THREE-DIMENSIONAL STRUCTURE OF PEPTIDOGLYCAN OF BACTERIAL CELL WALLS: INFRA RED INVESTIGATIONS

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1. Introduction

Peptidoglycan (fig.1), also called murein [1], is a derivative of chitin. The latter is a polymer consisting of β 1–4-linked *N*-acetylglucosamine residues. As distinguished from chitin, within peptidoglycan the OH group at C–3 of every second sugar residue forms an ether link to a D-lactyl residue. This derivative of *N*-acetylglucosamine is called *N*-acetylmuramic acid. A tetrapeptide consisting of alternating L- and D-aminoacid residues is bound to the carboxyl group of muramic acid. The peptides may be cross-linked between the ω-amino group of a diamino acid residue and the carboxyl group of a D-alanine residue of an adjacent peptidoglycan strand. In this way, the peptidoglycan forms a tight network around the bacterial cell. For the structure of the peptide moiety, two different models were suggested: parallel β-structure [2] and 2.2_7 helical structure [3].

A consideration of the amide I and V bands of infrared spectra has proven that the peptide moiety of peptidoglycan forms no β-structure. This is true for the peptide units bound to the carbohydrate moiety as well as for a synthesized peptide unit (table 1). In the carbohydrate moiety of peptidoglycan, as with chitin, interchain hydrogen bonds are formed between the *N*-acetyl groups of adjacent glycan strands. Furthermore, it is shown that the length of the interchain O–3H . . . . O–5 bonds is

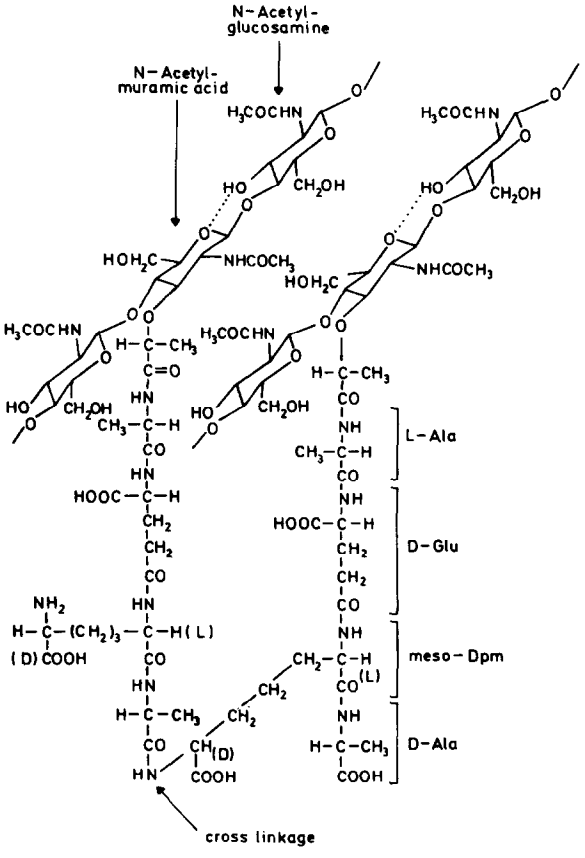

Fig.1. Fragment of the primary structure of peptidoglycan of *Escherichia coli*.

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Table 1
Infra data and the primary structure of the peptide moieties

Substance	Primary structure of peptide moieties	Amide I	Amide II	Amide V	Amide A	O–3H . . . O–5
Polypeptides with β-structure [9–11]		1630–32 vs	1530 s	about 700 m		–
tri L–Ala	L–Ala–L–Ala–L–Ala	1694 w 1637 vs	1551 s	692 m	3280 m–s 3262 m–s	–
Tetrapeptide	L–Ala–γ–D–Glu–L–Lys–D–Ala	1649 vs	1533 s		3265 s	–
Peptidoglycans of *Spirilum serpens*	N^{α}–L–Ala–γ–D–Glu–m–Dpm–D–Ala	1660 vs	1537 s	No pronounced band in the region at about 700 cm^{-1}	3300 s	very broad shoulder in the region 3450–3400 cm^{-1}
Lactocacillus plantarum	N^{α}–L–Ala–γ–D–GluNH_2–m–Dpm–D–Ala	1661 vs	1540 s		3305 s	
Staphylococcus aureus	N^{α}–(L–Ala–γ–D–GluNH_2)–N^{ϵ}–(Gly_5)–L–Lys–D–Ala	1657 vs	1540 s		3302 s	
Corynebacterium insidiosum	N^{α}–Gly–γ–(α–D–Glu–N–γ–D–Dab)–L–Dab–D–Ala	1655 vs	1535 s		3300 s	
Microbacterium lacticum	N^{α}–Gly–γ–(α–D–Glu–Gly–L–Lys)–L–Lys–D–Ala	1656 vs	1538 s		3300 s	
Micrococcus luteus	N^{α}–L–Ala–γ–(α–D–Glu–Gly)–N^{ϵ}–(L–Ala–γ–(α–D–Glu–Gly)–L–Lys–D–Ala)–L–Lys–D–Ala	1655 vs	1540 s		3295 s	
Chitin		1658 s 1623 m–s	1555 s		3300 s	3445 s

Abbreviations: vs = very strong; s = strong; m = medium; w = weak; m–DPM = meso Diaminopimelic acid; Dab = Diaminobytyric acid.

2.8–2.9 Å in the case of chitin. These hydrogen bonds are also formed in peptidoglycan, but in contrast to chitin, they are strongly disturbed with regard to length and bending.

2. Materials and methods

The peptidoglycans studied are listed in table 1. All peptidoglycans of gram-positive bacteria are prepared as described by Schleifer and Kandler [4]. The peptidoglycan of the gram-negative bacterium *Spirillum serpens* is prepared according to Weidel et al. [5]. The tetrapeptide (table 1) was synthesized as described by Schleifer and Krause [6]. The peptide tri-L-Ala was supplied by Bachem (Marina des Rey, USA).

Films are prepared on supports from suspensions of solutions, respectively, according to the method described by Hofmann and Zundel [7]. These films are studied either dried or hydrated in cells suitable for infrared investigations [8]. The spectra are plotted with a spectrophotometer model 325, supplied by Bodenseewerk Perkin–Elmer, Überlingen, West Germany (slit program 6.5, time response 2, registration speed 0.5–1.5 wave numbers/s).

3. Results and discussion

3.1. *Peptide moiety*

Infrared spectra of substances studied are shown in fig.2. The infrared bands taken from the spectra and used in the following discussion are also listed in table 1.

In the case of β-structure, the amide I band of polypeptides is observed at 1630 cm^{-1}, and when this structure is antiparallel, an additional weak band is found at about 1685 cm^{-1} [9,10]. The amide V band is observed with β-structures near 700 cm^{-1} [11]. The data for the position of the amide bands given in the literature are, however, usually data obtained for polymers. Therefore we have first determined whether these bands are shifted in the case of small peptides with β-structure. The dashed line in fig.2a shows the spectrum of tri-L-alanine. It is well known from X-ray crystallography that this peptide is present in antiparallel β-structure [12]. The

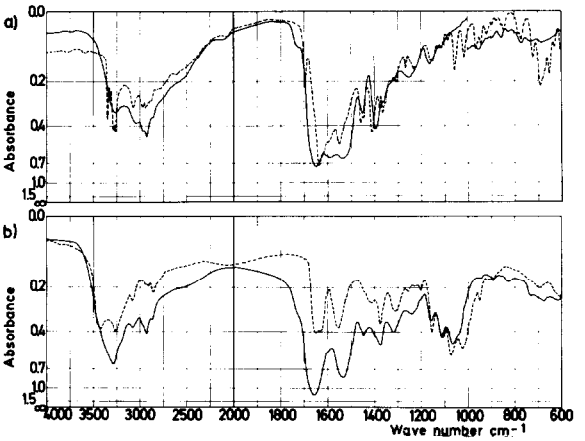

Fig.2. Infrared spectra of films (a) (- - - - - - - - -) of tri L-Ala; (———) of the tetrapeptide L-Ala-γ-D-Glu-L-Lys-D-Ala (b) (- - - - - - - -) of chitin; (———) of peptidoglycan *(Lactobacillus plantarum)*.

amide I band is found at 1637 cm^{-1}, and the additional band, indicating that this structure is antiparallel, is observed at 1694 cm^{-1}. The amide V band occurs at 692 cm^{-1}. Hence the amide I band of small peptides in β-structure is shifted a few cm^{-1} toward higher wave numbers compared with the position in the case of the polymer. Similar results were obtained by Sutton and Koenig [13].

The continuous line in fig.2a is the spectrum of a synthesized tetrapeptide subunit of peptidoglycan (table 1). With this peptide, the amide I band is observed at 1646 cm^{-1}, i.e., it is markedly different compared to the tripeptide in β-structure. Furthermore, no additional amide I band is found at 1694 cm^{-1}, and no amide V band is found near 692 cm^{-1}. These results demonstrate that the synthesized tetrapeptide of peptidoglycan is not present in β-structure. The spectrum drawn as a solid line in fig.2b is the spectrum of the peptidoglycan of *Lactobacillus plantarum*. In this case, one amide I band is observed at 1656 cm^{-1}. Thus , also a peptide bound to the glycan strand as in the intact peptidoglycan (fig.1), is not present in β-structure; though it is possible to build a molecular model with β-structure [2].

Which other conformation can be assumed for this tetrapeptide? α-helical conformation can be excluded for various reasons [3]: (1) This peptide is

too short for such a structure. (2) A peptide built up by alternating D- and L-amino acid residues cannot form such a structure. (3) Finally, α-helical structures do not fit in the lattice periodicity of peptidoglycan. As suggested in a previous publication [3], the 2.2_7 helical conformation is the most probable conformation of this tetrapeptide.

β-structure can not only be excluded for the peptide subunit of peptidoglycan of *Lactobacillus plantarum* but also for all peptidoglycans listed in table 1. The following points are particularly significant: The peptidoglycan of *Staphylococcus aureus* has pentaglycine peptides as interpeptide bridges [14] inserted between the ε-amino groups of L-Lys and the carboxyl group of D-Ala. The infrared spectrum shows that this interpeptide bridge is not in β-structure, either. The peptidoglycan of *Corynebacterium insidiosum* shows cross-linkage between the α-carboxyl group of D-Glu and the D-Ala of an adjacent peptidoglycan strand. This cross-linkage is mediated by a diaminobutyric acid residue [14]. Also in this case, no β-structure is induced due to this cross-linkage, as shown by the amide I band as well as by the fact that no amide V band is observed near 725 cm^{-1}. It is especially remarkable that the same structure is found with the multilayered peptidoglycan of gram-positive bacteria as with the thin layers of the gram-negative bacterium *(Spirilum serpens)*.

3.2. *Carbohydrate moiety*

The carbohydrate moiety of peptidoglycan is closely related to chitin. For comparison, the spectrum of chitin is also shown in fig.2b. The infrared spectra of chitin were studied earlier by various authors [15,16].

It was shown by X-ray analysis [17] that hydrogen bonds are formed between the *N*-acetyl groups of adjacent glycan strands. The stretching vibration of the NH groups in these hydrogen bonds is observed as a strong band at 3265 cm^{-1} [15,16]. In the case of peptidoglycan, this band is masked by the very strong amide A band of the peptide moiety which is observed at about 3300 cm^{-1}. However, no NH stretching vibration of free NH groups is found with peptidoglycan. With free NH groups, this band should occur at about 3430 cm^{-1} [18,19]. Thus, the NH . . . O interchain hydrogen bonds between *N*-acetyl groups should also be formed with peptidoglycan.

Chitin shows a strong band at 3445 cm^{-1} and a shoulder at 3480 cm^{-1}, as already observed by Marchessault et al. [15]. An OH . . . O hydrogen bond between O–3 and the ring O–5 was already suggested by Carlström [17] and by Ramachandran [20]. The band at 3445 cm^{-1} should probably be assigned to the stretching vibration of OH groups in this hydrogen bond. With regard to the relation between stretching vibration and bond length [21,22], the length of the O–3H . . . O–5 hydrogen bond is 2.8–2.9 Å.

With peptidoglycan, a very broad and therefore less pronounced shoulder is found in this region. In contrast to chitin, with peptidoglycan every second O–3H . . . O–5 interchain hydrogen bond cannnot be formed, since the O–3 of every second sugar residue forms an ether link to a lactyl residue. The broadening of the OH stretching vibration and the fact that no stretching vibration of free OH groups (when present, found about 3600 cm^{-1}) is observed suggest the following: The remaining O–3H . . . . . O–5 hydrogen bonds have a relatively broad distribution with regard to bond length and bond bending, due to slight distortions which are possible by slight rotation around the C–4–O and the C–1–O bonds. This explanation is in good agreement with the following X-ray result: With chitin, the 10.3 Å reflex is very pronounced, due to the periodic distance in the direction of the carbohydrate chain, whereas the same reflex is very weak with peptidoglycan [3,23]. The result that with peptidoglycan the lattice is distorted is confirmed by the kinetics of H–D exchange studied by infrared investigations. In the case of peptidoglycan, the H–D exchange at 25°C is almost complete within 3 days. This is indicated by the disappearance of the NH stretching vibration at 3300 cm^{-1} and the appearance of the ND stretching vibration at 2475 cm^{-1}. In the case of chitin, however, in 3 days only a H–D exchange of about 50% has taken place.

Infrared-spectra of peptidoglycan have shown that its carbohydrate moiety is similarly arranged in chitin and its peptide moiety does not occur in the β-structure.

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