THE AMINO ACID SEQUENCE OF A GLYCINE-CONTAINING CELL WALL PRECURSOR OF *MICROBACTERIUM LACTICUM*

K.H. Schleifer, R. Plapp and O. Kandler

Institut für Angewandte Botanik der Technischen Hochschule München und Bakteriologisches Institut der Südd. Versuchs- und Forschungsanstalt Freising-Weihenstephan

Received January 19, 1967

The cell wall of *Microbacterium lacticum* shows a very unusual amino acid composition (1). Besides muramic acid and glucosamine it contains the amino acids glycine, lysine, glutamic acid, D-alanine and a hitherto unidentified ninhydrin-positive compound. Unlike other bacterial cell walls, but similar to *Butyribacterium rettgeri* (2), L-alanine is missing and the amount of the diamino acid is increased. There are 3 moles lysine for one mole D-alanine. To elucidate the structure of this atypical murein (peptidoglycan), we isolated the incomplete murein precursor, which accumulates under the influence of D-cycloserine (3, 4) and determined its amino acid sequence. It can be written: UDP-mur-gly-glu-lys.

Experimental and results:

Microbacterium lacticum was isolated from milk and identified according to Bergey's Manual (5). The wall precursor was isolated as follows: 600 mg of D-cycloserine were added to 6 liters of a broth culture (1 % peptone from casein, 0,5 % yeast extract, 1,5 % glucose; pH 7,4) in the middle of the log phase. The cells were

harvested 1 hour after further incubation. The extraction was carried out by cold TCA (1 hour; 4° C); the TCA was removed by ether extraction and the neutralized extract was subjected to column chromatography on Dowex-I for the separation of the nucleotides according to Hurlbert et al. (6). The equipment used as well as the various modifications of the methods were described previously (7). As shown in Fig. 1, peak E of the extract from inhibited cells is considerably increased as compared to that of the control. These fractions were collected and the nucleotide was isolated by absorption on charcoal. Further purification was achieved by paper chromatography with isobutyric acid/ammonia as a solvent (4, 7, 8).

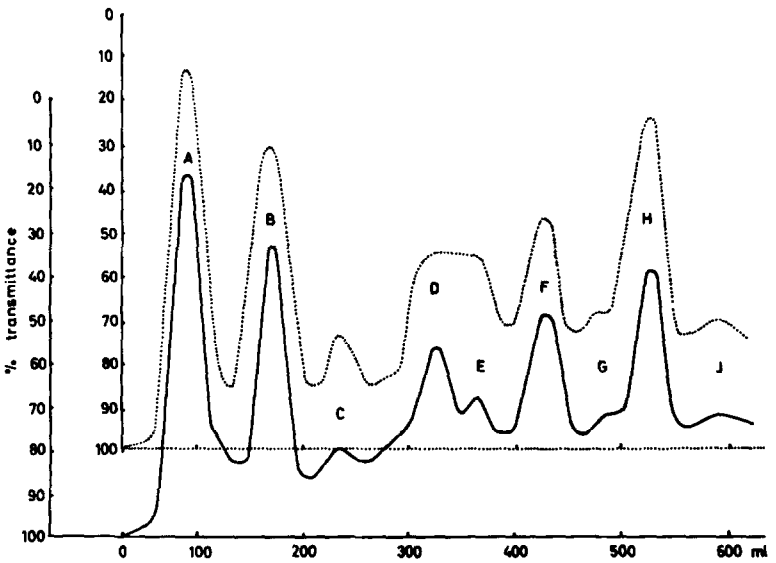

Fig. 1: Column chromatographic separation of nucleotides from the TCA extracts from D-cycloserine-treated (dotted line) and untreated cells (solid line). % transmission measured at 253 mμ.

The paper chromatogram showed only one zone which was ninhydrin-positive and also absorbed UV light. The compound from this zone was eluted and hydrolyzed (4 N HCl; 16 hrs.; 100° C). The amino

acids were determined by quantitative paper chromatography (9) as well as by an amino acid analyzer (Firma Bender and Hobein München). The following molar ratios were obtained: mur:glu:gly:lys 1,1:1,0:0,99:1,05.

The nucleotide was determined by measuring the absorption spectrum of the compound at pH 7,0 and 2,0. A maximum at 261,0 mμ was found at both pH values which is typical of uridine.

These data indicate that we isolated a UDP-muramyl tripeptide similar to that isolated from Staph. aureus (3) and from Proteus vulgaris (4). To determine the amino acid sequence, the peptide was at first dinitrophenylated (10) and hydrolyzed. The only DNP-derivative found by paper chromatography (solvents: 1,5 molar phosphate buffer pH 6,0 and propanol: 0,2 % ammonia 8:2) was ε-DNP-lysine. Hydrazinolysis (11) of the compound yielded lysine as the only free amino acid. These results indicate that lysine bound by its α-amino group is the C-terminal amino acid.

To determine the sequence of glycine and glutamic acid, the peptide was partially hydrolyzed (4 N HCl; 10 min; 100° C) and the resulting amino acids and peptides were identified after separation by two-dimensional paper chromatography (solvent systems: isopropanol:acetic acid:H_2O = 75:10:15; α-picoline:ammonia:H_2O = 70:2:28) as described previously (12). Besides the free compounds glycine, lysine, glutamic acid and muramic acid, two peptides were found (Fig. 2)

One peptide (Nr. 5) gave a bright yellow color on ninhydrin spray, which turned to brownish-violet after a few hours as it is typical of peptides with N-terminal glycine. It yields glycine and glutamic acid after hydrolysis and is chromatographically identical

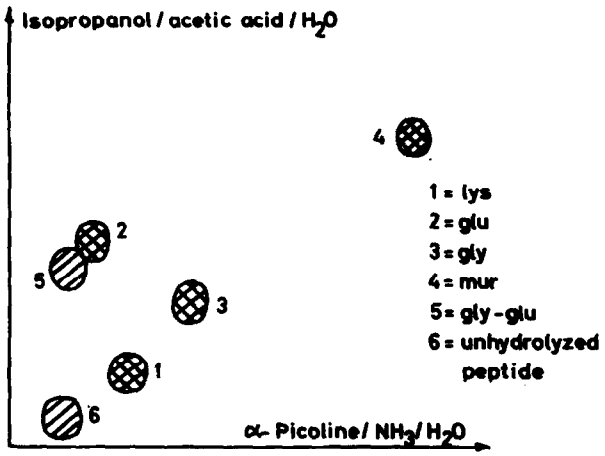

Fig. 2: Paper chromatogram of a partial hydrolysate of the UDP-muramyl-tripeptide

with authentical glycyl-glutamic acid. Another peptide spot (Nr.6) contained all 3 amino acids. It is probably a mixture of the tripeptide and the dipeptide glu-lys, which shows the same R_f value.

Based on these results, the accumulated cell wall precursor may be written: UDP-mur-gly-glu-lys, the ε-aminogroup of lysine being free. It is likely that muramic acid is N-acetylated, since no DNP-muramic acid was observed in the hydrolysate of the dinitrophenylated murein precursor.

Similar to the murein of Butyribacterium rettgeri, where L-alanine is replaced by serine, the murein of Microbacterium lacticum contains glycine which binds the peptide to muramic acid. This is in contrast with other glycine-containing mureins, where glycine is either a constituent of the crosslinking bridges as in Staph. aureus (13) or is bound to glutamic acid as a C-terminal side group as in Micrococcus lysodeikticus (14).

In partial hydrolysates of the TCA-extracted cell wall of Microbacterium lacticum the dipeptide lys-D-ala could be identified (unpublished results). The tetrapeptide of the murein therefore shows the sequence: gly-glu-lys-D-ala. It remains to be determined, what positions are taken by the 2 moles of additional lysine and by the unidentified amino acid mentioned in the beginning.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft. We are indebted to Hoffmann La Roche u. Co. A.G., Grenzach, for supplying D-cycloserine.

References

1. Schleifer, K.H., R.Plapp, and O.Kandler, in preparation
2. Miller,I., R.Plapp, and O.Kandler, Biochem.Biophys.Res.Comm.25, 415 (1966)
3. Strominger, J.L., R.H.Threnn, and S.S.Scott, J.Amer.chem.Soc.81, 3803 (1959)
4. Plapp,R. und O.Kandler, Arch.Mikrobiol.50, 282 (1965)
5. Breed,R.S., E.G.D.Murray, and N.R.Smith, Bergey's Manual of Determinative Bacteriology. The Williams & Wilkins Dompany (1957)
6. Hurlbert,R.B., H.Schmitz, A.F.Brumm, and V.R.Potter, J.Biol. Chem.209, 23 (1954)
7. Plapp,R. und O.Kandler, Arch.Mikrobiol.50, 171 (1965)
8. Ito,E., and J.L.Strominger, J.Biol.Chem.237, 2689 (1962)
9. Heilmann,J., J.Barrollier und E.Watzke, Hoppe-Seyler's Z.physiol.Chem. 309, 219 (1957)
10. Primosigh,J., H.Pelzer, D.Maass, and W.Weidel, Biochim. Biophys.Acta 46, 68 (1961)
11. Salton,M.R.J., Biochim.Biophys.Acta 52, 329 (1961)
12. Schleifer,K.H. und O.Kandler, Arch.Mikrobiol. (in press)
13. Ghuysen,J.M., D.J.Tipper, C.H.Birge, and J.L.Strominger, Biochemistry 4, 2245 (1965)
14. Mirelman,D., and N.Sharon, Biochem.Biophys.Res.Comm. 24, 237 (1966)