THE AMINO ACID SEQUENCE OF THE SERINE CONTAINING MUREIN OF BUTYBACTERIUM RETTGERI

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Studies on the chemistry of bacterial mureins (mucopeptides) have shown, that these polymers are built according to a common principle (1): The polysaccharide backbone consists of alternating ß (1-4) glycosidically bound N-acetyl-glucosamine (GlcNAc) and N-acetyl-muramic acid (MurNAc). The carboxyl group of muramic acid is substituted by a tetrapeptide of the general sequence L-ala-D-glu-diamino acid-D-ala. Therefore, the most simple chemical composition of a murein, expressed in molar ratios is: GlcNAc:MurNAc:Ala:Glu:diamino acid = 1:1:2:1:1. If a murein contains glycine, aspartic acid, serine, threonine or additional L-alanine, these amino acids are bound singly or in short peptides to the second amino group of the diamino acid and are involved in the cross-linking of the murein by forming a peptide bond with the C-terminal D-alanine of an adjacent tetrapeptide (2,3). The murein of Micrococcus lysodeikticus in which glycine is bound to glutamic acid represents the only exception so far (4).

In Butyribacterium rettgeri, however, a murein has been found, which differs considerably from this general principle in that it posesses two different diamino acids and only one mole of D-alanine per mole of glutamic acid but no L-alanine.

Experimental and Results

Bb. rettgeri ATCC 10825 was grown at 37°C in broth containing 0.5% glucose, 0.5% peptone from casein and 0.01% cystine. Cells were harvested in the stationary growth phase and the cell walls prepared as described by Cummins and Harris (5). Extraction of the cell walls with 10% trichloroacetic acid (TCA) in the cold (6) yielded no teichoic acid. TCA-extracted cell walls have been used for further analyses.

Amino acid composition. Paper chromatography[1] of a cell wall hydrolysate (4 N HCl, 100°C, 16 hrs) revealed the presence of muramic acid, glucosamine, alanine, glutamic acid, serine, and both ornithine and lysine. The results of a quantitative analysis[2] are shown in tab. 1.

Table 1. Amino sugar and amino acid composition of the cell walls of Butyribacterium rettgeri and Lactobacillus viridescens.

Components [a]	B. rettgeri		L. viridescens	
	µmole/mg	ratio [b]	µmole/mg	ratio [b]
Glucosamine	0.31	0.93	1.10	1.66 [c]
Muramic acid	0.33	0.97	0.71	1.07
L-Alanine	-	-	1.78	2.70
D-Alanine	0.31	0.92	0.56	0.85
Glutamic acid	0.34	1.00	0.66	1.00
Serine	0.31	0.91	0.63	0.95
Lysine	0.19	0.57	0.64	0.97
Ornithine	0.42	1.25	-	-

a. Values calculated include correction for losses during hydrolysis.
b. Ratio calculated assuming glutamic acid as 1.00.
c. Cell walls extracted with TCA and formamide.

[1] Solvent systems used: A) isopropanol:acetic acid:water = 75:10:15, B) a-picoline:ammonia:water = 70:2:28 and C) methanol:pyridine:water:formic acid = 80:10:19:1.

[2] amino acid analyzer of the Bender & Hobein Company, Munich.

The configuration of alanine (7) and ornithine (8) were determined enzymatically. Alanine showed the D-, ornithine the L-configuration.

The occurence of serine suggested a comparison with the murein of Lactobacillus viridescens (tab. 1) where L-serine is bound to the ε-amino group of lysine (3,9). In contrast, however, Bb.rettgeri contains no L-alanine, while L.viridescens contains 3 mole of L-alanine per mole of glutamic acid. The second difference is the occurence of two diamino acids in Bb.rettgeri, namely ornithine and lysine at a ratio of 1:0.5.

Digestion by lysozyme. The cell walls were digested by lysozyme and the solubilized components separated by paper chromatography in butanol:acetic acid:water (10). While most of the material remained at the origin or very close to it, one ninhydrine-positive band moved a few cm. This band, which was supposed to contain low molecular muropeptides, was eluted and hydrolysed (4 N HCl, 100°C, 16 hrs.). The amino acid analysis revealed the same composition as that of the whole cell wall. This indicates, that all the amino sugars and amino acids are present in the muropeptides and are therefore components of the lysozyme sensitive murein.

Determination of free amino groups of the murein and the muropeptides The cell walls as well as the muropeptides were dinitrophenylated (10) and hydrolysed (4 HCl, 100°C; 16 hrs.). The analysis of the hydrolysate by paper chromatography (1,5 M phosphate buffer, pH 6.0; Propanol:0.2% NH_3 = 8:2) showed the presence of δ-DNP-ornithine and α-DNP-lysine. The occurence of α-DNP-lysine contrasts with the findings in other lysine containing mureins, where dinitrophenylation yields ε-DNP-lysine (11,3). From this discrepancy one can conclude, that in the murein of Bb. rettgeri lysine is bound in a way different from the mureins investigated so far.

The quantitative determination of the DNP-derivatives (11) revealed

that DNP-ornithine and DNP-lysine are present at a ratio of 1:0.5 corresponding to the ratios of the total amount of both amino acids in the murein. The comparison of the amount of DNP-derivatives with the total amount of ornithine and lysine present before dinitrophenylation showed, that about 30% of the δ-aminogroups of ornithine and the α-aminogroups of lysine, respectively, are accessible to dinitrophenylation. This is much less than in E. coli, where 66% of the terminal-aminogroup of DAP may be dinitrophenylated (12).

Tentative determination of the amino acid sequence

Cell walls were partially hydrolysed at 100°C in 4 N HCl for 2 hrs. and the hydrolysate was subjected to two-dimensional paper chromatography using solvent systems A and B. Fig. 1 shows a typical chromatogram.

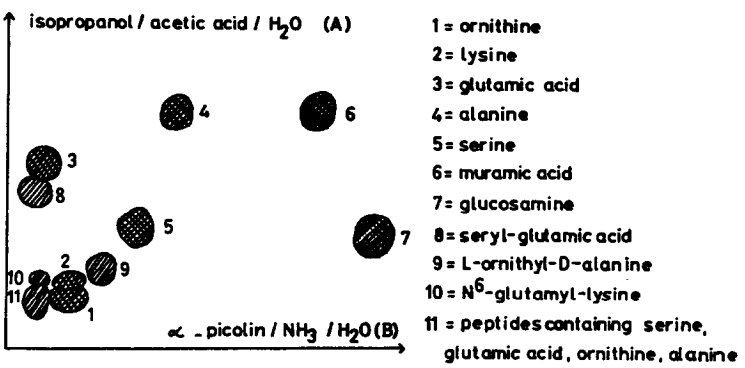

Fig.1 Chromatogram of a partial hydrolysate of Bb.rettgeri cell walls

The various peptides were separated and isolated at amounts of about 0.1 mg by repeated one-dimensional paper chromatography in solvent systems A and B and identified by quantitative estimation of the amino acid constituents and of the amino groups by dinitrophenylation as recently described in more detail (13).

In contrast with the murein of L. viridescens (3,9), serine is found to be bound to glutamic acid, but not to lysine. Obviously the peptide

serylglutamic acid replaces the peptide L-alanylglutamic acid which is found in all other mureins investigated so far (3,9,13,14). The position of ornithine is elucidated by the occurence of the dipeptide L-ornithyl-D-alanine. This observation together with the forementioned finding that dinitrophenylation of the murein yields δ-DNP-ornithine suggests the following amino acid sequence of the tetrapeptide attached to muramic acid: ser-glu-L-orn-D-ala. The configuration of serine and glutamic acid has not been determined so far.

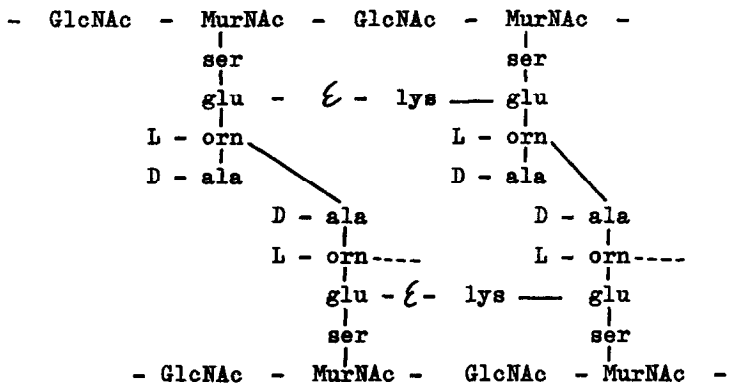

Fig. 2 Proposed amino acid sequence and cross-linkages of the murein of Bb. rettgeri.

Lysine is bound by its ε-aminogroup to glutamic acid, as indicated by the occurence of the dipeptide N^6-glu-lys. The substitution of glutamic acid by lysine resembles the binding of glycine to glutamic acid in Micrococcus lysodeicticus murein (4).

Since only 30% of the δ-amino group of ornithine and of the α-amino group of lysine are free, as already mentioned above, a high degree of cross-linking is to be expected.

The δ-amino group of ornithine might form a peptide bond with the carboxyl group of D-alanine of an adjacent tetrapeptide in analogy with the cross-linking between diaminopimelic acid and D-alanine in other mureins like that of E. coli (15). On the other hand lysine

could be attached by its α-amino group to the free carboxyl group of glutamic acid of an adjacent tetrapeptide. The cross linking of two glutamic acids by one lysine would explain, why lysine amounts to only about 0.5 mole per one mole of glutamic acid. The structure of the complete murein may be written as shown in fig. 2. However, no direct evidence for the proposed mode of cross-linking is available so far. The example of the Bb. rettgeri murein shows that in Gram-positive bacteria variations are not only found in the amino acids or peptides involved in the cross-linkage of murein, but also in the amino acid sequence of the tetrapeptide attached to muramic acid.

References

1. Martin, H.H., Ann.Rev.Biochem. 35, 457 (1966)
2. Ghuysen, J.M.,D.J.Tipper,C.H.Birge,and J.L.Strominger Biochemistry 4, 2245 (1965)
3. Plapp,R.,K.H.Schleifer,and O.Kandler,Fol.Microbiol. (in press)
4. Merilman,D.,and N.Sharon,Biochem.Biophys.Res.Commun.24, 237 (1966)
5. Cummins,C.S.,and H.Harris,J.gen.Microbiol.14, 583 (1956)
6. Armstrong,J.J.,J.Baddiley,J.G.Buchanan,B.Carss,and G.R.Greenberg J.Chem.Soc.1958, 4344
7. Bergmeyer, H.U.,Methoden der enzymatischen Analyse Verlag Chemie,Weinheim (1962)
8. Work,E.,Nature 201, 1107 (1964)
9. Kandler, O.,R.Plapp,and W.Holzapfel, in prep.
10. Primosigh,J.,H.Pelzer,D.Maass,and W.Weidel, Biochim.Biophys.Acta 52, 68 (1961)
11. Salton,M.R.J.,Biochim.Biophys.Acta 52, 329 (1961)
12. Takebe,J.,Biochim.Biophys.Acta 101, 124 (1965)
13. Schleifer,K.H.,and O.Kandler,Arch.Mikrobiol. (in press)
14. Jusic,D.,C.Roy,and R.W.Watson,Canad.J.Biochem.42, 1553 (1964)
15. Weidel,W.,and H.Pelzer,Advan.Enzymol.26, 193 (1964)