GLYCINE AS CROSSLINKING BRIDGE IN THE LL-DIAMINOPIMELIC ACID CONTAINING MUREIN OF *PROPIONIBACTERIUM PETERSSONII*

K. H. SCHLEIFER, R. PLAPP and O. KANDLER
Institut für Angewandte Botanik der Technischen Hochschule, München

Received 10 September 1968

1. Introduction

The amino acid composition of the cell wall of five strains of propionibacteria was studied qualitatively by Cummins and Harris [1]. They found that one strain (*P. shermanii*) contains only alanine, glutamic acid and meso-diaminopimelic acid (DAP), whereas four strains show LL-DAP instead of meso-DAP and, additionally, glycine. A quantitative analysis of the amino acid content of the cell walls of *P. peterssonii* and *P. rubrum* which contain LL-DAP and glycine was carried out by Allsop and Work [2]. The approximate molar ratios of the amino acids D-glutamic acid : D-alanine : L-alanine : LL-DAP : glycine were 1 : 1 : 1 : 1 : 1. No detailed investigation of the amino acid sequence of this murein (peptidoglycan) is known today.

The experiments described in the present paper show that the cross-linkage of the tetrapeptides (L-Ala-D-Glu-LL-DAP-D-Ala) is accomplished by glycine which is linked to the amino group of LL-DAP and the C-terminal D-alanine of an adjacent tetrapeptide.

2. Methods

P. peterssonii ATCC 4870 was grown in yeast extract-dextrose-broth (1% peptone from casein, 0.5% yeast extract, 0.5% glucose, pH = 7.0–7.2) or yeast extract-lactate medium (1% peptone from casein, 0.5% yeast extract, 0.5% sodium lactate, pH = 7.0–7.2) at 30°C and harvested in the stationary phase. Prior to harvesting the cell suspension was kept at 100°C for 30 min to inactivate autolytic enzymes and cell walls were prepared by the usual technique [3]. Paper chromatography was carried out on Schleicher and Schüll 2043b paper in the following solvent systems (v/v/v):

I. Isopropanol-acetic acid-water = 75:10:15
II. α-Picoline-ammonia solution 25%-water = 70:2:28
III. Methanol-pyridine-formic acid-H_2O = 80:10:1:19.

Quantitative amino acid analysis was carried out by an amino acid analyser. The determination of end-groups of the murein and of the peptides isolated from acid partial hydrolysates by paper chromatography were performed as described recently [3, 4].

3. Results

3.1. *Amino acid composition*

The quantitative amino acid composition of cell wall hydrolysates is summarized in table 1. Paper chromatography in solvent system III showed that only the LL-isomer of DAP was present. Both cell walls purified by tryptic digestion (CW-Tryp) as well as by extraction with trichloroacetic acid (CW-TCA) contained muramic acid, glucosamine, glutamic acid, LL-DAP, glycine and alanine at a molar ratio of approximately 0.5 : 0.5 : 1 : 1 : 1 : 2. One mole of ammonia was found per mole of glutamic acid, indicating that glutamic acid is probably amidated as in other mureins.

Table 1

Amino acid and amino sugar composition of cell walls of *P. peterssenii*

(CW-TRYP = cell walls purified by tryptic digestion,
CW-TCA = trichloroacetic acid extracted cell walls,
DNP-CW = dinitrophenylated cell walls.) – = not determined.

		CW-TRYP	DNP-CW-TRYP	CW-TCA	DNP-CW-TCA
μM/mg CW	Glu	0.330	0.285	0.395	0.370
	Gly	0.333	0.220	0.400	0.280
	Ala	0.547	0.500	0.630	0.590
	DAP	0.343	0.300	0.400	0.365
	Mur	0.140	–	0.205	–
	$GlcNH_2$	–	–	0.210	–
Molar ratio Glu = 1	Gly	1.01	0.77	1.01	0.76
	Ala	1.66	1.75	1.59	1.60
	DAP	1.04	1.05	1.01	0.98
	Mur	0.44	–	0.52	–
	$GlcNH_2$	–	–	0.53	–

3.2. *Determination of the free amino groups of the murein*

CW-Tryp and CW-TCA were dinitrophenylated and hydrolyzed (6 N HCl, 100°C, 6 hr). The analysis of the hydrolysates by paper chromatography (1.5 M phosphate buffer pH = 6.0; *n*-propanol-0.2% ammonia 8 : 2) yielded DNP-glycine and traces of mono-DNP-DAP. A comparison of the molar ratio of the amino acids of dinitrophenylated and normal cell walls (table 1) showed that approximately 25% of glycine are susceptible to dinitrophenylation. The decrease of DAP is not significant. The amount of DNP-DAP formed is probably less than 5% of the total DAP of the murein.

3.3. *Determination of the amino acids sequence*

CW-Tryp or CW-TCA were partially hydrolyzed (4 N HCl, 100°, 1 hr) and the hydrolysate was subjected to two-dimensional paper chromatography using solvent systems I and II (fig. 1).

The various peptides were separated and isolated by repeated one-dimensional paper chromatography in solvent systems I, II and III and subsequently analysed as described recently [3–5]. The occurrence of the peptides L-Ala-D-Glu, LL-DAP-D-Ala, D-Glu-LL-DAP-D-Ala, Mur-L-Ala and Mur-L-Ala-D-Glu after 20 min hydrolysis indicates that the tetra-

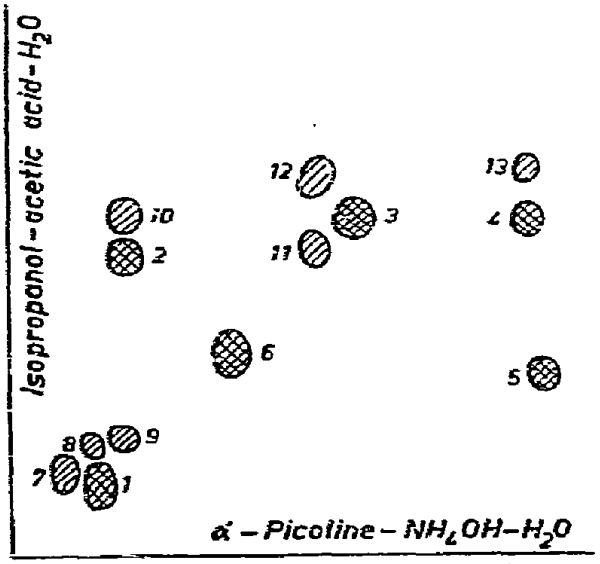

Fig. 1. Scheme of a chromatogram of an acid partial hydrolysate (4 N HCl, 100°C, 1 hr) of cell walls of *P. peterssonii* or *P. arabinosum*. 1 = LL-DAP, 2 = Glu, 3 = Ala, 4 = Mur, 5 = $GlcNH_2$, 6 = Gly; 7 = D-Glu-LL-DAP-D-Ala, 8 = Gly-LL-DAP-D-Ala, 9 = LL-DAP-D-Ala, 10 = L-Ala-D-Glu. 11 = D-Ala-Gly, 12 = Mur-L-Ala-D-Glu, 13 = Mur-L-Ala (Nos. 12 and 13 appear in 20 min hydrolysates only).

peptide attached to muramic acid has the same amino acid sequence as in the case of *Escherichia coli* [6] or

Lactobacillus plantarum [5], namely L-Ala-D-Glu-DAP-D-Ala. There is only a difference in the configuration of DAP. LL-DAP occurs instead of meso-DAP.

The tripeptide Gly-LL-DAP-D-Ala and the dipeptide D-Ala-Gly are most important for the elucidation of the cross-linkage. The occurrence of the two peptides shows that glycine is bound to the amino group of DAP as well as to the C-terminal D-alanine of an adjacent tetrapeptide. In contrast with the partial hydrolysates of cell walls of *Micrococcus lysodeikticus* [7] and *Microbacterium lacticum* [8] Glu-Gly was not found. Therefore, no glycine is attached to glutamic acid.

Fig. 2 shows a fragment of the murein of *P. peterssonii*. It has to be mentioned that the molar ratio of glutamic acid to muramic acid or glucosamine is not 1 : 1 (table 1) like in other mureins studied so far, but only 1 : 0.5. Therefore, the fragment shown in fig. 2 is probably not the only possible oligomer of the complete murein. Further studies are necessary to elucidate the deviation from the usual structure of the murein which is indicated by the low content of the amino sugars.

4. Discussion

The murein described in this paper is the first known case of an interpeptide bridge in a DAP-containing murein. The same type of cross-linkage occurs most likely in the murein of certain clostridia [9] and streptomyces [10]. In both cases the molar ratios of the amino acids are the same as in *P. peterssonii*. Furthermore we were able to isolate (unpublished) the peptides Gly-LL-DAP-D-Ala and D-Ala-Gly from the acid partial hydrolysate of the cell walls of *Clostridium perfringens*. These findings are in contrast with the proposal of Pickering [9] who assumed that glycine is bound to glutamic acid like in the murein of *M. lysodeikticus*.

Recently Arima et al. [11] reported some data on the enzymatic and chemical degradation of the cell walls of *Streptomyces* which indicate that glycine is involved in the cross-linkage of the murein in these organisms too.

Besides *P. peterssonii*, which was investigated extensively, the cell walls of several other species were isolated to determine the amino acid composition.

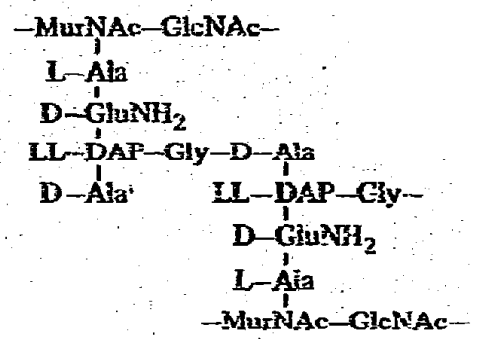

Fig. 2. Amino acid sequence of a fragment of the murein of *P. peterssonii*.

P. shermanii * and *P. freudenreichii* ATCC 6207 contained meso-DAP in addition to alanine and glutamic acid. All the other species (*P. rubrum* ATCC 4871, *thoenii* ATCC 4872, *zeae* ATCC 4964, *jensenii* ATCC 4867, *pentosaceum* ATCC 4875, *arabinosum* ATCC 4965) contained glycine in addition and meso-DAP was replaced by LL-DAP as in the case of *P. peterssonii*. The two dimensional paper chromatograms of the acid partial hydrolysate of *P. shermanii* and *freudenreichii* were identical to those of the cell walls of *L. plantarum* [5] while those of the other species are identical with those of *P. peterssonii*. Obviously, two types of murein exist within the genus *Propionibacterium*. The distribution of these two types agrees with the different morphology within the genus. It is well known that the two meso-DAP containing species *P. shermanii and freudenreichii* are more or less coccoid, while all the other species form coryneform rods.

Acknowledgement

We are indebted to Miss L. Weidermann and Miss R. Keck for careful technical assistance and to the Deutsche Forschungsgemeinschaft for financial support.

* From the culture collection of the Inst. für Bakteriologie, Südd. Versuchs-u. Forschungsanstalt für Milchwirtschaft, Freising-Weihenstephan.

References

[1] C. S. Cummins and H. Harris, J. Gen. Microbiol. 18 (1958) 173.
[2] J. Allsop and E. Work, Biochem. J. 87 (1963) 512.
[3] K. H. Schleifer and O. Kandler, Arch. Mickrobiol. 57 (1967) 335.
[4] O. Kandler, R. Plapp and W. Holzapfel, Biochim. Biophys. Acta 147 (1967) 252.
[5] N. Weiss, R. Plapp and O. Kandler, Arch. Mikrobiol. 58 (1967) 313.
[6] W. Weidel and H. Pelzer, Advanc. Enzymol. 26 (1964) 193.
[7] K. H. Schleifer and O. Kandler, Biochem. Biophys. Res. Commun. 28 (1967) 965.
[8] K. H. Schleifer, R. Plapp and O. Kandler, Biochim. Biophys. Acta 154 (1968) 573.
[9] B. T. Pickering, Biochem. J. 100 (1966) 430.
[10] T. Nakamura, G. Tamura and K. Arima, J. Ferment. Technol. 45 (1967) 869.
[11] K. Arima, T. Nakamura and G. Tamura, Agr. Biol. Chem. 32 (1968) 530.