ISOLATION OF AN ORNITHINE-CONTAINING CELL WALL PRECURSOR OF *LACTOBACILLUS CELLOBIOSUS*

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The chemical analysis of the cell walls of lactic acid bacteria has shown that the murein (peptidoglycan) contains either lysine or diaminopimelic acid as a diamino acid (1,2,3). In the hydrolysate of the cell wall of *L. cellobiosus*, however, ornithine was found (4) like in some other bacteria (5,6,7,8). To show that ornithine is in fact a constituent of the murein we isolated nucleotide-activated murein precursors containing ornithine. They were obtained by inhibiting *L. cellobiosus* by vancomycin and D-cycloserine.

Vancomycin is known to interfere with the utilization of a lipid-phosphodisaccharide-pentapeptide during murein synthesis in cell-free preparations from *Staph. aureus* and *M. lysodeikticus* (9). In vivo it also leads to the accumulation of a UDP-muramyl-pentapeptide in many bacteria (10,11,12). On the other hand, D-cycloserine is an inhibitor of alanine racemase and causes the accumulation of a UDP-muramyl-tripeptide in *Staph. aureus* (13) and *Proteus mirabilis* (14).

Experimental and results:

L. cellobiosus ATCC 11739 was grown in MRS-medium (15) at 30° C.

Cells of the logarithmic growth phase (4 lit. medium) were harvested by centrifugation, washed with saline and resuspended in 1.5 l MRS-medium. Mg^{++} and Mn^{++}-salts were omitted, since they are known to abolish the growth inhibitory effect of vancomycin (16).

Vancomycin (20 µg/ml) were added and after 45 min incubation at 30° C, the cells were harvested and extracted two times by cold 10 % (w/v) trichloroacetic acid. After removing the TCA by ether extraction the neutralized extract was subjected to column chromatography (Dowex 1; formic acid/ammonium formiate gradient as described previously (17).

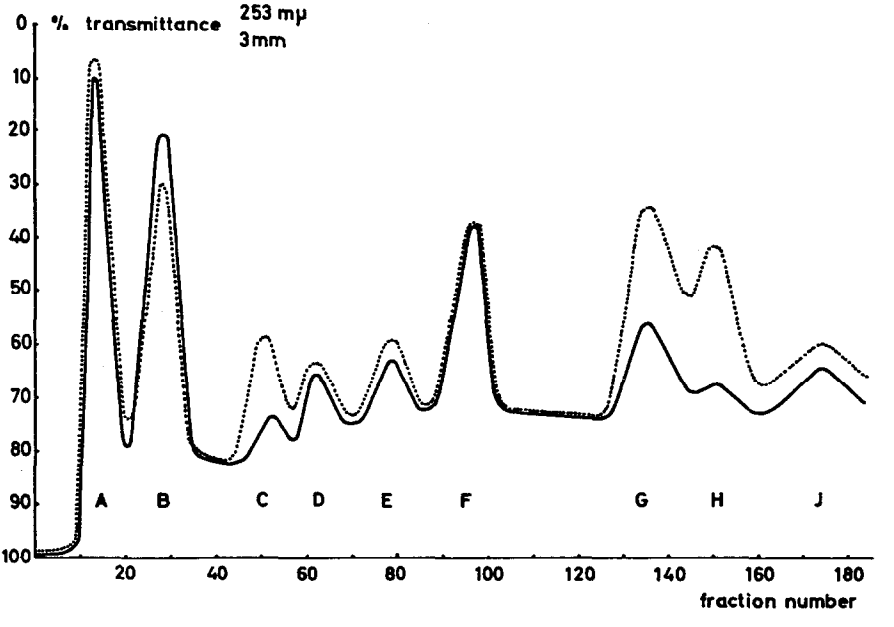

Fig. 1: Column chromatographic separation of nucleotides from the TCA extracts from vancomycin-treated (dotted line) and untreated cells (solid line). % transmission measured at 253 mu.

Fig. 1 shows a chromatogram of an extract from untreated and vancomycin-treated cells. Peak C, normally containing 5'-UMP, is considerably increased in extracts from inhibited cells. The nucleo-

tides from this peak were isolated (17) and chromatographed on paper (isobutyric acid/ammonia/water 66/1/33). Two UV-absorbing spots were detected, one of which was identical with 5'-UMP, the other gave a positive ninhydrin reaction. This compound, which was assumed to be a UDP-linked murein precursor, was isolated for further analysis.

The UV-absorption spectrum of the compound, measured at pH 2 and pH 7 is identical with that of UDP at these pH-values. The result of the phosphate analysis (18) showed that the compound contains 1 mole labile phosphate and 2 moles of total phosphate per mole of uridine which was determined photometrically. N-acetyl-hexosamine was determined by a modified Elson-Morgan reaction (19) after hydrolysis of the compound in 0,1 N HCl at 100° C for 10 min. 0,96 mole N-acetyl-hexosamine was found to be present per mole of uridine. The amino acid analysis (amino acid analyzer Bender u. Hobein, München) after hydrolysis of the compound in 4N HCl at 100° C for 16 hrs. showed that muramic acid, glutamic acid, ornithine and alanine are present at a molar ratio of 1:1:1:3. Tab. 1 contains the molar amounts of the various compounds derived from the cell wall precursor, which obviously is a UDP-NAc-muramyl-pentapeptide.

To characterize the compound further, the configuration of the amino acids was determined. The enzymatic determination of D- and L-alanine (20) in the hydrolysate showed a ratio L-: D-alanine = 1:1,9. To determine the configuration of glutamic acid, the amount of L-glutamic acid was determined by L-glutamate dehydrogenase. Since only 5 % of the total glutamic acid gave a positive reaction, the glutamic acid of the precursor belongs to the D-series. Ornithine had already been shown to belong to the L-series in recent experiments (4).

compound	µM/0,5 ml solution	molar ratio glu = 1
uridine	0,70	1,01
labile phosphate	0,67	0,97
total phosphate	1.40	2.03
N-acetylhexosamine	0,71	1,03
muramic acid	0,66	0,95
alanine	1,99	2,88
glutamic acid	0,69	1,00
ornithine	0,67	0,97

Tab. 1: Total amount of the various components of the UDP-muramyl-pentapeptide.

To elucidate the amino acid sequence, the precursor was partially hydrolyzed (4 N HCl; 1 hr; 100° C) and the resulting peptides were isolated by paper chromatography and analyzed as described recently (21). Two main peptides were found:

1. L-alanyl-D-glutamic acid, 2. L-ornithyl-D-alanine.

Furthermore an incomplete cell wall precursor was isolated by inhibiting a growing culture with D-cycloserine. Isolation and identification were performed in the same way as in the experiments with vancomycin. Besides UDP and muramic acid, the precursor **contained L-alanine, D-glutamic acid and L-ornithine at a molar ratio of 1:1:1. Dinitrophenylation of this UDP-muramyl-tripeptide as well as that of the UDP-muramyl-pentapeptide yielded -DNP-ornithine as the only DNP-derivative. All these data are compatible with the known amino acid sequence of the murein precursor found in Staph. aureus, but L-lysine is replaced by L-ornithine. Therefore the precursor of L. cellobiosus can be written: UDP-murNAc-L-ala-D-glu-L-orn-D-ala-D-ala.**

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