HYDRAZINOLYSIS OF PURIFIED MUCOPEPTIDE FROM THE WALL OF AEROBACTER CLOACAE

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In a study of the composition of purified mucopeptide (MP) from Aerobacter cloacae NRC 492, molar ratios of glucosamine (GN), muramic acid (Mur), alanine (Ala), glutamic acid (Glu), and diaminopimelic acid (Dap) of 2:2:3:2:2 respectively were reported (1). On this basis it was postulated that two types of peptide were present, (I) containing two moles of Ala along with Glu and Dap and (II) containing only one alanine residue, Glu and Dap. The amino acid sequence in (I) was assumed to be Ala. Glu. Dap. Ala (2, 3) and in (II) Ala. Glu. Dap., each with the corresponding alanine covalently bound to a muramic carboxyl (4). Since Salton (5) and Perkins and Rogers (6) had reported carboxypeptidase to be inactive toward bacterial mucopeptide, hydrazinolysis (7) remained as the only direct method to test the hypothesis that C-terminal Ala and Dap were present in purified mucopeptide from A. cloacae.

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A procedure for the hydrazinolysis of MP was modified from Akabori (7) and Bradbury (8). Two mgm. of purified MP (1) were thoroughly mixed with seven mgm. of hydrazine sulphate and the mixture dried in vacuo for four hr. in a glass tube provided with a stopcock. Anhydrous hydrazine (0.1 ml) was introduced with a capillary pipette and the reaction mixture frozen in an acetone-dry ice bath. The tube was then evacuated, sealed and heated for 16 hr. at 60°C. Following removal of excess hydrazine in vacuo over conc. H_2SO_4, the residue was dissolved in hot water, 0.4 ml. freshly distilled benzaldehyde was added, and after shaking for two hr. the mixture was separated into two layers by centrifuging for 10 min. at 5000 G. Two successive water extracts of the benzaldehyde layer were combined with the original aqueous layer, concentrated and divided into three aliquots: (a) for analysis in the Beckman-Spinco amino acid analyzer (9): (b) for paper chromatography and (c) for reaction with fluorodinitrobenzene in $NaHCO_3$ buffer for 3 hr. at pH 9 (10).

Integration of peaks in the effluent curve from the amino acid analyzer showed a molar ratio of Ala to Dap of 1.7 to 1. Beyond a trace of Glu too small to measure no other amino acids were present. The presence of free Ala, Dap and a trace of Glu in the aqueous layer after hydrazinolysis of MP was confirmed by chromatographing an aliquot in n-butanol:pyridine:water:acetic acid (11). After dinitrophenylation and extraction of excess fluorodinitrobenzene with ether, the aqueous layer was extracted with ethyl acetate. Chromatography of concentrates from both the aqueous and ethyl acetate layers on Whatman No. 1 paper in

the tertiary amyl alcohol-phthalate system at pH 6 (12) showed only DNP-Ala and DNP-Dap. Two yellow DNP-Dap spots corresponding (1) to mono-DNP-Dap and (2) to the slower of the two di-DNP-Dap spots were visible. After applying the Reindel-Hoppe procedure to the paper (13) the spot corresponding to mono-DNP-Dap turned blue. The DNP amino acids were reconstituted by the method of Lowther (14) and chromatographed in methanol-water-10 N HCl-pyridine (15). After spraying with ninhydrin two spots appeared, one corresponding to LL-Dap and the other to Ala.

It seems reasonable to assume from these results that C-terminal Ala and Dap are present in purified MP from A. cloacae and that they are released as free amino acids by controlled hydrazinolysis. If this assumption is correct, the results provide evidence in support of the view that two types of peptide chain are present, one ending in Ala and the other ending in Dap. Assuming that the destruction of Ala during hydrazinolysis is approximately equal to that of Dap, the two chains are present in a ratio of about 2 to 1. This ratio is identical with that estimated by a different method and reported by Weidel for the two equivalent mucopolymer sub-units from E. coli B (3). In the native wall terminal amino acids may be electrovalently bonded to positively charged groups in adjacent chains. Ionic bonds may then be broken during isolation of the MP or under the conditions for hydrazinolysi

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