at 23°C, 76% of the α-isomer of the Phe dipeptide (10 mM) described above was hydrolyzed, while the ε-dipeptide was completely resistant to the enzymatic hydrolysis under these conditions.

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8. Obtained as a crystalline solid with mp 86-88°C, $[\alpha]_D^{25}$ +19.8° (c 0.4, MeOH). Anal. Calcd for $C_{21}H_{33}N_3O_4$: C, 64.42; H, 8.50; N, 10.73. Found: C, 64.66; H, 8.35; N, 10.67.
9. Structure determination was accomplished by 250 MHz ^{1}H NMR. As inferred from the NMR spectrum of independently synthesized N-Ac-L-Phe-α-L-Lys-O-tert-Bu (using chymotrypsin as a catalyst in water[5] with N-ε-CBZ-L-Lys-O-tert-Bu as the nucleophile), formation of the peptide bond through the α-NH_2 group resulted in a downfield shift of the α-proton from 3.31 to 4.27 ppm (and no appreciable effect on the ε-protons). In contrast, the peptide synthesized using subtilisin in tert-amyl alcohol showed a downfield shift only for the ε-protons from 2.74 to 3.08 ppm (and no other significant changes in the NMR spectrum of the dipeptide's lysine moiety).
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11. The same conditions as for subtilisin, except that the alanine rather than phenylalanine ester was used and the reaction catalyzed by 100 mg/mL lipase was carried out for 3 days. The oily product, purified by silica gel column chromatography, was converted to a HCl salt and crystallized (mp 172-173°C (dec), $[\alpha]_D^{25}$ -6.2° (c 0.4, MeOH)). Anal. Calcd for $C_{15}H_{30}ClN_3O_4$: C, 51.20; H, 8.59; N, 11.94. Found: C, 51.17; H, 8.64; N, 11.83.

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