

Elasticity of the Rod-Shaped Gram-Negative Eubacteria

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We report a theoretical calculation of the elasticity of the peptidoglycan network, the only stress-bearing part of rod-shaped Gram-negative eubacteria. The peptidoglycan network consists of elastic peptides and inextensible glycan strands, and it has been proposed that the latter form zigzag filaments along the circumference of the cylindrical bacterial shell. The zigzag geometry of the glycan strands gives rise to nonlinear elastic behavior. The four elastic moduli of the peptidoglycan network depend on its stressed state. For a bacterium under physiological conditions the elasticity is proportional to the bacterial turgor pressure. Our results are in good agreement with recent measurements.

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Bacteria are examples of cellular organisms in which many biological processes depend upon the mechanical state of their surfaces [1]. Bacteria are classified, broadly, into eubacteria and archaeobacteria. The former include most bacteria (examples of which are *Escherichia coli* and *Pseudomonas aeruginosa*) while the latter live in unusual environments [2]. Depending on their ability to be stained by the Gram technique, bacteria have been divided into two classes: the so-called Gram-negative and Gram-positive. Here we focus on the mechanical properties of the cell walls of Gram-negative eubacteria. This wall contains two surface membranes. The inner one, the plasma membrane, serves as the major permeability barrier. The outer membrane consists of phospholipids on the inner leaflet and lipopolysaccharides on the outer one and contains porins, proteins which form aqueous channels that render the membrane permeable to molecules. The third component of the cell envelope, the peptidoglycan network, lies in the so-called periplasmic space between the outer and the inner membranes and is linked to the outer membrane via lipoproteins. This network is thin (about 30 to 80 Å) [3]. Many Gram-negative bacteria have a nearly cylindrical shape. The internal volume of bacteria is filled with a cytoplasm containing a high concentration of macromolecules together with small solutes which confer to it a high osmotic pressure relative to the environment of the cell. Unlike animal cells, bacteria possess no bulk cytoskeleton. While the shape of a bacterium is determined by the structure of the peptidoglycan network, it is maintained by the turgor pressure p , i.e., the osmotic pressure difference between the inside p_{in} and the outside p_{out} pressures with $p = p_{in} - p_{out} > 0$. For Gram-negative bacteria the turgor pressure is reported to range from ~ 1 to 5 atm [1,4,5]. The turgor pressure is counterbalanced by the tension of the peptidoglycan network which, therefore, bears a high lateral stress. This network can also experience large deformations [6,7].

Until recently, information about the mechanical properties of bacterial envelopes was poor. Applications of

new techniques, however, have provided new insights into these systems and has permitted the development of testable models of the mechanical properties of bacterial envelopes. The experiments have used either whole bacteria or their isolated peptidoglycan networks. Among the former were the optical tweezers technique applied to manipulate *E. coli* [8] and atomic force microscopy (AFM) used for the qualitative characterization of several bacteria [9], for studying *E. coli* exposed to antibiotics [10], for measuring a force between a bacterium and a surface [11], for characterizing the effect of protamine on bacteria [12], for measuring the elasticity of a sheath of *Methanospirillum hungatei* [13] and for defining a turgor pressure of *Magnetospirillum gryphiswaldense* [14]. Recently, AFM was used to measure the elastic properties of the peptidoglycan network of *E. coli* and *P. aeruginosa* [15]. Here we concentrate on the mechanical properties of the latter inside living bacteria.

Yao *et al.* [15] isolated the peptidoglycan networks of the rod-shaped bacteria *E. coli* and *P. aeruginosa*, suspended them over a groove etched into a silicon surface, and, probing the sample with an AFM tip, measured the elastic moduli of both hydrated and dried networks. These were found to be 2.5×10^7 and $\sim 4 \times 10^8$ Pa, respectively.

A model of the configuration of the peptidoglycan network in rod-shaped bacteria was proposed by Verwer *et al.* [16] and was recently extended by Koch [17]. In this model the network is formed by inextensible, flexible glycan strands composed of disaccharide monomers cross-linked by elastic peptides (Fig. 1A). Two nearest cross-links are separated by two disaccharide groups of a glycan strand. The peptides are oriented along the bacterial cylindrical axis, while the glycan strands zigzag around the circumference of the cylindrical bacterial shell. The knots of the peptidoglycan network represent a distorted hexagonal lattice (Fig. 1A).

In this Letter making use of the above model, we report the calculation of the elastic modulus along the cylindrical

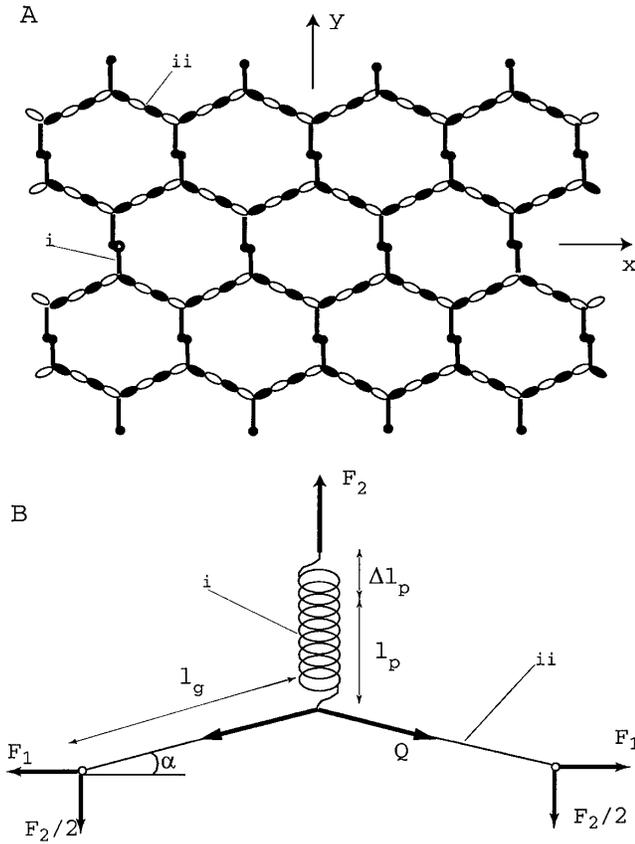


FIG. 1. (A) Schematic representation of a peptidoglycan network. Peptide cross-links are indicated by (i) while the ellipses represent *N*-acetylglucosamine (light) and *N*-acetylmuramic acid (dark) residues forming the glycan strand (ii). Although each *N*-acetylmuramic acid group possesses a peptide moiety, only 50% of them are oriented so that they can form cross-links. In practice, between 35% and 50% of the total peptide moieties are cross-linked at a given time in the cell's growth cycle [18]. (B) Structural element of the model of a peptidoglycan network. Spring (i) represents a peptide cross-link and heavy line (ii) represents a segment of a glycan strand. F_1 and F_2 are the forces acting on the structural element while Q is the tension in the glycan strand segment. l_p and Δl_p are the unstretched length and the length change of a peptide spring, l_g is the length of the inextensible glycan segment, and α is the zigzag angle.

axis of the peptidoglycan network of Gram-negative rod-shaped eubacteria and relate it to recent experiments.

In accord with their chemical structure, we model the mechanical behavior of the glycan strands between successive cross-links as inextensible filaments (length l_g between two cross-links) under tension and the peptide cross-links as elastic springs with spring constant κ and length l_p in the unextended state. These filaments and springs form the lattice shown in Fig. 1A. Loading of this network forces the glycan filaments to take a zigzag shape. This shape, together with the large deformability of the peptide cross-links, represents the possibility for large deformations of the network and gives rise to an intrinsic geometrical nonlinearity. We consider a flat

rectangular sheet of the network representing an involute of the cylindrical shell (radius R), the peptide cross-links being aligned along the Oy axis with the average orientation of the glycan strands being parallel to the Ox axis. We assume that the sheet is subjected to the stresses $\sigma_1 = pR/h$ and $\sigma_2 = pR/2h$ characteristic of a cylindrical shell. Here h is the peptidoglycan thickness. In the following we write the stress tensor as $\sigma_{xx} \equiv \sigma_1$, $\sigma_{yy} \equiv \sigma_2$, $\sigma_{xy} = \sigma_{yx} \equiv \sigma_3$ and the elastic moduli as $\lambda_{11} \equiv \lambda_{xxxx}$, $\lambda_{22} \equiv \lambda_{yyyy}$, $\lambda_{12} \equiv \lambda_{xxyy}$, $\lambda_{33} \equiv \lambda_{xyxy}$. The Oy axis coincides with the cylindrical axis in the cylindrical coordinate system (φ, y) and x is related to the angular coordinate φ of this system as $x = R\varphi$.

Consider one knot of this network (Fig. 1B). The forces acting on this element depend upon the state of the bacterium and are represented by F_2 and F_1 acting on the peptides and glycan strands of the element shown in Fig. 1B. They stretch the peptide cross-link by Δl_p and give rise to the zigzag angle α with the coordinate of the knot given by y . One finds that $F_2 = 2Q \sin \alpha$; $F_1 = Q \cos \alpha$, where Q is the tension of the glycan filament with the extension of the peptide given by $\Delta l_p = F_2/\kappa$ (Fig. 1B).

Let N_p denote the number of the peptide cross-links attached to two neighboring glycan strands, N_g denote the total number of the glycan strands, L_y is the cylinder length, and $L_x = 2\pi R$ is the sheet width. One finds

$$L_x = 2l_g N_p \cos \alpha; \quad L_y = N_g \left(l_p + l_g \sin \alpha + \frac{F_2}{\kappa} \right). \quad (1)$$

The total force $F_1^{(\text{tot})}$ applied to the sheet along the Ox axis and $F_2^{(\text{tot})}$ applied along the Oy axis can be expressed as $F_1^{(\text{tot})} = \sigma_1 L_y h$ and $F_2^{(\text{tot})} = \sigma_2 L_x h$. On the other hand, one finds $F_1^{(\text{tot})} = F_1 N_g$ and $F_2^{(\text{tot})} = F_2 N_p$. This yields the forces F_1 and F_2 acting on the structural element and the tension force Q :

$$F_1 = pR \left(l_p + l_g \sin \alpha + \frac{F_2}{\kappa} \right); \quad F_2 = \frac{\pi p R^2}{N_p}; \quad (2)$$

$$Q = \frac{\pi p R^2}{2N_p \sin \alpha}.$$

The latter result allows one to establish the implicit relation between the bacterial turgor pressure and the zigzag angle:

$$\frac{pR}{\kappa} = \frac{l_g(3 \cos 2\alpha - 1) - 4l_p \sin \alpha}{2l_g \sin 2\alpha}. \quad (3)$$

One can see that the angle α decreases with decreasing turgor pressure and, as $p \rightarrow 0$, tends to a finite value $\sin \alpha_0 = l_g [(l_p^2 + 3l_g^2)^{1/2} + l_p]^{-1}$ rather than to zero (in other words, the zigzag does not straighten itself as $p \rightarrow 0$). The reason for this is that, independently of p , the ratio of circumferential tension to the longitudinal tension of a cylindrical shell is $\sigma_1/\sigma_2 = 2$.

Note that Eqs. (1)–(3) show that the peptidoglycan elasticity is nonlinear. This stems from the geometric nonlinearity related to the zigzag shape of the glycan filaments.

From the chirality and structure of the peptidoglycan network one finds that its symmetry group is C_2 . Within this group the two-dimensional tensor of elastic constants λ_{ij} has four independent elements λ_{11} , λ_{22} , λ_{12} , and λ_{33} [19]. Accordingly, the structural element of the network shown in Fig. 1B can be characterized by four spring constants κ_{11} , κ_{22} , κ_{12} , and κ_{33} . In this Letter we limit ourselves by calculating the longitudinal elastic constant λ_{22} describing the elasticity of the peptidoglycan along the cylindrical axis.

Let us assume that the force F_1 is a constant and study the reaction of the structural element on increasing the force F_2 . Under these conditions the spring (Fig. 1B) describing the elasticity of the peptide cross-link works in series with the zigzag string. One finds the spring constant of the structural element to be $\kappa_{22} = 2\kappa Q / (2Q + \kappa l_g \cos^2 \alpha)$. Since the whole peptidoglycan sheet has N_p equal structural elements working in parallel and N_g working in series, one finds its total spring constant in the form $K_{22} = \kappa_{22} N_p / N_g$. One can express the perturbation of the total force $\Delta F_2^{(\text{tot})}$ acting along the Oy axis as $\Delta F_2^{(\text{tot})} = \sigma_2 L_x h = K_{22} \Delta L_y$, where the stress tensor component σ_2 has the form $\sigma_2 = \lambda_{22} u_2$ with $u_2 = \Delta L_y / L_y$. One finds the elastic modulus to be $\lambda_{22} = \kappa_{22} N_p L_y (N_g L_x h)^{-1}$. Making use of Eqs. (1) and (3) one finds the expression for the elastic constant λ_{22} to be

$$\lambda_{22} = \frac{\kappa Q}{2(2Q + \kappa l_g \cos^2 \alpha) h} \cot \alpha, \quad (4)$$

which, together with Eqs. (1), (2), and (3), yields its dependence on the turgor pressure in a parametric form.

The typical size of *E. coli* is $R \approx 5 \times 10^{-7}$ m, $L_y \approx 2$ to 6×10^{-6} m, and $l_g \approx l_p \approx 10^{-9}$ m [20]. Its turgor pressure is 2 to 3 atm [21] and the thickness of its peptidoglycan network is $h = 30 \text{ \AA}$ [22]. The rigidity of the peptide cross-link, κ , is unknown. One can estimate it as follows: $\kappa = \kappa_{\text{ent}} + \kappa_{\text{int}}$ is composed of the entropic contribution $\kappa_{\text{ent}} \approx k_B T / l_p^2$, and κ_{int} stemming from the attraction between its components, yielding $\kappa_{\text{ent}} \approx 4.1 \times 10^{-3}$ N/m. Our simulation of the peptide energy due to electrostatics and hydrogen bonding yields roughly the same value for κ_{int} . One finds $\kappa \sim 10^{-2}$ N/m and the ratio pR/κ of the order of 10. This implies a regime of small values of the angle $\alpha \approx 3\pi\kappa(4pl_g N_p)^{-1}$ of the order of $\alpha \sim 0.1$ rad and enables one to find a simple expression for the tension: $Q \approx p^2 l_g^3 N_p^2 / \pi^2 \kappa$. In this regime one finds $\kappa l_g / Q \ll 1$. This yields a simple approximate expression for the elastic constant

$$\lambda_{22} \approx \frac{pR}{2h}. \quad (5)$$

Another interesting case is that of a bacteria with no turgor pressure, as might result from antibiotic treatment.

In the case $p \approx 0$, the relationship between F_1 and F_2 is broken and Eq. (4) no longer holds. Now the peptides represent a set of parallel springs working in series and the longitudinal elastic constant λ_{22}^* is

$$\lambda_{22}^* = \kappa \frac{l_p + l_g}{l_0 h}, \quad (6)$$

where l_0 is the distance between two second neighbor knots along a glycan strand when $\alpha \approx \pi/2$ so that $l_0 \sim 10^{-9}$ m. As yet there have been no measurements of the elasticity of Gram-negative bacteria with $p \approx 0$.

Substituting the values of the parameters into the expression (5) one finds the elastic modulus of the peptidoglycan network to be $\lambda_{22} \approx 3 \times 10^7$ Pa. This is good correspondence with the value obtained by Yao *et al.* [15]. Making use of Eq. (6) one obtains an estimate $\lambda_{22}^* \sim 10^6$ to 10^7 Pa. A value of 3×10^7 Pa for the elastic modulus of a bacterial wall was also measured for *Bacillus subtilis* by Thwaites *et al.* [23]. For two reasons, however, these experiments cannot be directly interpreted in terms of our results. First, *B. subtilis* is a Gram-positive bacterium and therefore possesses a wall with a thickness of about 20 cross-linked peptidoglycan layers. Second, the measurements were carried out in air, and it is unknown to what extent the turgor pressure was decreased by the process of dehydration [23]. If one assumes that the turgor pressure is completely removed, then, by roughly estimating that the wall of the *B. subtilis* as ~ 10 peptidoglycan layers working in parallel $\kappa_{\text{ef}} \sim 10\kappa$, one finds $\lambda_{22}^* \sim 10^7$ Pa which is consistent with the measured value. Note that our result is in a good agreement with the value recently reported for a living *B. subtilis* [24].

It should be noted that Obermann and Höltje as well as Harz *et al.* [25] showed that the glycan strands of *E. coli* exhibit a length distribution with a maximum occurring at length ~ 10 disaccharide units (~ 10 nm) and decreasing monotonically to near zero at the shortest length. This distribution is equivalent to introducing a set of breaks (defect structures) into the above peptidoglycan network. Calculations show that, if the defect structure could be modeled by random bond breaking, then restrictions had to be placed upon which bonds could be cut in the neighborhood of bonds already cut [26]. The result of this was to conclude that such defect structures are cuts, running approximately perpendicular to the orientation of the glycan strands. It is, however, unknown what material fills these cuts or even whether they are filled at all. Lacking this information it is premature to discuss the effects of the defect structures upon peptidoglycan network elasticity. However, the agreement between the results reported here and the experimental data suggests that these defects play a minor role with regard to the elastic properties of such peptidoglycan networks.

To summarize, we have derived equations for the longitudinal elastic constant of a model of the (maximally

cross-linked) peptidoglycan network of Gram-negative eubacteria in the shape of a cylindrical shell. On the basis of both general considerations and our own computer simulations of the peptide cross-links, we found that our equations yielded $\sim 10^7$ Pa in accord with recent measurements. We raise the question regarding the importance of slitlike defects in the cylindrical network and conclude that these might play only a minor role in determining the elastic properties.

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