Opinion TRENDS in Microbiology Vol.13 No.12 December 2005

Towards a comprehensive view of the bacterial cell wall

Boris Dmitriev[1], Filip Toukach[2] and Stefan Ehlers[3]

[1]N.F. Gamaleya Institute for Epidemiology and Microbiology, Gamaleya str. 18, Moscow 123098, Russia
[2]N.D. Zelinsky Institute for Organic Chemistry, Leninsky Prosp. 33, Moscow 119991, Russia
[3]Division of Molecular Infection Biology, Research Center Borstel, Parkallee 22, D-23845 Borstel, Germany

Direct *in vivo* visualization, in full atomic detail, of the microbial cell wall and its stress-bearing structural architecture remains one of the prime challenges in microbiology. In the meantime, molecular modeling can provide a framework for explaining and predicting mechanisms involved in morphogenesis, bacterial cell growth and cell division, during which the wall and its major structural component – murein – have to protect the cell from osmotic pressure and multiple tensile forces. Here, we illustrate why the scaffold concept of murein architecture provides a more comprehensive representation of bacterial cell wall physiology than previous models.

Introduction

The cell wall of bacteria represents a structural unity of variable thickness, which is located outside the plasma membrane and completely covers the cell. It is both firm-to-rupture and elastic, thereby preventing the cell from disintegration by the intracellular osmotic pressure [1].

The major stress-bearing component of the bacterial cell wall is murein, a chemical synonym of which is peptidoglycan (PG). In 1964, Weidel and Pelzer [2] proposed that PG strands within the 'bag-shaped' murein (sacculus) run parallel to the plasma membrane. Being cross-linked by peptide bridges, glycan chains were thought to make thin networks (layers). Glycan chains were assumed to run perpendicular to the long axis of the cell, whereas peptide bridges were arranged in parallel [3,4]. The major obstacle for direct experimental proof of this postulated structure is that a living cell does not possess a fixed cell-wall structure because the cell wall is in a state of permanent biosynthesis, assembly, disassembly and turnover. This makes the cell-wall architecture of each individual cell within a population both heterogeneous and irregular.

During the past four decades, several experimental observations have accumulated that tend to contradict the adopted structural paradigm of PG layers (reviewed in [5,6]). It is, therefore, timely and necessary to readdress the crucial question of how the murein tertiary structure copes *in vivo* with the major processes and tensile forces of bacterial cell physiology. Recently, we proposed a radically different concept for the architecture of bacterial cell walls, known as the scaffold model [5–8]. As a result of re-evaluating experimental evidence, this model shows glycan chains within murein of either Gram-negative or Gram-positive bacteria running perpendicular to the plasma membrane. Cross-linked by peptide bridges, they produce a continuous sponge-like matrix (not layers) that can function as an elastic external cytoskeleton.

Obviously, the traditional and novel models are mutually exclusive. However, because the chemical parameters of the two models are closely related, and because there is no direct experimental approach to distinguish between them, a stimulating, if controversial, discussion of the two models has been initiated in the literature [4,6,9]. In our opinion, it is time to put the models to use for explaining and predicting basic facets of bacterial cell physiology. Here, we show how the scaffold model fulfills this demand in the paradigmatic case of Gram-negative *Escherichia coli*.

Morphological metamorphoses

Murein determines the shape of the bacterial cell. Although intuitively evident for symmetrical cells that possess a classical (round or rod-like) morphology, this statement must also be true for the branched type of morphology, when cells exhibit Y-, X- or Ж-like shapes caused by mutations of specific genes encoding the synthesis of murein-assembling enzymes. The morphological transformations observed for the rod-like *E. coli* cells [10,11] are particularly impressive: after a set of mutations they grew either as filaments or branched dendrites or large spheres, the spheres dividing like *Neisseria* or *Staphylococcus* (i.e. with a successive alteration of the division plane orientation). These radical morphological transformations are solely possible if the stress-bearing murein possesses a kind of universal tertiary structure, which effortlessly tolerates and enables major morphological perturbations. The major structural principle of murein architecture has to be universal and valid for all types of bacterial cell morphology.

General chemical and physical principles of murein architecture

Although the primary chemical structure of murein might vary in different organisms, the material, regardless of the taxonomy and morphology of a given bacterial cell,

Corresponding author: Ehlers, S. (sehlers@fz-borstel.de).
Available online 19 October 2005

 0966-842X/$ - see front matter doi:10.1016/j.tim.2005.10.001

Box 1. Characteristic structural elements of murein

(i) A peptidoglycan (PG) strand is both a regular and symmetrical molecule. Every strand consists of alternating disaccharide-peptide units; disaccharide fragments are connected into regular glycan chains, and peptide substituents project outward from each odd monosaccharide residue (Figure I). The disaccharide-repeating unit consists of N-acetylmuramic acid (blue disk, beginning of the chain) and N-acetylglucosamine (red disk).

(ii) According to X-ray diffraction data, conformation of the strand represents a right-handed helix with the symmetry order +4, each turn of the helix consisting of four disaccharide units with four peptide side-chains oriented outward [35,36]. A strand with two turns of the helix is shown in the insert.

(iii) Strands are of different lengths: oligomers comprising 8–12 disaccharide units predominate [14–17] but short chains and long polymers are also present.

(iv) The formation of peptide bridges is readily possible because each peptide arm possesses both free amino (filled circles) and carboxyl (empty circles) groups.

(v) Not all adjacent peptides are bridged, therefore, the degree of cross-linking is variable.

(vi) Some crucial physical parameters are as follows: the length and the width of one disaccharide unit is 1.0 and 1.1 nm, respectively [6], the lengths of the peptide arm and the expanded bridge are 2.2 and 4.36 nm, respectively [20].

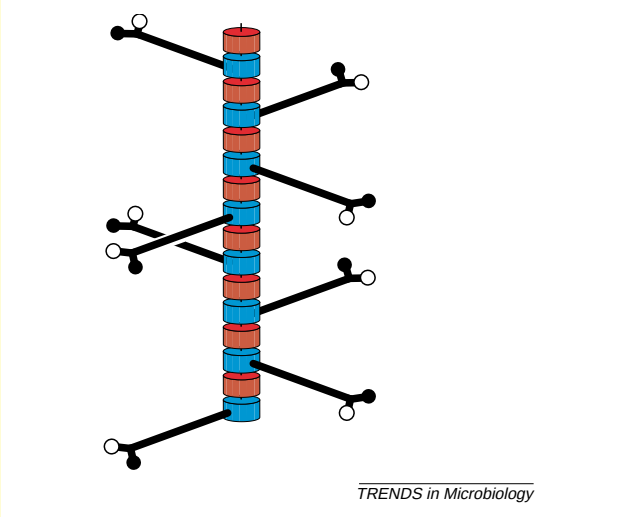

Figure I. General view of a separate peptidoglycan (PG) strand.

invariably comprises PG strands cross-linked by peptide bridges [12]. The chemical features of PG strands are summarized in Box 1.

The murein sacculus is a cocoon-like construction, which has to contain and oppose the rupturing forces, therefore, its architecture must correspond to the mechanical principles of safe engineering and constructing. Therefore, stress-bearing elements within murein of the rod-like bacterium must be arranged differently than suggested by the classical model and, if we remain within the confines of this model, these elements should adopt a hexagonal architecture (Figure 1a). Analogous considerations have led Koch to propose the term 'chicken-wire' network [13]. It is clear that the stress-bearing properties of the 'chicken-wire' architecture directly depend on the lengths of PG-strands. These, however, have been demonstrated experimentally to be rather short [14–17]. The predominant occurrence of short chains translates into numerous cuttings within the network. The number of large holes increases dramatically when the degree of murein cross-linking is reduced to the levels observed in nature, and in this case the network becomes dysfunctional [6].

The situation is radically different in the case of the scaffold model, which does not crucially depend on the strand lengths and readily accommodates both the short chains and a lower degree of cross-linking (Figure 1b). The crucial questions are: (i) how are the glycan strands oriented relative to the plasma membrane and each other and (ii) what is the murein architecture like?

Structural paradigm for murein architecture in rod-like Gram-negative bacteria

The murein of all Gram-negative bacteria belongs to the simplest chemical type [12]. The cell wall of *E. coli* has been studied comprehensively by electron microscopy and biochemical methods. The actual distance between the inner and outer membranes (i.e. the periplasm height in hydrated *E. coli* cells deeply frozen at ambient pressure) was reproducibly measured as 33 nm [18]. Furthermore, the observed width of the major murein body was 8 nm, and the material was located close to the outer membrane. Here, it was centered around the ends of the peptide moieties of lipoprotein molecules, whose protein core is 8 nm [19]. The bulk of murein extended toward the plasma membrane and gradually became less dense; the overall thickness of the whole murein mass was ~18 nm [20]. Evidently, this material is a major structural component of the periplasm and represents the resilient 'periplasmic gel' of the bacterial envelope [21].

Combining the principles of the scaffold-like murein architecture and the experimentally determined parameters of the *E. coli* periplasm detailed previously, we now present the first graphical in-scale depiction of the Gram-negative envelope (Figure 2a). Traditional cartoons of the Gram-negative envelope depict the periplasmic space as essentially empty with a thin murein layer inside [22,23]; therefore, our presentation in Figure 2a is radically different from all previous models. Figure 2a, however, readily illustrates that the periplasmic space is prone to compression. It is therefore easy to understand that, when *E. coli* cells were rapidly frozen at a high pressure, the height of the periplasm dropped to 20 nm and the visible zone of murein was reduced to 6 nm [24].

The murein architecture resembles a sponge-like matrix, the height of the matrix being proportional to the glycan-chain lengths. How can the scaffold-like murein architecture be assembled during continuous bacterial cell growth and division? There are two peculiarities that compound this problem: (i) the murein-assembling enzymes are membrane-bound proteins that use precursors from the cytoplasm, and (ii) the wall, which is being assembled, is located in the periplasm at a substantial distance from the membrane. Before answering the question, we would like readers to recall that cylindrical and pole regions of the rod-like cell are synthesized by two distinct mechanisms: (i) patch-insertion mode of growth and (ii) zonal mode of growth

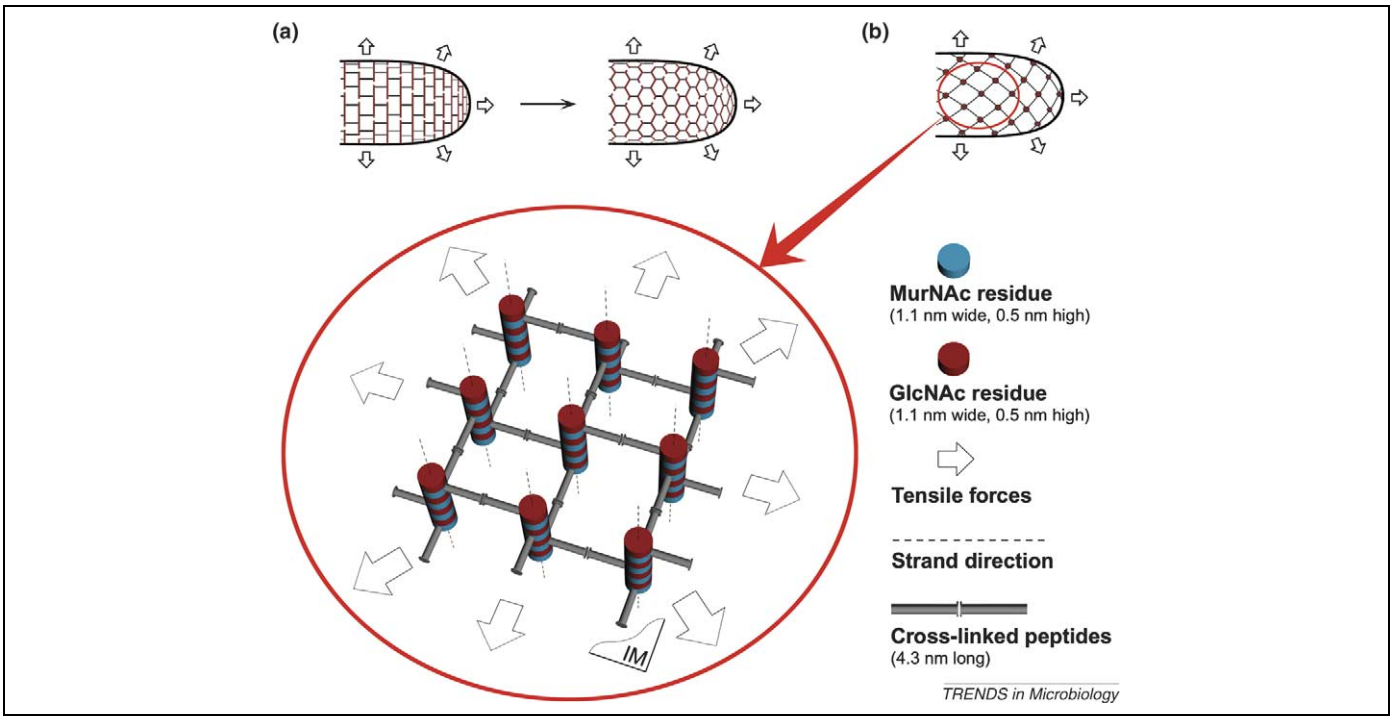

Figure 1. Arrangement of the stress-bearing elements within the cell wall of a rod-like bacterium. **(a)** Glycan chains run as postulated by the classical model, that is, parallel to the plasma membrane and the short axis of the cell (not along the tensile forces). Transformation to the 'chicken-wire' architecture is most probable. **(b)** Glycan chains are arranged perpendicular to the plasma membrane, peptide bridges being the stress-bearing elements of the construction (scaffold model). The orientation of bridges is in accord with the direction of tensile forces. A small fragment of the murein architecture is enlarged in the circle. Nine cross-linked peptidoglycan (PG) strands are clearly seen, plasma membrane (IM) being underneath the strands. For the purpose of clarity, only one turn of each strand is depicted, otherwise numerous crossed lines obscure the picture. In fact, strands are longer and the number of bridges is bigger. Four helical pores (channels) are seen round the central strand.

via attachment of the nascent strands to the leading edge [25].

To explain the mode of cylindrical growth, the concept of membrane-adhesion zones developed by Bayer is appropriate [26]. According to this concept, the inner membrane (IM) is able to bulge and approach the outer membrane (OM), effectively making a kind of OM–murein–IM multi-enzyme complex [27]. The initial local hollow space within the murein can be produced, for example, by the soluble transglycosylase Slt70 and endopeptidase; both enzymes are known to participate in gradual murein degradation [28]. The cavity is expected to expand by the turgor pressure, thus enabling the inner membrane to bulge. If the murein-synthesizing complexes assemble on top of the protrusion, they can simultaneously use precursors coming from the cytoplasm and be in contact with the pre-existing murein, which functions as the acceptor for the nascent strands. As the newly synthesized murein starts to fill the expanded cavity, the convex membrane gradually returns to its original position. As soon as the perforated murein is mended and a large piece of new material is inserted, the current round of murein growth is completed and the inner membrane bulges appear in other places to repeat new cycles. Metaphorically speaking, the membrane loaded with the murein-synthesizing complexes functions as a sewing machine, new murein being assembled in a direction from the OM to the IM (Figure 2b). To the best of our knowledge, this is the only mechanism that explains the random patchiness of murein *en masse* insertions into the sidewall of *E. coli* [25].

Regarding a secure mechanism for cell-wall division, we propose that, after chromosome segregation, intensive synthesis of murein is triggered, culminating in septum formation. During the constriction of the cell, the leading edge of the murein structure and the curved bend of the plasma membrane are clearly exposed, both of them adopting the form of concentric rings. The septum grows strictly centripetally, like the iris diaphragm of a camera, from the peripheral edges of the murein to the center [29]. In the case of septum formation, the murein-synthesizing complexes, such as the members of FtsZ-ring and associated counterparts [30], are probably located not at the top of membrane bulges (as is the case for cylindrical growth) but at the invaginated membrane curve.

The proposed concept of bacterial cell-wall morphogenesis is in agreement with well-documented observations that the process of a gradual *E. coli* cells lysis is paralleled by the release of simple muropeptides and peptides with concomitant increase in the cross-linking index of the remaining cell walls [28]. Moreover, in the course of lysis, walls become progressively thinner from inside to outside [31], whereas no visible holes and long cuts were observed on the isolated sacculi [32]. It is clear from Figure 2a that lytic degradation of the murein from inside by Slt70 and endopeptidase will result in the release of simple degradation products, and the surviving walls become thinner but relatively more cross-linked in comparison to the original murein. It is difficult to explain these

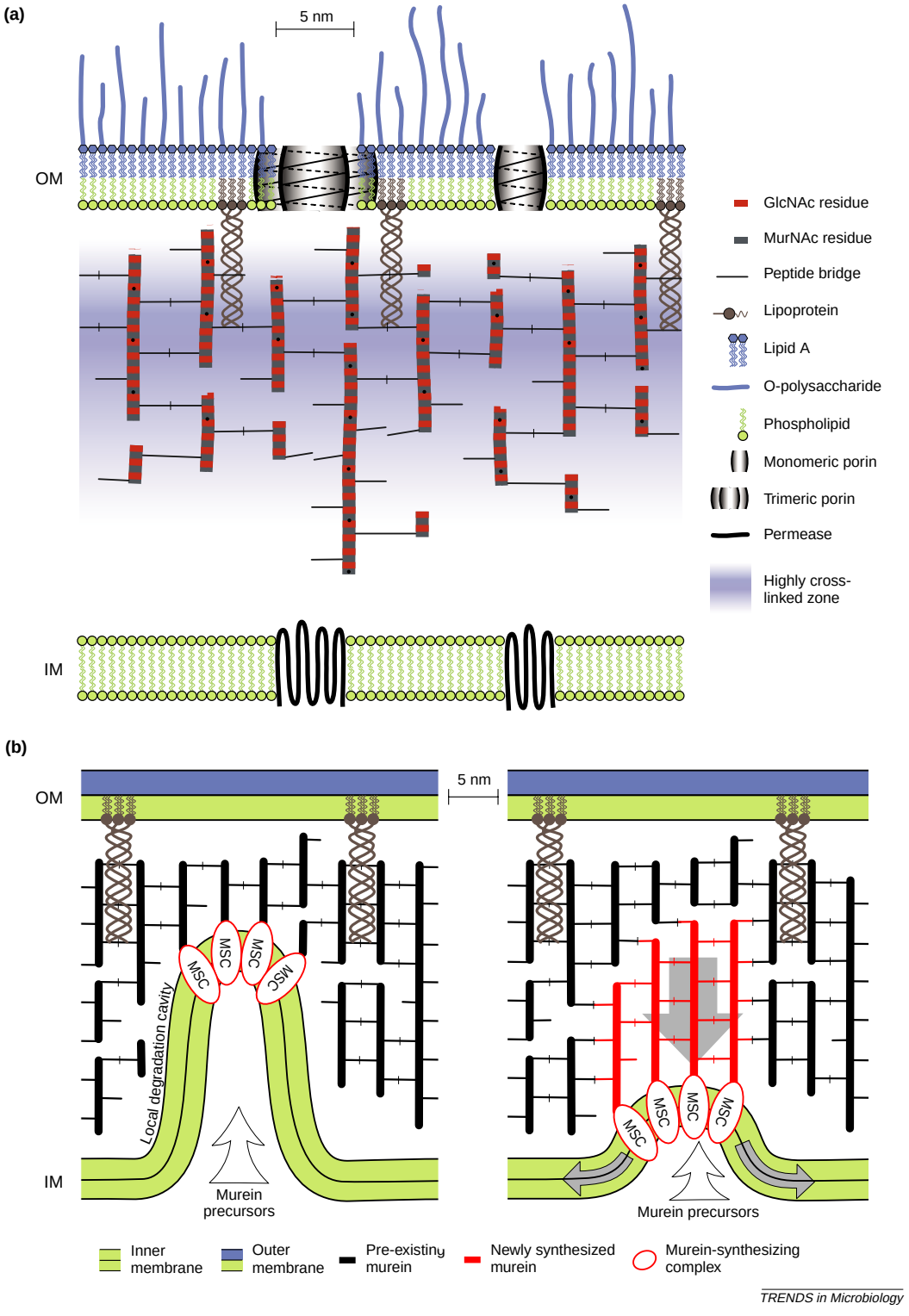

Figure 2. Proposed molecular architecture of a Gram-negative envelope and a tentative mechanism of bacterial cell wall growth. **(a)** The asymmetric outer membrane (OM) consists of two leaflets, the external one comprising lipopolysaccharide molecules (blue) and the internal leaflet comprising phospholipids (green). The essential component of the internal leaflet is lipoprotein (brown), the peptide moiety of which protrudes into the periplasm. Different transmembrane porins, either trimeric or monomeric, are common components of the OM. The inner membrane (IM, green) comprises phospholipids and is penetrated by different transmembrane proteins, of which two permeases are presented (black serpentine). Permeases are thought to locate strictly beneath the corresponding porins. The major component of the periplasm is the hydrated 'periplasmic gel'(blue diffuse zone) consisting of murein, the bridged PG strands of which run perpendicular to the IM. Certain central bridges are covalently connected to the ends of lipoprotein molecules that protrude into the periplasm. The height of the periplasm is variable and depends on external conditions. **(b)** Dynamics of the lateral murein growth by the patch-insertion mechanism is clearly seen from two 'snap-shot' pictures. On the left, the plasma membrane locally bulges to fill the cavity produced within the old cell-wall by a lytic enzyme bound to the periplasm. The existing turgor pressure is a driving force for both the cavity expansion and membrane bulging. The murein-synthesizing complexes (MSC ovals) locate on the bulge top, therefore having the possibility of translocating the biosynthetic murein precursors from the cytoplasm across the membrane with concomitant synthesis of the novel PG strands and their immediate attachment to the old wall edges. On the right, the novel murein patch is growing by elongation of the inserted strands, thus filling the cavity and pushing the membrane bulge downward until the plasma membrane returns to its original state.

Table 1. Experimental observations and murein models: compatibility

Observation and properties	Scaffold model	Network model	Refs
Arrangement of the strands and peptide bridges along tensile forces	Agreement	Conflict (classical), Agreement (chicken-wire)	
Branched-cell morphology	Agreement	Conflict	
Successive alteration of the division plane orientation	Agreement	Conflict	[37]
Release of 100-kDa proteins by hypo-osmotic shock	Agreement	Conflict	[6,38,39]
In silico modeling of murein assembly from separate strands to form stress-bearing matrix	Possible	Problematic	[6,34]
Reduction by 50% of murein content per unit of the surface area	Compatible	Problematic	[6,40]

observations from the position of the classical model because it implies that the cell wall is already thin and that lytic enzymes cut it along the glycan strands, much like scissors cut paper.

Current status of the scaffold model

We have also tentatively simulated the layered murein architecture according to the traditional network-model, and a comparison of the two models is presented in Table 1. Although the scaffold concept seems to have clear advantages over the traditional model, we do not wish to imply that it is unconditionally superior. Repeated experimental feedback and modeling input from cell wall experts of divergent opinions will be necessary to refine the model to fully reflect reality.

Recently, critics of the model argued that all our calculations are based on an average glycan-chain length of 8–12 disaccharide units, whereas (according to the critics) this average length is 33 units [4]. It is important to realize, however, that there are two methods for determining glycan chain lengths: (i) the direct measurement of glycan chains distribution in cell walls digests with L-alanine amidase followed by HPLC quantitative analysis [14], and (ii) an indirect method that determines the amount of terminal units relative to the amount of all fragments [33]. The first method is rather tedious, but highly accurate, whereas the second method is simpler but prone to overestimation. Evidently, the data obtained by the first method were our prime choice for creating an accurate model. Several researchers [17,20,25,34] interpret the data in the same way as we do here, namely to imply that the average glycan chain length is in the order of 8–12 disaccharides.

Concluding remarks

The proposed scaffold-like principle of bacterial murein architecture fulfills the requirements of a stress-bearing construction, enabling it to be simultaneously porous and elastic, compact and stretchable. The porous matrix retains a large amount of water, thus exhibiting the properties of a periplasmic gel, which is able to shrink and swell. The proposed principle is also in agreement with the idea of periplasm compartmentalization. The high viscosity gel prevents convection and enables only facilitated diffusion under the guidance of specific substrate-binding proteins and the plasma membrane potential, charge gradients being built up within the murein. The existence of both the compartmentalization and the gradient of electrical potential is a prerequisite for the effective influx of necessary nutrients across the periplasm; this is also true for the trafficking of polymers via distinct secretion pathways. These features of the proposed murein architecture offer new links to further biophysical and biochemical studies of the functions of bacterial cytoplasm and envelope, particularly those high-resolution technologies that are aimed at unravelling the problem of how cells are able to control precisely both the predetermined form and the constancy in length and width of their envelopes.

Acknowledgements

B.D. and F.T. are recipients of a scholarship from the Research Center Borstel. S.E. holds a grant from the Deutsche Forschungsgemeinschaft (SFB470-C9).

References

1 Stanier, R.Y. and van Niel, C.B. (1962) The concept of a bacterium. *Arch. Mikrobiol.* 42, 17–35
2 Weidel, W. and Pelzer, H. (1964) Bagshaped macromolecules – a new outlook on bacterial cell walls. *Adv. Enzymol. Relat. Areas Mol. Biol.* 26, 193–232
3 Höltje, J-V. (1998) Growth of the stress-bearing and shape-maintaining murein sacculus of *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* 62, 181–203
4 Vollmer, W. and Höltje, J-V. (2004) The architecture of the murein (peptidoglycan) in Gram-negative bacteria: vertical scaffold or horizontal layer(s)? *J. Bacteriol.* 186, 5978–5987
5 Dmitriev, B.A. *et al*. (1999) Layered murein revisited: a fundamentally new concept of bacterial cell wall structure, biogenesis and function. *Med. Microbiol. Immunol. (Berl.)* 187, 173–181
6 Dmitriev, B.A. *et al*. (2003) Tertiary structure of bacterial murein: the scaffold model. *J. Bacteriol.* 185, 3458–3468
7 Dmitriev, B.A. *et al*. (2000) Molecular mechanics of the mycobacterial cell wall: from horizontal layers to vertical scaffolds. *Int. J. Med. Microbiol.* 290, 251–258
8 Dmitriev, B.A. *et al*. (2004) Tertiary structure of *Staphylococcus aureus* cell wall murein. *J. Bacteriol.* 186, 7141–7148
9 Matias, V.R. and Beveridge, T.J. (2005) Cryo-electron microscopy reveals native polymeric cell wall structure in *Bacillus subtilis* 168 and the existence of a periplasmic space. *Mol. Microbiol.* 56, 240–251
10 Young, K.D. (2003) Bacterial shape. *Mol. Microbiol.* 49, 571–580
11 Varma, A. and Young, K.D. (2004) FtsZ collaborates with penicillin binding proteins to generate bacterial cell shape in *Escherichia coli*. *J. Bacteriol.* 186, 6768–6774
12 Schleifer, K.H. and Kandler, O. (1972) Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol. Rev.* 36, 407–477
13 Koch, A.L. (1998) Orientation of the peptidoglycan chains in the sacculus of *Escherichia coli*. *Res. Microbiol.* 149, 689–701

14 Harz, H. *et al*. (1990) Isolation and separation of the glycan strands from murein of *Escherichia coli* by reversed-phase HPLC. *Anal. Biochem.* 190, 120–128
15 Obermann, W. and Höltje, J-V. (1994) Alterations of murein structure and penicillin-binding proteins in minicells from *Escherichia coli*. *Microbiology* 140, 79–87
16 Ishidate, K. *et al*. (1998) Analysis of the length distribution of murein glycan strands in *fts*Z and *fts*I mutants of *Escherichia coli*. *FEMS Microbiol. Lett.* 168, 71–75
17 Boneca, I.G. *et al*. (2000) Characterization of *Staphylococcus aureus* cell wall glycan strands, evidence for a new beta-N-acetylglucosaminidase activity. *J. Biol. Chem.* 275, 9910–9918
18 Dubochet, J. *et al*. (1983) Electron microscopy of frozen-hydrated bacteria. *J. Bacteriol.* 155, 381–390
19 Shu, W. *et al*. (2000) Core structure of the outer membrane lipoprotein from *Escherichia coli* at 1.9Å resolution. *J. Mol. Biol.* 299, 1101–1112
20 Koch, A.L. (2000) Simulation of the conformation of the murein fabric: the oligoglycan, pentamuropeptide, and cross-linked nona-muropeptide. *Arch. Microbiol.* 174, 429–439
21 Hobot, J.A. *et al*. (1984) Periplasmic gel: new concept resulting from the reinvestigation of bacterial cell envelope ultrastructure by new methods. *J. Bacteriol.* 160, 143–152
22 Neidhardt, F.C. *et al.* (1990) Structure and function of bacterial cell parts. In *Physiology of the Bacterial Cell: A Molecular Approach*. pp. 30–61, Sinauer Associates
23 Koronakis, V. *et al*. (2004) Structure and function of TolC: the bacterial exit duct for protein and drugs. *Annu. Rev. Biochem.* 73, 467–489
24 Matias, V.R. *et al*. (2003) Cryo-transmission electron microscopy of frozen-hydrated sections of *Escherichia coli* and *Pseudomonas aeruginosa*. *J. Bacteriol.* 185, 6112–6118
25 De Pedro, M.A. *et al*. (2003) Patchiness of murein insertion into the side wall of *Escherichia coli*. *Microbiol.* 149, 1753–1761
26 Bayer, M.H. and Keck, W. (1990) Localization of penicillin-binding protein 1b in *Escherichia coli*: immunoelectron microscopy and immunotransfer studies. *J. Bacteriol.* 172, 125–135
27 Ishidate, K. *et al*. (1986) Isolation of differentiated membrane domains from *Escherichia coli* and *Salmonella typhimurium*, including fraction containing attachment sites between the inner and outer membranes and the murein skeleton of the cell envelope. *J. Biol. Chem.* 261, 428–443
28 Kitano, K. *et al*. (1986) Transglycosylase and endopeptidase participate in the degradation of murein during autolysis of *E. coli*. *J. Bacteriol.* 167, 759–765
29 Nanninga, N. (1998) Morphogenesis of *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* 62, 110–129
30 Errington, J. *et al*. (2003) Cytokines is in bacteria. *Microbiol. Mol. Biol. Rev.* 67, 52–65
31 Leduc, M. *et al*. (1985) Correlation between degradation and ultrastructure of peptidoglycan during autolysis of *Escherichia coli*. *J. Bacteriol.* 161, 627–635
32 Burman, L.G. *et al*. (1983) Evidence for multisite growth of *Escherichia coli* murein involving concomitant endopeptidase and transpeptidase activities. *J. Bacteriol.* 156, 386–392
33 Glauner, B. (1998) Separation and quantitation of muropeptides with high-performance liquid chromatography. *Anal. Biochem.* 172, 451–464
34 Pink, D. *et al*. (2000) On the architecture of the Gram-negative bacterial murein sacculus. *J. Bacteriol.* 182, 5925–5930
35 Burge, R.E. *et al*. (1977) Structure of the peptidoglycan of bacterial cell wall. *J. Med. Biol* 117, 927–953
36 Labischinski, H. *et al*. (1979) On the secondary and tertiary structure of murein. Low and medium X-ray evidence against chitin-based conformation of bacterial peptidoglycan. *Eur. J. Biochem.* 95, 147–155
37 Cooper, S. (1997) Division pattern of a round mutant of *Escherichia coli*. *J. Bacteriol.* 179, 5582–5584
38 Demchick, P. and Koch, A. (1996) The permeability of the cell wall fabric of *Escherichia coli* and *Bacillus subtilis*. *J. Bacteriol.* 178, 768–773
39 Vazquez-Laslop, N. *et al*. (2001) Molecular sieve mechanism of selective release of cytoplasmic proteins by osmotically shocked *Escherichia coli*. *J. Bacteriol.* 183, 2399–2404
40 Prats, R. and De Pedro, M.A. (1989) Normal growth and division of *Escherichia coli* with a reduced amount of murein. *J. Bacteriol.* 171, 3740–3745