A STUDY OF A TUBERCULIN-ACTIVE PEPTIDE FROM MYCOBACTERIA OF STRAIN VALLEE-58

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We have determined the molecular weight, C-terminal residue, and N-terminal sequence of peptide (VI) isolated from a hydrochloric acid extract of mycobacteria of the tuberculosis strain Vallee-58. The determination of the primary structure of the tuberculin-active fragment is of interest for understanding the molecular mechanisms of allergenic activity.

The N-terminal amino acid was identified by means of the DNP derivative using two-dimensional chromatography according to Levy [1]. This showed the presence of 0.75 μmole of alanine per μmole of peptide. The N-terminal sequence was studied by Edman's method of stepwise cleavage [2]. The amino acids regenerated from the PTH derivatives [3] were identified by a combination of high-voltage electrophoresis at pH 1.9 [4] and paper chromatography in the butan-1-ol–pyridine–acetic acid–water (15:10:3:12) system.

A check on the amino acid split off was performed by comparing the amino acid composition of the peptide before and after each stage of degradation. The results obtained permitted the conclusion that glutamic acid is located before the N-terminal alanine.

The C-terminal residue was split off by hydrazinolysis according to Akabory [5]. For the more complete removal of the hydrazides, the reaction mixture was exhaustively extracted with benzaldehyde, isovaleraldehyde, and enanthaldehyde. The free C-terminal amino acid was determined by the two-dimensional electrophoresis-chromatography method in an amino acid analyzer and by chromatography according to Levy after the performance of the reaction with fluorodinitrobenzene [1]. In each case, arginine and ornithine, which is formed from arginine on hydrazinolysis, were found.

The peptide concerned contains two semicystine residues and has a tendency to form a tetramer with mol.wt. 21,880. The molecular weight determined by the gel-filtration method according to Whitaker (of the peptide previously oxidized with performic acid) was 5495.

By comparing the results on the molecular weight and amino acid composition with the results of the determination of the N- and C-terminal residues, the structure of the tuberculin-active peptide can be represented provisionally in the following way: Ala-Glu-(Asp_5, Thr_3, Glu_5, Pro_5, Gly_3, Ala_3, $1/2Cys_2$, Val_3, Met_1, Ile_2, Leu_4, Tyr_1, Lys_1, His_2, Arg_4, Try_1)-Arg.

The above facts indicate that peptide VI is a convenient material for the investigation of the problem with the structure and biological activity of allergens.

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