Stereoconfiguration of Amino Acids in Peptides from a Mycobacillin-Synthesizing Cell-Free System

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The stereoconfiguration of amino acids, as determined by treatment with L-amino acid oxidase, D-amino acid oxidase and L-glutamate decarboxylase (containing L-aspartate decarboxylase activity), in the peptides from a mycobacillin-synthesizing cell-free system is identical with that of the growing mycobacillin peptide chain if its synthesis starts from L-proline and is interrupted at various points by amino acid deprivation.

It has been reported (Sengupta & Bose, 1972) that a mycobacillin-synthesizing cell-free system synthesizes a series of peptides when deprived of certain component amino acids of this cyclic peptide antibiotic. These peptides correspond in composition to fragments of mycobacillin starting from L-proline at the N-terminal end. Mycobacillin, like other peptide antibiotics, contains D-amino acids, i.e. D-glutamic acid and D-aspartic acid (Banerjee & Bose, 1963; Sengupta & Bose, 1971). We therefore decided to ascertain the stereoconfiguration of the aspartic acid and glutamic acid residues in the peptides formed in incomplete systems.

Peptides from the mycobacillin-synthesizing cell-free systems were isolated as described by Sengupta & Bose (1972). These peptides were then hydrolysed with 5M-HCl at 100°C for 5 h. The acid was removed *in vacuo* and the acid-free hydrolysate was dissolved in 1 ml of water. Portions (0.5 ml) of the amino acid mixture so obtained were incubated separately with hog kidney D-amino acid oxidase, snake-venom L-amino acid oxidase and L-glutamate decarboxylase (containing L-aspartate decarboxylase activity) of *Clostridium welchii* (Sigma Chemical Co., St. Louis, Mo., U.S.A.). The amino acid mixture after incubation was deionized on a column of Dowex-50 (H⁺ form) and subjected to paper chromatography to determine the disappearance, if any, of amino acids from the mixture. Solvent systems used in paper chromatography were (a) butan-1-ol-acetic acid-water (4:1:1, by vol.) and (b) aq. 70% (v/v) ethanol. The spots of amino acids when obtained were quantitatively assayed by the ninhydrin method (Giri *et al.*, 1952).

Table 1 shows that the glutamic acid content of the di-, the tri-, the hexa-, the octa- and the undeca-peptide remains unaltered by the treatment with L-glutamate decarboxylase. The glutamic acid of these

peptides is thus in the D-configuration. The aspartic acid of the di- and the tri-peptide is completely oxidized by D-amino acid oxidase, whereas that of the hexa-, the octa- and the undeca-peptide remains unchanged to the extent of 47, 32 and 24% respectively. On the other hand the aspartic acid of the di- and the tri-peptide remains unaltered by glutamate decarboxylase (containing aspartate decarboxylase activity) treatment, whereas that of the hexa-, the octa- and the undeca-peptide remains unchanged to the extent of 48, 62 and 75% respectively by the same treatment. So it appears that the aspartic acid in the di- and the tri-peptide is of the D-configuration, and, since the D/L-isomer ratio of aspartic acid in the hexa-, the octa- and the undeca-peptides is approx. 1:1, 2:1 and 3:1 respectively, these peptides contain 1, 2 and 3 residues of D-aspartic acid and 1, 1 and 1 residue of L-aspartic acid respectively. Table 1 also shows that the other amino acids occurring in the peptide chain of mycobacillin are of the L-configuration. Thus the stereoconfiguration of amino acids in these peptides is identical with that of the growing mycobacillin peptide chain if its synthesis starts from L-proline and is interrupted at various points by amino acid deprivation.

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Table 1. *Stereoconfiguration of amino acids of peptides isolated from a mycobacillin-synthesizing enzyme system*

The incubation mixture for treatment with D- or L-amino acid oxidase consisted of 1.8 ml of 20 mM-sodium pyrophosphate buffer, pH 8.3 (containing 0.02 ml of type C-100 catalase; Sigma Chemical Co.), 0.5 ml of enzyme solution (5 mg of either D- or L-amino acid oxidase/ml of the pyrophosphate buffer) and 0.5 ml of the peptide hydrolysate in the pyrophosphate buffer. The reaction was carried out at 37°C for 180 min in a Warburg apparatus and then terminated by the addition of 2 vol. of ethanol. Amino acids in the supernatant were recovered and determined as described by Sengupta & Bose (1971). The L-amino acid oxidase does not act on L-proline, L-aspartic acid, L-glutamic acid and L-serine (Bender & Krebs, 1950). The incubation mixture for the L-glutamate decarboxylase treatment consisted of 1.50 ml of 0.5 M-sodium acetate buffer, pH 4.2, 0.5 ml of the peptide hydrolysate in the same acetate buffer and 0.5 ml of enzyme (24 mg of enzyme/ml of buffer). The reaction was conducted in a Warburg apparatus at 37°C for 240 min and terminated with 4 vol. of ethanol. Amino acids in the supernatant were recovered and determined as described by Sengupta & Bose (1971).

Peptides from mycobacillin-synthesizing system	Amino acids after L-amino acid oxidase treatment	Amino acids after D-amino acid oxidase treatment (with % recovery)	Amino acids after L-glutamate decarboxylase treatment (with % recovery)	Configuration of amino acids and their molar proportions
(Pro ₁ , Asp ₁)	Pro, Asp	Pro	Pro, Asp (90%)	D-Asp (1), L-Pro (1)
(Pro ₁ , Asp ₁ , Glu ₁)	Pro, Asp, Glu	Pro, Glu (94%)	Pro, Asp (90%), Glu (89%)	D-Asp (1), D-Glu (1), L-Pro (1)
(Pro ₁ , Asp ₂ , Glu ₁ , Tyr ₂)	Pro, Asp, Glu	Pro, Asp (47%), Glu (95%), Tyr	Pro, Asp (48%), Glu (87%), Tyr	L-Pro (1), D-Glu (1), D-Asp (1), L-Asp (1), L-Tyr (2)
(Pro ₁ , Asp ₃ , Glu ₁ , Tyr ₂ , Ser ₁)	Pro, Asp, Glu, Ser	Pro, Asp (32%), Glu (93%), Tyr, Ser	Pro, Asp (62%), Glu (91%), Tyr, Ser	L-Pro (1), L-Tyr (2), L-Ser (1), D-Asp (2), L-Asp (1), D-Glu (1)
(Pro ₁ , Asp ₄ , Glu ₂ , Tyr ₂ , Ser ₁ , Leu ₁)	Pro, Asp, Glu, Ser	Pro, Asp (24%), Glu (91%), Ser, Leu, Tyr	Pro, Asp (75%), Glu (88%), Tyr, Ser, Leu	L-Pro (1), L-Tyr (2), L-Ser (1), L-Leu (1), D-Asp (3), L-Asp (1), D-Glu (2)