Isolation of a tripeptide containing α-aminoadipic acid from the mycelium of *Penicillium chrysogenum* and its possible significance in penicillin biosynthesis

Following the observation that L-cystinyl-L-valine is available for penicillin biosynthesis by a pathway not involving breakdown to free amino acids[1], it seemed of interest to examine the mycelium of *Penicillium chrysogenum* for the presence of this compound or of related peptides containing both cystine and valine.

Washed mycelium of *P. chrysogenum* WIS 51-20 F3 from a culture 63 h old which had been grown in the absence of phenylacetate was incubated[2] for 1 h at 24° on a rotary shaker in the presence of phenylacetic acid, giving 10 units penicillin/ml/h. L-[^{14}C]valine was added to the incubation medium in order to facilitate the subsequent isolation of metabolic products derived from this amino acid. After incubation, the mycelium was washed with phosphate buffer and boiled with 75 % ethanol. The extracted amino acids and peptides were purified by adsorption on Zeo-Karb 225 cation-exchange resin in the H^+ form and displacement with dil. ammonia. The eluted material was then treated with performic acid which oxidized cystine and cystine-containing peptides to the corresponding sulphonic acids. These acidic compounds were separated from unchanged material by a second column of Zeo-Karb 225. The aqueous eluate from this column was concentrated to dryness and fractionated by paper chromatography (phenol–water, 5 : 2 *v/v*, with ammonia in the tank) into two ninhydrin-positive bands, A, non-radioactive (R_F, 0.07) and B, radioactive (R_F, 0.20). Electrophoresis on Whatman 3 mm paper in 2 *N* acetic acid resolved band A into cysteic acid and the sulphonic acid corresponding to glutathione (GSO_3H). Similarly, band B was found to contain β,β-dimethylcysteic acid (penicillaminic acid, derived from penicillin by the oxidation procedure and hence labelled after incubation with [^{14}C]valine[3]) and an unknown compound, which was also labelled and had a similar electrophoretic mobility to GSO_3H. Hydrolysis of this compound with 6 *N* HCl for 18 h gave α-aminoadipic acid, cysteic acid and valine in equimolar proportions, the amino acids being identified by 2-dimensional chromatography (butanol–acetic acid–water, 126 : 20 : 54, *v/v/v*) and electrophoresis (0.175 *M* pyridine acetate, pH 5) and estimated on paper[4]. The amount of amino acids liberated on hydrolysis indicated that the peptide was isolated in a yield of about 10 μg/g of fresh mycelium. Free α-aminoadipic acid has also been found in the amino acid fraction of the mycelium.

Reaction of the peptide with 2 : 4-dinitrofluorobenzene[5], followed by partial hydrolysis with 12 *N* HCl at 37° for 48 h yielded the following 6 degradation products (in order of increasing electrophoretic mobility in 0.08 *M* pyridine-acetate buffer at pH 5): (1) valine, (2) cysteicylvaline, (3) DNP-α-aminoadipic acid, (4) cysteic acid, (5) unchanged DNP-peptide, and (6) DNP-α-aminoadipylcysteic acid. Compounds 1–4 were identified by comparison with authentic specimens; the structure of compound 6 is inferred from its electrophoretic mobility, which was very similar to that of DNP-γ-glutamylcysteic acid, and from the absence of radioactivity, indicating that valine had been removed from the starting material. There was, unfortunately, insufficient material available for further degradation of the product.

Abbreviation: DNP-, dinitrophenyl-.

The above evidence indicates that the amino acid sequence of the peptide is α-aminoadipylcysteicylvaline. The change in electrophoretic mobility of the peptide on increasing the pH from 2.1 to 3.5 is identical with that of synthetic γ-glutamylcysteicylvaline whereas the mobility of α-glutamylcysteicylvaline is relatively unchanged. This behaviour shows that the α-carboxyl group of the α-aminoadipic residue is free and hence the structure of the material originally present in the mycelium is δ-(α-aminoadipyl)-cyst(e)inylvaline (I). The configuration of the amino acid residues is not yet known, but the possible biogenetic relationship of I to cephalosporin N[6] is obvious. Although cephalosporin N has not yet been detected in fermentations of *Penicillium chrysogenum*, it is not impossible that it may occur intracellularly in small concentrations. One may speculate further that penicillin biosynthesis involves the cyclisation of the tripeptide I to cephalosporin N followed by an exchange of side-chain for a carboxylic acid, such as phenylacetic acid in the case of benzylpenicillin. An exchange reaction of this type has recently been reported[7]. On this basis, it is likely that the mould *Cephalosporium*, which synthesises cephalosporin but is unable to produce benzylpenicillin even in the presence of an external supply of phenylacetic acid, may lack the appropriate transferase to carry out the last reaction in penicillin biosynthesis.

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Tetraiodothyroacetic acid, triiodothyroacetic acid and oxidative phosphorylation

Since the demonstration by PITT-RIVERS[1] that the acetic acid analogues of thyroxine and triiodothyronine had definite biological activity, investigators have attempted to assess the possible physiological importance of these compounds. THIBAULT[2] has demonstrated that both tetraiodothyroacetic acid and triiodothyroacetic acid produce an immediate rise in oxygen consumption when injected into intact animals. However, LARDY *et al.*[3] have shown that the acetic acid analogues are generally less effective than thyroxine or triiodothyronine. Finally, the identification by GALTON AND PITT-RIVERS[4] of tetraiodothyroacetic acid and triiodothyroacetic acid in liver and kidney tissue from mice makes possible the consideration of a physiological role for