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Cell Wall Mechanical Properties as Measured with Bacterial Thread Made from *Bacillus subtilis*

NEIL H. MENDELSON[1]* AND J. J. THWAITES[2]

Department of Molecular and Cellular Biology, University of Arizona, Tucson, Arizona 85721,[1] and Department of Engineering, Cambridge University, Cambridge CB2 1PZ, England[2]

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Engineering approaches used in the study of textile fibers have been applied to the measurement of mechanical properties of bacterial cell walls by using the *Bacillus subtilis* bacterial thread system. Improved methods have been developed for the production of thread and for measuring its mechanical properties. The best specimens of thread produced from cultures of strain FJ7 grown in TB medium at 20°C varied in diameter by a factor of 1.09 over a 30-mm thread length. The stress-strain behavior of cell walls was determined over the range of relative humidities between 11 and 98%. Measurements of over 125 specimens indicated that cell wall behaved like other viscoelastic polymers, both natural and man-made, exhibiting relaxation under constant elongation and recovery upon load removal. This kinetic behavior and also the cell wall strength depended greatly on humidity. The recovery from extension observed after loading even up to a substantial fraction of the breaking load indicated that the properties measured were those of cell wall material rather than of behavior of the thread assemblage. Control experiments showed that neither drying of thread nor the length of time it remained dry before testing influenced the mechanical properties of the cell walls. Specimens drawn from TB medium and then washed in water and redrawn were found to be stiffer and stronger than controls not washed. However, tensile properties were not changed by exposure of cells to lysozyme before thread production. This suggests that glycan backbones are not arranged along the length of the cell cylinder. The strength of the cell wall in vivo was estimated by extrapolation to 100% relative humidity to be about 3 N/mm^2. Walls of this strength would be able to bear a turgor pressure of 6 atm (ca. 607.8 kPa), but if the increase in strength of water-washed threads was appropriate, the figure could be 24 atm (ca. 2,431.2 kPa).

Very little information is currently available about the mechanical properties of bacterial cell walls, although such information would be useful in a number of areas ranging from fundamental studies of bacterial growth and shape determination to applied systems in which bacteria are used for industrial processes. The dimensions of individual bacterial cells preclude direct measurements similar to those made in the study of other materials. Nevertheless, it has been shown that cell walls, in particular the load-bearing polymer peptidoglycan, stretch appreciably and recover (1, 5, 6, 10, 11, 19) and that cell filaments contract when the osmotic pressure is eliminated, indicating that the wall is stretched in living cells (7). The flexibility of the cell wall can be seen by observing the swimming motions of either long cells or cell separation-suppressed filaments. The degree to which wall can stretch and compress is also evident in the shape of cells found at the hairpin loop formed by folding of cell filaments during macrofiber formation (14).

We recently described a new system, bacterial thread, in which it is possible to measure the mechanical properties of *Bacillus subtilis* cell walls directly by standard techniques used in the study of textile threads (22). Bacterial threads should not be confused with macrofibers, although both can be produced from the same strains. Threads are man-made, can range to a meter in length, and contain about 50,000 cellular filaments in parallel alignment, whereas macrofibers produce themselves and are helically twisted aggregates of tens to hundreds of cellular filaments, rarely exceeding 10 mm in length. Significant improvements have now been made in the production of thread specimens and in their measurement by using a specially constructed tensile-testing instrument. The results obtained with this instrument from measurements of over 100 thread specimens are described in this report.

* Corresponding author.

MATERIALS AND METHODS

Bacteria. *B. subtilis* 168 FJ7, a cell separation-suppressed (*lyt*) mutant that carries *lyt-2* and *metC* markers, was used to produce the cultures from which bacterial threads were made. It is the same strain studied previously as bacterial thread (22) and also as macrofibers (15, 16).

Media and growth conditions. Cultures were grown in the complex medium TB consisting of the following (in grams per liter of water): tryptose (Difco Laboratories), 10; beef extract (Difco), 3; and NaCl, 5. This medium was supplemented with uracil (final concentration, 20 μg/ml) and thymine (final concentration, 20 μg/ml). Cultures were grown at 20°C in plastic petri dishes (100 by 15 mm) containing 8 ml of fluid TB medium. The cultures were inoculated by toothpick transfer from previous fluid-grown cultures of FJ7. When the cultures were grown overnight without agitation, the long cellular filaments produced formed a unique textile weblike structure. All of the cells in an entire culture appeared to belong to a single web.

Production of bacterial thread. Threads were produced by drawing out the web from the culture. A short nickel-chrome wire was bent into an S-shaped hook, one end of which was attached to a length of standard cotton thread. This thread was wound around a small drum that was attached to a quartz clock mechanism. The free end of the hook was inserted into the center of the web and raised by the clock mechanism at the rate of 22 mm/min. Individual cell filaments were thus drawn radially into the forming thread that

was apparently compressed by surface tension at the fluid-air interface into a compact bundle. The forming process was identical to that described earlier for hand-drawn specimens (18). Although the clock mechanism advanced the thread in 1-s pulses, the final bacterial thread produced bore no evidence of such pulsations. Uniform threads of up to 1 m in length and 180 μm in diameter were routinely produced. The largest specimens contained about 50,000 cellular filaments in cross-section and a total number of cells in excess of 10^{10}. Most specimens were produced by being drawn into the room atmosphere (~24°C, ~45% relative humidity). Some specimens, however, were produced and maintained at specific relative humidities throughout their testing. These threads were drawn into a controlled atmosphere within an AtmosBag (Aldrich Chemical Co., Inc.) by placing the entire culture and drawing apparatus inside the chamber. The atmosphere within the chamber was brought to a particular relative humidity by passing air through solutions of H_2SO_4 according to formulas given in reference 24 before the air was introduced into the chamber. The actual relative humidity within the chamber was determined with a probe attached to a hygrometer (Hanna Instruments; HI 8064, certified to ±3%, obtained from Markson).

Mounting thread specimens and measuring their uniformity. Threads were cut into specimens 30 mm long. These were glued to cards similar to that shown in Fig. 2 of reference 21. The new cards differed in that they contained mounting holes punched above and below to match posts on the jaws of the instrument used to measure the mechanical properties of the specimens. The uniformity of specimen diameter along the 30-mm length was determined by light microscopy. The mounted specimens were examined at a magnification of ×100 by taking measurements with an ocular micrometer about every 0.3 mm along the length. The ratio of maximum to minimum diameter for the most uniform specimens was 1.09.

Treatments of specimens before testing. Two kinds of treatment protocols were used: one in which the treatment was administered to the cellular web before drawing, and another in which drawn thread was rehydrated. In 12 cases, lysozyme (chicken egg white, crystallized three times; Sigma Chemical Co.) was added (final concentration, 40 μg/ml) to cellular webs which were then incubated at 24°C for 90 min before being drawn in the standard manner. These digestion conditions provided the maximum possible degradation of cell wall, leaving web structure in a form that could be drawn into thread. Continued incubation in lysozyme beyond this stage led to rapid reduction in cell filament length, disorganization of the web, and eventually liberation of spheroplasts. The rehydration procedure involved lowering a thread slowly into a beaker containing a liter of fluid so that it did not touch the walls. About 10 cm of thread remained dry above the fluid during the rehydration and was discarded when specimens were made. The rehydrated structures were allowed to swell fully before being redrawn in the standard manner. Specimens were rehydrated either in distilled water (reagent grade, produced by a Milli-Q system; Millipore Corp.) or in TB medium as described below.

Scanning electron microscopy. Thread specimens were fixed to aluminum stubs by using double-sided tape and were coated with gold. They were examined with an ISI scanning electron microscope model DS-130.

Tensility-testing instrument and test protocol. An instrument similar to the standard machines used in textile testing (e.g., Instron) was constructed from an optical bench slideway, the lead screw of which was driven by a stepper motor.

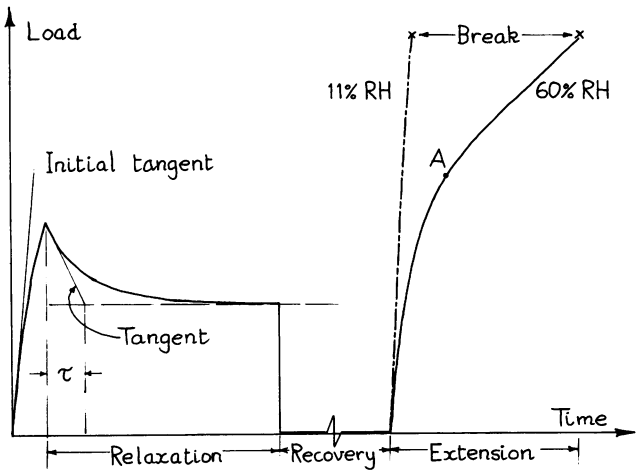

FIG. 1. Record of a typical *B. subtilis* FJ7 bacterial thread tensility test. During the first part of the test, the specimen is extended at constant rate. It is then held at constant length for a period of relaxation. It is then returned to its original (gauge) length and allowed to recover. Finally it is extended to break. The difference between high and low relative humidities (RH) (low relative humidity values shown with compressed "load" scale) is shown only for final loading. Point A represents the breaking point for hydrated threads at the corresponding relative humidity. The time scale of relaxation is indicated by τ, the time which would be required, at the initial rate of relaxation, for the fully relaxed state to be reached. (Reprinted from reference 16.)

The support cards on which thread specimens had been mounted were attached to upper and lower jaws, so that the thread was held in a vertical position. The sides of the support card were then cut, and the upper jaw was made to traverse at one of a set of fixed speeds, thus extending the thread. The most frequent rate of stretching used was 1 mm/min. The thread extension could be measured to ±2.5 μm. The lower jaw was fixed to a load cell consisting of a stiff double-cantilever spring, the displacement of which was measured by a linear variable displacement transducer. The output signal from the linear variable displacement transducer was amplified and transferred to an OmniScribe pen-chart recorder (Houston Instruments). The variations in load with extension or simply as a function of time were recorded. The system was enclosed at constant relative humidity within an Atmos-Bag. The standard test protocol and parameters measured for calculations of cell wall properties are shown in Fig. 1. The specimen was mounted so that when the support card was cut away, the thread was slightly slack. The motor was then turned by hand until the thread was just taut; the motor position was noted. The specimen was then extended. Following the increase in tension shown in Fig. 1, extension was allowed to continue until a target value of approximately half the breaking load was reached. Extension was then stopped, and the specimen was kept at constant length while tension relaxation was recorded until the value became asymptotic, which usually took between 20 and 30 min. The stretched specimen was then returned to its precise initial (gauge) length by manual rotation of the motor. The bowed thread was then allowed to recover for at least 30 min. Any residual extension was then noted in a final extension during which the stress was recorded to break. The breaking extension was determined from the point at which the tension suddenly fell to zero. The relative humidity was recorded on the chart at the beginning of the test, at the end of the relaxation period, at the end of the recovery

TABLE 1. Mechanical property values of cell walls derived from *B. subtilis* threads at different relative humidities

Specimen type and relative humidity (%)	Avg values for[a]:				
	ε_b (%)	σ_b (N/mm^2)	E_i (kN/mm^2)	E_r/E_i	τ (min)
Standard					
11	0.5–1.3	320	18	0.80	1.4
50	5–30	62	1.7	0.23	0.7
65	20–60	21	0.23	0.25	0.7
98	50–70	3.0	0.01	0.40	2.3
Water washed					
50	0.5–1.6	240[b]	15[b]	—[c]	—

[a] Properties: ε_b, extensibility as indicated by the range of observed values; σ_b, maximum strength observed; E_i, initial modulus; E_r, relaxed modulus; τ, time scale of load relaxation. The moduli and the τ value are averages obtained by drawing the (visually) best curves through the respective plots of property versus relative humidity.

[b] Averages for 10 specimens tested in the range of 48 to 56% relative humidity.

[c] —, Not measured.

period, and at the time of break. A variation involved making repeated extension-retraction cycles to investigate recovery from repeated loading. At the end of each test, the relative humidity was again measured. After being tested, the specimen was removed, and the diameter of the thread at the break point was measured by light microscopy by the method described above.

Calculated properties. Thread extensibility was determined by the geometric strain at break (breaking extension divided by initial length, given as a percent), and its strength was determined by the tensile stress at break (breaking tension divided by the area of the fracture cross section, in newtons per square millimeter [i.e., megapascals]). Elasticity was given by the initial (Young) modulus (incremental stress divided by incremental strain, in kilonewtons per square millimeter), which was measured from the initial tangent to the tension-extension curve (Fig. 1) and an average cross-sectional area based on the geometric mean of the maximum and minimum thread diameters. The viscoelastic nature was determined by the relaxed modulus (equilibrium stress divided by imposed strain) and the time scale of relaxation by the time τ (Fig. 1) that would be taken to reach the equilibrium tension at the initial rate of relaxation. When the cross-sectional area was calculated, the ratio of wall area to thread area was taken to be 0.2, which corresponds to a wall thickness of 40 nm.

RESULTS

Cell wall properties. Of all 125 specimens, the following were tested: (i) 73 standard threads (drawn into room atmosphere from TB cultures), tested in atmospheres of from 11 to 98% relative humidity; (ii) 20 threads which were never dried (produced and stored at the same relative humidity as that used when testing), tested in the range of 48 to 94% relative humidity; (iii) 20 washed threads (hydrated in either water or TB medium and then redrawn), tested in the range of 48 to 56% relative humidity; and (iv) 12 threads from lysozyme-treated cultures, tested over the same range as standard threads. For most of them, the following properties were derived from tests following the protocol shown in Fig.

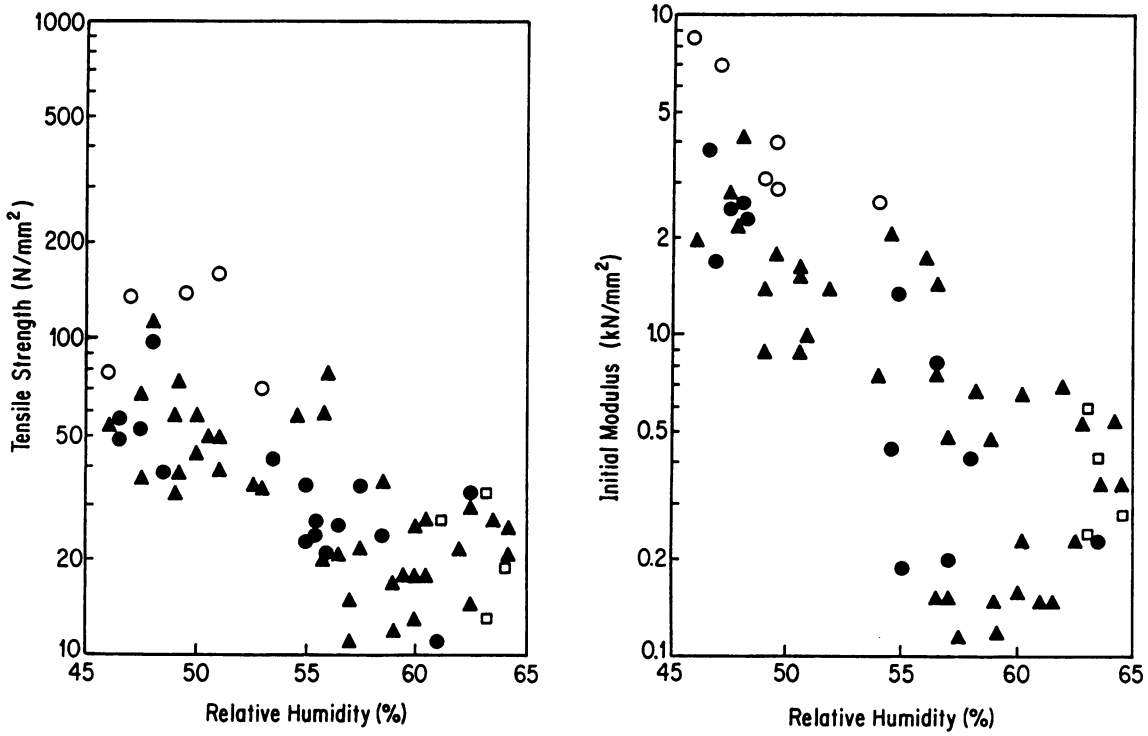

FIG. 2. Mechanical properties of cell walls measured in *B. subtilis* FJ7 thread in the range between 45 and 65% relative humidity. (A) Tensile strength; (B) initial (Young) modulus. Symbols: ▲, standard threads tested after being drawn from TB medium into room atmosphere; ●, threads that were not dried, tested after being drawn from TB medium into an atmosphere of the same relative humidity as that present during the test; □, threads drawn from lysozyme-digested cultures; ○, threads that were washed and then redrawn from TB medium.

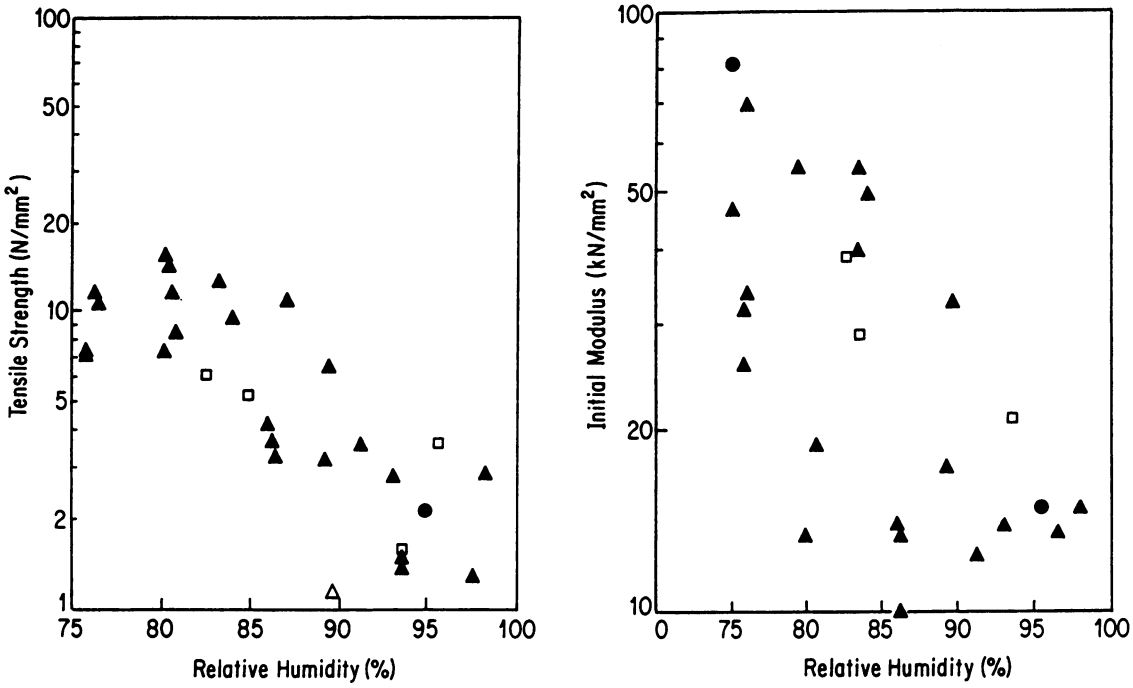

FIG. 3. Mechanical properties of cell walls measured in *B. subtilis* FJ7 thread in the range between 75 and 98% relative humidity. (A) Tensile strength; (B) initial (Young) modulus. Symbols are as described in the legend to Fig. 2.

1: extensibility, tensile strength, initial elastic modulus, the relaxed modulus, and the characteristic relaxation time τ.

Property variation as a function of relative humidity. Results for standard threads are summarized in Table 1. The full set of data (not shown here but to be published elsewhere) shows continuous variation in each property over the entire relative humidity spectrum. Previously we found that cell wall mechanical properties changed greatly as a function of the amount of water present (22). The new measurements, which because of the improved technique exhibited less spread, strengthen this conclusion. The extensibility rose rapidly (with rising relative humidity) up to about 60% (at 60% relative humidity) and then remained constant. Both tensile strength and elastic moduli fell continuously with increasing relative humidity; strength fell by a factor of about 100, and the moduli fell by a factor of about 2,000.

Figure 2 shows the results for the range of 45 to 65% relative humidity. This includes the region of the relative humidity spectrum (48 to 56%) in which threads that had been rehydrated in water and then redrawn were tested. Figure 3 shows results in the range of 75 to 100% relative humidity. Results from standard threads, threads that were never dried, and threads produced after lysozyme treatment of the culture webs are included in both ranges. There were no significant differences in properties between standard and never dried threads, nor did the lysozyme treatment have any effect. This was clear in the range of 60 to 65% relative humidity (Fig. 2) and above 80% relative humidity (Fig. 3). It was also true for the four remaining lysozyme-treated threads tested in the range of 11 to 15% relative humidity. So far as we can establish, the age of the threads, between formation and testing, up to 20 days, had no effect either.

Estimation of in vivo properties. Under normal physiological growth conditions in the laboratory, cells are fully hydrated. Direct measurements of such cells are not currently within the capabilities of our instrumentation, but extrapolation from results at high relative humidity is possible. These results are shown in Fig. 3. A clear trend is evident, indicating what the approximate values must be like in growing cells. Extrapolation of the tensile strength values are particularly significant, since the strength of the cell wall places limitations on the amount of turgor pressure that could be borne by cells. The data shown in Fig. 3 suggest a value of about 3 N/mm^2 at 100% relative humidity. Assuming that the cylindrical part of the cell wall is stronger in the hoop direction than in the axial direction and that the poles are stronger than the cylinder, the extrapolated value of 3 N/mm^2 means that cells growing in TB medium could be subject to a turgor pressure of 0.6 MPa (about 6 atm) without bursting. It should be remarked that, while the assumption of a particular ratio for wall thickness to cell diameter affects the calculation of breaking stress and modulus, it does not affect this conclusion, for the ratio appears in the same way in the calculation of wall stress both from turgor pressure and in our experiments.

Hydrated-redrawn-thread structure. A number of unusual phenomena were noted in measurements of water-hydrated threads. First of all (Table 1), the results of water hydration were an increase in strength at about 50% relative humidity by a factor of approximately 4 and an increase in stiffness by a factor of approximately 9, whereas the effect of rehydration in TB medium was negligible (Fig. 2). The strength increase was obtained despite the fact that breaking extensions were significantly less than those for standard threads, the breaking point in the final extension always appearing to correspond to a point such as point A in Fig. 1. Examination of the diameter of broken ends in such threads revealed that the diameter at the place of break was almost always appreciably greater than the minimum diameter along the specimen length. This was never the case in standard

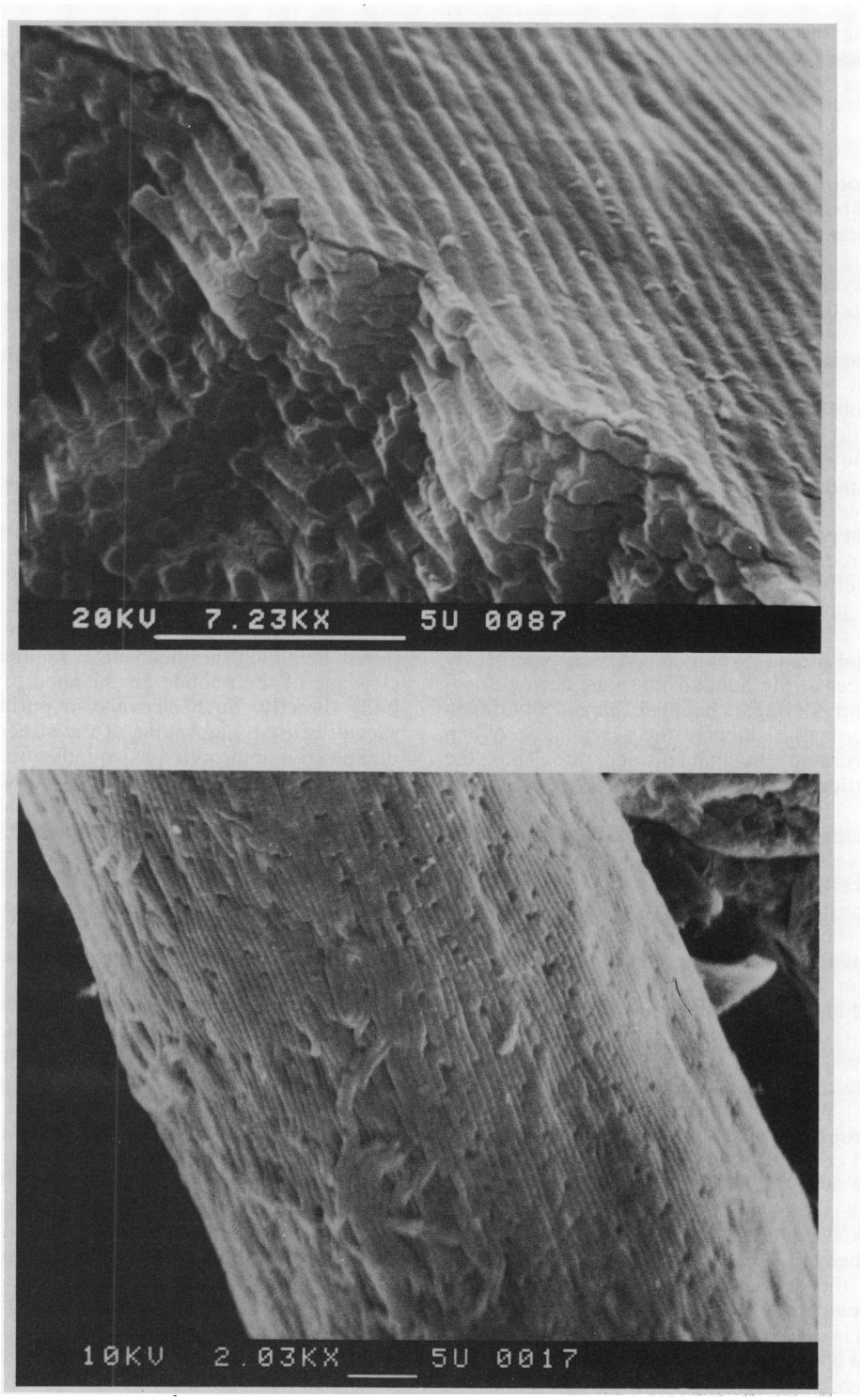

FIG. 4. Scanning electron micrographs showing differences between *B. subtilis* thread drawn directly from TB medium (top) and those redrawn after being washed in water (bottom). The top panel shows the surface and interior at a fracture cross section. Coating of individual cellular filaments is evident at the surface of the thread. The bottom panel shows gaps in the surface of a water-washed thread that apparently lacks the coating material. Bar, 5 μm.

threads, and it suggests to us that breakage may arise in water-hydrated specimens at faults in the assemblage rather than in the wall material itself. We therefore examined threads which had been water-hydrated and then redrawn by scanning electron microscopy and found that the surface of such specimens is very different from that of standard threads. Standard threads contain a surface-coating material (probably residual TB medium) that apparently fills all gaps between cells on the surface and within the thread, whereas the surfaces of threads which have been water-hydrated and then redrawn are free of this coating and contain many gaps between cell and cellular filament ends (Fig. 4). The align-

ment of cells along the thread axis is not as perfect in water-hydrated threads as it is in those drawn directly from TB medium (compare also Fig. 3 in reference 22).

DISCUSSION

To understand both the way in which bacterial cell walls form structures responsible for determination and maintenance of cell shape and for protection of the cell membrane from rupture by turgor pressure and the way in which they may influence cell cycle regulation, we require knowledge of their material properties. We used fiber science engineering approaches to measure these properties in bacterial thread. It should be emphasized that our measurements do indeed pertain to the cell wall material and not to the thread assemblage. Two observations indicate that the individual cellular filaments do not slide along one another during stretching: first, the shape of the stress-strain curves observed (Fig. 1) is not like that found when multistranded textile fibers pull out but is exactly like those found in the true measurement of polymer fibers. Secondly, sliding would be an irreversible process leading to permanent deformation, whereas the recovery of bacterial thread from very large extensions is also just like that of polymer materials. It appears that there must be very strong adhesion between filaments in bacterial thread. When compressed together by surface tension forces at the fluid-air interface during drawing and subsequent drying, chemical bonds (hydrogen bonds) probably form that stabilize the assemblage. When threads are fully hydrated, the filaments separate. Thus, the viscoelastic properties observed must be those of the cell wall material.

It is conceivable that, just at the point of break, filaments slide, but we believe that they do not. It is not possible to identify with any certainty actual cell fracture in scanning electron micrographs, but the extent to which individual filaments protrude beyond the median plane of break is clearly visible in light and scanning electron micrographs. The protrusion is on the order of tens of micrometers. The length of individual filaments as observed in cultures before drawing is on the order of millimeters. If filament sliding accounted for thread failure, it would be expected that filaments would protrude by such amounts. (The specimen length was 30 mm.) We therefore believe that not only the viscoelastic properties but also the fracture properties observed are those of cell wall material.

In gram-positive walls such as those we studied, the structure of the wall is known to consist of a porous network of highly cross-linked peptidoglycan to which teichoic acid polymers are attached (12, 20). Neither the orientation of the peptidoglycan nor the degree of order in the structure is known. Available evidence suggests, however, that a crystalline organization is unlikely and that there are no α-helices or β-sheets but that a completely unordered conformation is also unlikely (3, 4, 8, 9, 18, 23). The way in which macrofibers grow indicates that the wall must be assembled in a highly organized manner and that its final form is likely to reflect in some way the geometry of assembly (15).

The stress-strain curves obtained from bacterial thread indicate that the stress-bearing polymer peptidoglycan behaves like other viscoelastic polymers, both natural and synthetic. At low relative humidity, the almost linear stress-strain relation is typical of a glassy polymer. The nonlinear behavior for relative humidity greater than about 60% (Fig. 1) is like that of a rubbery polymer. The kinetics of stress relaxation at a constant level of extension and the ratio of the relaxed modulus to the initial modulus are similar to those of other highly cross-linked polymers. The most likely mechanism to account for the deformation of peptidoglycan is therefore, as for other polymers, a combination of chain unfolding and disentangling which is, as in our experiments, to a large extent reversible. For most polymers, the chain in question is the backbone of the polymer, but peptidoglycan is much less polymerized than cellulose or chitin (approximately 100 disaccharide units compared with about 10,000 for cellulose) and its side chains are much longer. It is arguable that the peptide chains are those that unfold. This is supported by the evidence of data from threads drawn from lysozyme-treated cultures. For even when the degree of digestion was increased to the maximum possible compatible with thread production, no differences could be found in the properties of the wall material compared with controls not subjected to cleavage of the glycan backbone.

It is possible that the highly cross-linked nature of the wall may result in a nonlinear relationship between glycan length and stress-strain behavior so that a measurable effect would require digestion beyond the point at which threads could still be drawn from the digested web, but it is more likely that the glycans are oriented predominantly in the hoop direction rather than parallel to the cell axis and that unfolding of the peptides is the major feature of our experiments. Experiments involving the mechanical ramifications of enzymatic cleavage of the peptide are planned to examine this possibility directly. Such cleavage in purified *Escherichia coli* sacculi caused a loosening of the structure and revealed an organization interpreted as an indication that the glycans lie predominantly in the hoop direction (23). Molecular structure modeling of peptidoglycan also led to the suggestion that glycans lie in this orientation in *B. subtilis* (10).

Models of peptide structure based on X-ray crystallography studies have suggested that the minimum energy conformation in isolation consists of a ringlike structure lying close to the glycan backbone (2). If extended to its maximum length, the peptide would project about three times as far from the glycan as in the ring configuration (9). This would define the maximum length to which the structure could be stretched before covalent bonds would be broken. Therefore, if the peptides were oriented parallel to the cell cylinder axis and were not subject to other interactions, a 200% length extension should be possible. If the average orientation of the peptides was at an angle to the cell axis, less extension of the cylinder length would be possible with maximum extension of the peptides. Other factors which must affect the deformation are (i) electrostatic interactions between charged groups in the wall such as those at other regions of the peptidoglycan, on accessory polymers such as teichoic acid, on other macromolecules in the wall such as proteins, or on counterions; and (ii) hydrophobic-hydrophilic interactions that involve peptidoglycan groups and water in the wall. Our measurements indicate that the degree of extension possible in peptidoglycan is highly dependent on the amount of water present; maximum extension on the order of 60 to 70% was found for all relative humidities above about 60%. This is clearly much less than theoretical values but it is approximately three times the degree to which peptidoglycan is believed to be stretched from its relaxed conformation in live cells of *E. coli* because of turgor pressure (7). At present we do not know which factors limit the degree to which our specimens can be extended.

The results obtained from testing standard threads indicate that the cell wall extensibility, except at low humidity, is comparable to that of cellulose but much less than that of

other biological polymers such as elastin (21, 25). On the other hand, the tensile strength of cell walls is comparable to that of cellulose only when dry. When wet, cell walls are about 15 times weaker than cellulose and 5 times weaker than chitin (21). Walls containing a greater amount of water were progressively weaker than those with less water. Because cell wall has a much larger number of possible H-bond sites than cellulose, this weakening most probably reflects conformational differences in the wall polymers that contribute to the strength of drier walls, but it could be due to hydrophilic interactions of constituent groups that are stabilized when the amount of bulk water increases. The variation in elastic modulus with relative humidity is even more dramatic. When dry, cell wall has approximately the same modulus as cellulose, but the modulus falls by a factor of about 2,000 when the cell wall is wet, compared with a factor of about 4 for cellulose. The results support quantitatively the supposition of Marquis that because of its shorter-chain, noncrystalline structure, peptidoglycan is less stiff than cellulose (8, 11, 19). But the results refer only to axial deformation; cell wall may be much stiffer in the hoop direction (1, 9, 10). Indeed, it would be surprising if it is not, for cells maintain their diameter very constantly during growth. We intend to test this by modifying our apparatus so that the anisotropic properties of bacterial thread can be measured.

When bacterial threads were washed by hydration in distilled water and then redrawn, the properties of the wall material changed. The washed specimens appeared stiffer and stronger than controls which were either not washed or washed in the growth medium TB. We believe that this change in properties reflects the removal from the walls of ions such as sodium and chloride that are present in high concentration in TB medium. Water-washed threads also differed from controls in the point at which they ruptured during final extension to break. Many broke prematurely, suggesting that faults in the thread surfaces might be responsible for effectively reducing the thread diameter. Scanning electron micrographs showed that washed threads had many gaps in their surfaces at which breakage could initiate. Comparable spaces in standard threads either were not present or were apparently filled by residual TB medium. It is not unrealistic to assume that the viscoelastic properties of peptidoglycan would be influenced by the ionic environment. Previously, Marquis showed that the degree of contraction of cell walls was governed by electrostatic interactions in the wall and thus was strongly influenced by ions (11). A systematic examination of ion effects on the stress-strain behavior of bacterial threads is currently in progress and will be reported elsewhere.

For threads washed in water and tested in the relative humidity range of 48 to 56%, the tensile strength was about four times that of standard threads. If this factor also applied at very high relative humidity, our extrapolated estimate of washed-wall strength would be 12 N/mm^2 (12 MPa). Cells with such walls would be able to withstand a turgor pressure of some 24 atm (ca. 2,431 kPa), which is on the same order as that obtained by indirect techniques some years ago (13, 17). Clearly, further experimentation is required to clarify this point. It is not at all obvious what state of cell walls, in the form of thread, should be considered in order to make a valid comparison with cells in vivo.

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LITERATURE CITED

1. **Baldwin, W. W., M. J.-T. Sheu, P. W. Bankston, and C. L. Woldringh.** 1988. Changes in buoyant density and cell size of *Escherichia coli* in response to osmotic shocks. J. Bacteriol. **170**:452–455.
2. **Barnickel, G., D. Naumann, H. Bradaczek, H. Labischinski, and P. Giesbrecht.** 1983. Computer aided molecular modelling of the three-dimensional structure of bacterial peptidoglycan, p. 61–66. *In* R. Hackenbeck, J.-V. Holtje, and H. Labischinski (ed.), The target of penicillin. W. de Gruyter & Co., Berlin.
3. **Burge, R. E., R. Adams, H. H. M. Balyuzi, and D. A. Reaveley.** 1977. Structure of the peptidoglycan of bacterial cell walls. II. J. Mol. Biol. **117**:955–974.
4. **Burge, R. E., A. G. Fowler, and D. A. Reaveley.** 1977. Structure of the peptidoglycan of bacterial cell walls. I. J. Mol. Biol. **117**:927–953.
5. **Isaac, L., and G. C. Ware.** 1974. The flexibility of bacterial cell walls. J. Appl. Bacteriol. **37**:335–339.
6. **Knaysi, G., J. Hillier, and C. Fabricant.** 1950. The cytology of an avian strain of *Mycobacterium tuberculosis* studied with the electron and light microscopes. J. Bacteriol. **60**:423–447.
7. **Koch, A. L., S. L. Lane, J. A. Miller, and D. G. Nickens.** 1987. Contraction of filaments of *Escherichia coli* after disruption of cell membrane by detergent. J. Bacteriol. **169**:1979–1984.
8. **Labischinski, H., G. Barnickel, H. Bradaczek, and P. Giesbrecht.** 1979. On the secondary and tertiary structure of murein. Eur. J. Biochem. **95**:147–155.
9. **Labischinski, H., G. Barnickel, and D. Naumann.** 1983. The state of order of bacterial peptidoglycan, p. 49–54. *In* R. Hackenbeck, J.-V. Holtje, and H. Labischinski (ed.), The target of penicillin. W. de Gruyter & Co., Berlin.
10. **Labischinski, H., G. Barnickel, D. Naumann, and P. Keller.** 1985. Conformational and topological aspects of the three-dimensional architecture of bacterial peptidoglycan. Ann. Inst. Pasteur Microbiol. **136A**:45–50.
11. **Marquis, R. E.** 1968. Salt-induced contraction of bacterial cell walls. J. Bacteriol. **95**:775–781.
12. **Marquis, R. E.** 1988. Turgor pressure, sporulation, and the physical properties of cell walls, p. 21–32. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
13. **Marquis, R. E., and E. L. Carstensen.** 1973. Electric conductivity and internal osmolality of intact bacterial cells. J. Bacteriol. **113**:1198–1206.
14. **Mendelson, N. H.** 1982. Dynamics of *Bacillus subtilis* macrofiber morphogenesis: writhing, folding, close packing, and contraction. J. Bacteriol. **151**:438–449.
15. **Mendelson, N. H., D. Favre, and J. J. Thwaites.** 1984. Twisted states of *Bacillus subtilis* macrofibers reflect structural states of the cell wall. Proc. Natl. Acad. Sci. USA **81**:3562–3566.
16. **Mendelson, N. H., and J. J. Thwaites.** 1988. Studies of *Bacillus subtilis* macrofiber twist states and bacterial thread biomechanics: assembly and material properties of cell walls, p. 109–125. *In* P. Actor, L. Daneo-Moore, M. L. Higgins, M. R. J. Salton, and G. D. Shockman (ed.), Antibiotic inhibition of bacterial cell surface assembly and function. American Society for Microbiology, Washington, D.C.
17. **Mitchell, P., and J. Moyle.** 1956. Osmotic function and structure in bacteria. Symp. Soc. Gen. Microbiol. **6**:150–180.
18. **Naumann, D., E. Fischer, W. Ronspeck, G. Barnickel, H. Labischinski, and H. Bradaczek.** 1983. The conformational behaviour of the peptide part of murein—an infrared and raman spectroscopic study, p. 73–78. *In* R. Hakenbeck, J.-V. Holtje, and H. Labischinski (ed.), The target of penicillin. W. de Gruyter & Co., Berlin.

19. **Ou, L.-T., and R. E. Marquis.** 1970. Electrochemical interactions in cell walls of gram-positive cocci. J. Bacteriol. **101:** 92–101.
20. **Rogers, H. J., H. R. Perkins, and J. B. Ward.** 1980. Microbial cell walls and membranes. Chapman & Hall, Ltd., London.
21. **Thor, C. J. B., and W. F. Henderson.** 1940. The preparation of alkali chitin. Am. Dyestuff Rep. **29:**461–464.
22. **Thwaites, J. J., and N. H. Mendelson.** 1985. Biomechanics of bacterial walls: studies of bacterial thread made from *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **82:**2163–2167.
23. **Verwer, R. W. H., N. Nanninga, W. Keck, and U. Schwarz.** 1978. Arrangement of glycan chains in the sacculus of *Escherichia coli*. J. Bacteriol. **136:**723–729.
24. **Weast, R. C., and M. J. Astle (ed.).** 1980. Handbook of chemistry and physics, 60th ed., p. E-46. CRC Press, Inc., Boca Raton, Fla.
25. **Weis-Fogh, T.** 1960. A rubber-like protein in insect cuticle. J. Exp. Biol. **37:**889–906.