Penicillin-Binding Proteins and Peptidoglycan of
Treponema pallidum subsp. pallidum

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Penicillin-binding proteins (PBPs) of Treponema pallidum subsp. pallidum (T. pallidum) were characterized by using [3H]penicillin G and a conjugate consisting of ampicillin and 125I-labeled Bolton-Hunter reagent. Both antibiotics specifically radiolabeled proteins with molecular masses of 94, 80, 63, and 58 kilodaltons (kDa); 125I-labeled Bolton-Hunter reagent-ampicillin also radiolabeled several polypeptides with lower molecular masses. The 94- and 58-kDa proteins demonstrated the highest binding affinities for [3H]penicillin G and were radiolabeled at concentrations of 8 and 40 nM, respectively. Radiolabeling of PBPs was detectable after 1 min of incubation in 1 nM [3H]penicillin G and was nearly maximal within 10 min. The rapidity of penicillin binding contrasted with the observation that only 40% of virulent treponemes became immobilized during prolonged incubation in vitro with a much higher concentration (1 mM) of unlabeled penicillin. Two lines of evidence indicated that most, if not all, of the PBPs are integral cytoplasmic membrane proteins: (i) preincubation of organisms in 0.1% Triton X-100 solubilized nearly all of the outer membranes but did not affect radiolabeling of PBPs, and (ii) except for the 80-kDa proteins, the PBPs partitioned into the detergent phase following extraction with the nonionic detergent Triton X-114. The presence of peptidoglycan in T. pallidum was confirmed by the detection of muramic acid in the sodium dodecyl sulfate-insoluble, protease K-resistant residue obtained from Triton X-114-extracted organisms.

The exquisite sensitivity of Treponema pallidum subsp. pallidum (T. pallidum) and the related pathogenic treponemes to penicillin was recognized nearly 40 years ago (11, 22). Nevertheless, the basis for the treponemical activity of beta-lactam antibiotics is poorly understood. In both gram-positive and gram-negative bacteria, these agents inactivate membrane-bound enzymes (designated penicillin-binding proteins [PBPs]) that are essential for peptidoglycan biosynthesis (30, 34), and it has been presumed that they kill treponemes via a similar mechanism. In support of this hypothesis, Cunningham et al. (8) recently identified putative PBPs in T. pallidum. On the other hand, direct biochemical evidence for the existence of peptidoglycan in T. pallidum has not been reported; its presence has been inferred from electron micrographs (17) and from biochemical analysis of cultivatable, nonpathogenic treponemes (16, 18, 28).

Considerations relevant to both experimentally produced and acquired human syphilis prompted our investigations of T. pallidum PBPs and peptidoglycan. First, either or both of these substances could provide biochemical markers in ongoing efforts to localize specific protein immunogens to either the cytoplasmic or outer membranes of the organism. Second, elucidation of the molecular basis for the treponemical activity of beta-lactams may expedite selection of antibiotics for clinical evaluation. This is particularly important in view of the alarming increases in acquired and congenital syphilis recently reported in the United States (6) and the fact that penicillin allergy frequently complicates the therapy of patients with syphilis (35). Last, most treponemal proteins investigated in recent years have been characterized on the basis of their reactivities with monoclonal or polyclonal antibodies (24). PBPs are logical candidate molecules for characterization by biochemical rather than immunological criteria.

The present study, therefore, had the following objectives: (i) to define the properties of the putative PBPs of T. pallidum, (ii) to determine their cellular locations, and (iii) to provide direct evidence for the presence of peptidoglycan.

MATERIALS AND METHODS

Source and preparation of treponemes. The virulent Nichols strain of T. pallidum was propagated by intratesticular passage in New Zealand White rabbits without the use of cortisone acetate injections. Ten to twelve days after inoculation, the rabbits were sacrificed by intravenous injection of 1 ml of T-61 euthanasia solution (American Hoescht Corp., Somerville, N.J.), and the testes were aseptically removed. Treponemes were extracted on a rotary shaker in either phosphate-buffered saline (PBS [pH 7.4]) or, when intended for extended incubation, in a highly enriched medium supplemented with 10% heat-inactivated normal rabbit serum (32). Methionine and cysteine were omitted from media used for 35S radiolabeling of T. pallidum proteins (26). Freshly extracted treponemes were centrifuged twice for 10 min at 500 x g to remove gross testicular debris. When required, organisms were purified by Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) density gradient centrifugation (15).

Antibiotics. Unlabeled potassium penicillin G and sodium ampicillin were obtained from Sigma Chemical Co., St. Louis, Mo. [3H] penicillin G (27 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, Ill. Upon receipt, 50-μCi portions were evaporated to dryness on a Speed-Vac concentrator (Savant Instruments, Inc., Farmingdale, N.Y.) and stored at −70°C. The portions were reconstituted in 50 mM sodium phosphate buffer (pH 7.4) immediately prior to PBP radiolabeling experiments. Ampicillin was conjugated to 125I-labeled Bolton-Hunter reagent by the method of Schwarz et al. (29). Briefly, 500 μCi of 125I-labeled Bolton-Hunter reagent (Amersham) was reacted at 0°C for 30 min

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with 25 μg of sodium ampicillin in 0.1 M sodium borate buffer (pH 8.5). The [125I]ampicillin conjugate was purified on a 5-ml column of Bio-Gel P2 (Bio-Rad Laboratories, Richmond, Calif.). For calculation purposes, it was presumed that the peak representing the 125I-labeled Bolton-Hunter reagent-ampicillin contained the entire 25 μg of starting material.

**Radiolabeling of *T. pallidum* PBPs.** Two different protocols, both modifications of the method described by Spratt (31), were used to radiolabel PBPs.

(i) **Extended incubation.** Organisms cleared of gross testicular debris were collected by high-speed centrifugation at 20,000 × g for 15 min and suspended in enriched medium at a concentration of 10^7/ml. Unlabeled penicillin G from a 20-mg/ml aqueous stock solution was added to a 2-ml portion to give a final penicillin concentration of 1 mM. After a 10-min preincubation at 34°C, 1 nmol of [3H]penicillin per ml (1 μM final concentration of [3H]penicillin) was added to this and to an identical portion that had not been preincubated with unlabeled penicillin. Additional incubation mixtures contained either no antibiotics or 1 mM unlabeled penicillin G. They then were incubated for 18 h at 34°C in an atmosphere of 95% N₂-5% CO₂. Organisms were harvested in 0.5-ml portions by high-speed centrifugation as described above. The pellets were solubilized by the addition of 25 μl of 2% (wt/vol) sodium lauryl sarcosinate (sarcosyl; Sigma) and stored at -70°C prior to analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and fluorography (described below).

(ii) **Short-term incubation.** PBPs also were radiolabeled by using short incubation periods. [3H]penicillin G or 125I-labeled Bolton-Hunter reagent-ampicillin was added to 3 × 10^8 to 5 × 10^9 freshly extracted or Percoll-purified organisms in microcentrifuge tubes, and the mixtures were incubated for up to 20 min at 34°C in a water bath. Identical portions were preincubated for 10 min with a 1,000-fold excess of unlabeled antibiotic prior to the addition of radiolabeled antibiotic. At the end of the incubation period, a 1,000-fold excess of unlabeled antibiotic was added to mixtures containing only radiolabeled antibiotic. The mixtures were immediately placed on ice and, depending on the volume of the incubation mixture, either diluted 1:1 with 2% sarcosyl or centrifuged at 20,000 × g at 4°C for 15 min followed by the addition of 25 μl of 2% sarcosyl to each pellet. To determine the concentration dependence of penicillin binding, 0.25, 1.2, 6, and 30 pmol of [3H]penicillin G were added to microcentrifuge tubes containing 3 × 10^4 Percoll-purified treponemes in 30 μl of PBS (final [3H]penicillin G concentrations of 8, 40, 200, and 1,000 nM, respectively). After a 20-min incubation period, specimens were processed as described above. The time course of penicillin binding was determined by adding 30 pmol of [3H]penicillin G to portions containing 3 × 10^4 Percoll-purified organisms in 30 μl of PBS (final concentration, 1 μM) and quenching the reaction by the addition of a 1,000-fold excess of unlabeled penicillin at 1, 5, 10, and 30 min. For localization of PBPs, freshly extracted organisms were suspended at a concentration of 10^9/ml in PBS. Portions (0.5 ml) containing 5 × 10^6 treponemes were preincubated for 15 min at 34°C in either PBS or 0.1% Triton X-100 (Aldrich Chemical Co., Inc., Milwaukee, Wis.) prior to the addition of [3H]penicillin G to a final concentration of 1 μM.

**Intrinsic radiolabeling of *T. pallidum* polypeptides.** *T. pallidum* proteins were 35S radiolabeled by the protocol of Stamm and Bassford (32), with minor modifications (26). Organisms at an approximate concentration of 10^9/ml were incubated for 18 h at 34°C with 0.2 mCi of [35S]methionine (Amersham) per 10^9 organisms.

**Phase partitioning and immunoprecipitation of *T. pallidum* polypeptides.** Triton X-114 extraction and phase partitioning were performed as previously described (4, 26). Portions (0.5 ml) containing 5 × 10^6 treponemes were added to an equal volume of ice-cold 4% Triton X-114 (Sigma) in PBS and extracted overnight on a rotary shaker. Insoluble material was removed by centrifugation at 20,000 × g at 4°C for 20 min. The supernatant was incubated for 10 min in a 37°C water bath, and the phases were separated by centrifugation for 5 min in a microcentrifuge at room temperature. The detergent phases were washed five times with ice-cold PBS, after which they were either acetone precipitated or diluted 10-fold in PBS to an approximate detergent concentration of 1% for immunoprecipitation with pooled human syphilitic sera (26).

**SDS-PAGE, autoradiography, and fluorography.** Samples for SDS-PAGE were boiled for 10 min in final sample buffer composed of 62.5 mM Tris hydrochloride (pH 6.8), 10% glycerol, and 2% SDS. Proteins were separated by electrophoresis on 2.5% stacking and 12.5% separating gels, both with 2.6% cross-linking (19). The gels were stained with Coomassie brilliant blue and either soaked in fluorographic enhancer (Autofluor; National Diagnostics, Somerville, N.J.) or directly dried under high vacuum for exposure on XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) at -70°C.

**Preparation of *T. pallidum* sacculi and analysis of peptidoglycan.** Percoll-purified organisms (4 × 10^10) were extracted overnight at 4°C with 2% Triton X-114 in PBS. The Triton X-114-insoluble material was collected by centrifugation at 20,000 × g at 4°C for 20 min and washed three times in ice-cold PBS. The pellet was suspended in 0.5 ml of final sample buffer and boiled for 10 min. After the suspension was cooled, 100 μg of proteinase K (Sigma) was added, and the mixture was incubated at 55°C for 1 h. The insoluble material was collected by centrifugation for 10 min at 130,000 × g at room temperature in an Air-Fuge (Beckman Instruments, Inc., Palo Alto, Calif.) and washed four times with distilled water. The resulting material was hydrolyzed in 6 N HCl at 110°C for 20 h and derivatized with phenylisothiocyanate. Derivatized amino acids were analyzed with a Waters PicoTag System (Waters Associates, Inc., Milford, Mass.) on a 30-cm C18 column by the general method of Bidlingmeyer et al. (3).

**Electron microscopy.** Specimens were prepared for whole-mount electron microscopy by the single-droplet method (33) on Parlodion (Ted Pella, Inc., Tustin, Calif.) and carbon-coated 400-mesh copper grids (Ted Pella). Specimens were negatively stained with 1% uranyl acetate (Sigma) and examined at 60 kV of accelerating voltage on a JEOl 100C electron microscope.

**RESULTS**

**Identification of PBPs.** Proteins with apparent molecular masses of 94, 80, 68, 63, and 58 kilodaltons (kDa) were radiolabeled during extended incubation of virulent treponemes with 1 μM [3H]penicillin G (Fig. 1A, lane 2). Preincubation in a 1,000-fold excess (1 mM) of unlabeled penicillin inhibited radiolabeling of all but the 68-kDa protein (lane 3). This protein also was not detected during short-term incubation of Percoll-purified organisms with the same concentration of radiolabeled penicillin G (lane 4). To characterize further the putative PBPs, experiments were con-
dected with a conjugate consisting of 125I-labeled Bolton-Hunter reagent and ampicillin, an antibiotic presumed to have potent treponemical activity (27). At an approximate concentration of 1 \( \mu \)M, 125I-labeled Bolton-Hunter reagent-ampicillin labeled the same four proteins in Percoll-purified organisms as \( ^{3}H \)penicillin, although the differences in the relative intensities of the proteins were less apparent than with \( ^{3}H \)penicillin (Fig. 1B, lane 2). In addition, this reagent also labeled a broadly migrating 47- to 45-kDa protein (or doublet) and a 38-kDa protein (Fig. 1B, lane 2). Preincubation with an approximate 1,000-fold excess (1 mM) of unlabeled ampicillin inhibited to some extent the radiolabeling of both the lower- and the higher-molecular-mass proteins (Fig. 1B, lane 3). Most of the PBPs appeared to correspond to proteins in the whole-cell lysates stained with Coomassie brilliant blue (Fig. 1A and B, lanes 1).

Concentration dependence and time course of penicillin binding. The relative binding affinities of the PBPs were investigated by determining the concentration dependence and kinetics of \( ^{3}H \)penicillin binding. The 94- and 58-kDa PBPs were radiolabeled at extremely low concentrations, 8 and 40 nM, respectively (Fig. 2A, lanes 3 and 4). Radiolabeling of the 80- and 63-kDa PBPs required 1 \( \mu \)M \( ^{3}H \)penicillin G (lane 1). The PBPs rapidly bound radiolabeled penicillin. Radiolabeling of the 94- and 58-kDa proteins was detected after 1 min of incubation in 1 \( \mu \)M \( ^{3}H \)penicillin G (Fig. 2B, lane 1). All four PBPs were radiolabeled within 10 min (lane 3), with some additional penicillin binding occurring during the remainder of the 30-min incubation period (lane 4). The rapidity of penicillin binding contrasted with the slow rate at which virulent treponemes became immobilized during prolonged in vitro incubation. In two separate 18-h experiments, approximately 60% of the organisms remained motile in the presence of either 1 \( \mu \)M \( ^{3}H \)penicillin or 1 mM unlabeled penicillin (as opposed to more than 90% active motility among organisms incubated without antibiotic). Neither concentration of antibiotic produced gross morphological alterations observable by dark-field microscopy (data not shown).

Localization and hydrophobicity of PBPs. Freshly extracted treponemes were preincubated either in PBS or in 0.1% Triton X-100 to solubilize the outer membranes before the addition of \( ^{3}H \)penicillin G. The PBP profiles were identical regardless of the presence or absence of the outer membranes, as confirmed by electron microscopy (data not shown). Phase partitioning with the nontoxic detergent Triton X-114 (4) was used to determine whether any of the PBPs were hydrophobic and, therefore, likely to be integral membrane proteins. The 94-, 63-, and 58-kDa proteins labeled with either \( ^{3}H \)penicillin or 125I-Bolton-Hunter reagent-ampicillin partitioned into the detergent phase (Fig. 3, lanes 1 and 2). The amounts of these proteins detected in both the Triton X-114-insoluble material and the detergent phase, however, were small compared with the amounts in whole-cell lysates (data not shown). The 47- and 45-kDa and 38-kDa proteins labeled exclusively by the 125I-ampicillin conjugate were readily detected in the detergent phase (lane 2). Two broad 17- and 18.5-kDa bands, both of which were apparent in overexposed autoradiographs of whole-cell lysates labeled with 125I-Bolton-Hunter reagent-ampicillin (data not shown), also appeared in the 125I-Bolton-Hunter reagent-ampicillin detergent phase (lane 2).

Detection of the higher-molecular-mass PBPs within the Triton X-114 detergent phase was somewhat unexpected; proteins of this size were not identified in previous analyses based upon phase partitioning (26). This may have reflected the fact that the earlier experiments investigated membrane protein antigens rather than all of the proteins that partitioned into the detergent phase. For this reason, PBPs within the detergent phase were compared with 35S-labeled detergent-phase proteins before and after immunoprecipitation with pooled human syphilitic sera. Proteins that appeared to correspond to the 94-, 63-, and, perhaps, 58-kDa PBPs were detected among the 35S-labeled detergent-phase proteins (Fig. 3, lane 3). However, only trace amounts of the 94- and 63-kDa 35S-labeled proteins were immunoprecipitated (lane 4). The 38- and 17-kDa PBPs appeared to comigrate with...
3S-labeled detergent-phase antigens, whereas the 45- to 47-kDa PBP migrated more slowly than the antigen of similar size (lanes 2 and 4). Interestingly, a number of other 3S-labeled detergent-phase proteins also were not immunoprecipitated (lanes 3 and 4).

**Analysis of peptidoglycan.** Percoll-purified *T. pallidum* was extracted extensively with 2% Triton X-114 in order to remove most of the membrane-associated proteins prior to the isolation of peptidoglycan. The Triton X-114-insoluble material consisted of ghostlike cell bodies with attached endoflagella and no discernible cytoplasmic membranes (Fig. 4). These cell bodies, or sacculi, retained much of the original spiral configuration of the treponemes. Glutamine (or glutamic acid), glycine, alanine, and ornithine constituted the major components of the SDS-insoluble, proteinase K-resistant material isolated from them (Fig. 5). Muramic acid (along with a minor muramic acid derivative designated mur'), but not diaminopimelic acid, also was detected (Fig. 5). The respective molar ratios for glutamine (or glutamic acid), muramic acid, glycine, alanine, and ornithine in *T. pallidum* peptidoglycan were 1.0:1.17:1.29:1.85:1.94. Calculation of the molar ratio of muramic acid was based upon the finding that only 22.3% of commercially obtained material was recovered following hydrolysis (data not shown).

**DISCUSSION**

Despite the considerable progress made in recent years in the characterization of native and recombinant *T. pallidum* protein antigens, biological or physiological functions have been assigned to only a few of the polypeptides of the organism (for a review, see reference 24). Nevertheless, it is likely that many *T. pallidum* polypeptides share biochemical and physiological activities with proteins of other well-studied, cultivatable microorganisms. The ubiquity of PBPs in the cell membranes of diverse gram-positive and gram-negative bacteria (13, 34), in conjunction with the demonstration that pathogenic treponemes are exquisitely sensitive to penicillin (11, 22), suggested that PBPs could be identified in *T. pallidum* by methods developed for their identification in other microorganisms (13, 31).

Identification of PBPs in *T. pallidum* may be complicated by the presence of rabbit testicular contaminants that nonspecifically bind antimicrobial agents. For this reason, we used several different experimental protocols to identify proteins that satisfied the generally accepted criteria for PBPs (13, 34). Polypeptides with molecular masses of 94, 80, 63, and 58 kDa were radiolabeled during both short-term and extended incubation with [3H]penicillin G. A 68-kDa protein was labeled during extended incubation experiments, but it was not detected following short-term incubation of Percoll-purified organisms. Radiolabeling of this protein during extended incubation also was not competitively inhibited by an excess of unlabeled penicillin. This protein was probably a rabbit testicular contaminant, most likely serum albumin, rather than a true PBP. Nonspecific binding of penicillin and other beta-lactam antibiotics to serum albumin is well recognized (23). The fact that the radiolabeled material remained associated with the 68-kDa protein after boiling in SDS suggested that 3H-labeled penicilloyl groups bound covalently to it during the prolonged incubation (20).

Differences in the relative intensities of the radiolabeled PBPs, particularly the 58-kDa protein, also were occasionally seen (for example, compare figures 1A and 2A). Nevertheless, the results with [3H]penicillin G were, overall, quite consistent. The same four proteins also were radiolabeled specifically by 125I-Bolton-Hunter reagent-ampicillin, further confirming their identity as true PBPs. In contrast to Cunningham et al. (8), we did not observe labeling of proteins smaller than 58 kDa with radiolabeled penicillin G. However, specific labeling of lower-molecular-mass proteins occurred with the 125I-ampicillin conjugate.

The 94- and 58-kDa PBPs demonstrated the highest binding affinities for [3H]penicillin G. These proteins bound radiolabeled antibiotic more rapidly and at much lower concentrations than either the 80- or 63-kDa protein. In fact, the concentrations at which the 94- and 58-kDa PBPs became radiolabeled were close to the minimum treponemical concentrations determined in two different in vitro systems (22, 25). It is possible, therefore, that either the 94- or 58-kDa protein or both represent the lethal targets for penicillin. The failure of [3H]penicillin G to radiolabel the lower-molecular-mass proteins suggests that, whatever their role in peptidoglycan synthesis, they probably are unnecessary for the treponemical activity of beta-lactam antibiot-
ics. Although ampicillin is considered to be an effective antimicrobial agent for the treatment of incubating syphilis (27), neither studies of its effects on treponemal motility and virulence nor adequate clinical trials of its efficacy in the treatment of human syphilis have been reported. However, in vitro animal and human studies with amoxicillin, a closely related antibiotic, indicate that ampicillin should have potent treponemical activity (12, 21). Our data provide further support for this, since 125I-Bolton-Hunter reagent-ampicillin radiolabeled the higher-molecular-mass PBPs at concentra-

tions readily achievable in a clinical setting (23). Experiments with other beta-lactam antimicrobial agents will determine whether binding to the 94- or 58-kDa protein or both is a prerequisite for treponemical activity.

Nell (22) noted that penicillin concentrations below 0.01 μg/ml failed to immobilize a significant proportion of treponemes during an 18-h incubation period in vitro. Even at extremely high penicillin concentrations, immobilization of treponemes did not occur for 4 h; complete immobilization required 12 h of incubation (22). We also observed a significant proportion of motile treponemes after extended incubation with both radiolabeled and unlabeled penicillin G.

The discrepancy between the rapidity of penicillin binding and the slow rate of treponemal immobilization may be a result of limited diffusion of antibiotic through the permeability barrier of the intact outer membrane, the relatively slow generation time of virulent treponemes (7), or both. Determination of the percentage of the treponemal population that binds penicillin at various time points will be necessary to resolve this issue. These observations emphasize the findings of both clinical and laboratory investigators that successful cure of syphilis requires prolonged treponemical concentrations of penicillin (27).

Except in the study by Barbas et al. (1), PBPs in gram-negative bacteria have been localized exclusively to the cytoplasmic membrane (31, 34). A major objective of the current investigation was to establish, as precisely as possible, their location in T. pallidum. Penicillin binding was unaffected by a concentration of Triton X-100 that selectively solubilizes the T. pallidum outer membrane. Additionally, most of the PBPs were sufficiently hydrophobic to
partition into the Triton X-114 detergent phase. Taken together, these experiments provided strong, albeit indirect, evidence that most, if not all, of the PBPs are integral cytoplasmic membrane proteins. Definitive localization must await experiments with isolated cytoplasmic membranes.

The immunoprecipitation results indicate that a number of the detergent-phase proteins, including the higher-molecular-mass PBPs, are poorly antigenic. For this reason, proteins with molecular masses corresponding to the larger PBPs were not identified in a recent study of *T. pallidum* membrane immunogens based upon the phase-partitioning technique (26). Recovery of the higher-molecular-mass PBPs following extraction with Triton X-114 was particularly poor. If these proteins correspond to the 35S-labeled proteins of similar molecular masses, then inefficient solubilization alone cannot explain their poor recovery because the 35S-labeled proteins were readily solubilized. Release of bound antibiotic during prolonged extraction under nondenaturing conditions (31) and the relatively low specific activities of the labeled PBPs may have contributed to their limited detectability within the Triton X-114 detergent phase. Recently, Cunningham et al. (9) reported that they were unable to solubilize PBPs from intact *T. pallidum* by using Triton X-114. The discrepancy between their results and those reported here probably reflects differences in extraction conditions, as well as the limited detectability of solubilized PBPs even under optimal conditions.

The observation that some of the lower-molecular-mass PBPs appeared to comigrate during SDS-PAGE with 35S-labeled detergent-phase antigens raises the possibility that some of these membrane immunogens may participate in peptidoglycan biosynthesis. Although highly speculative, this is consistent with the fact that PBPs and the detergent-phase antigens both appear to be located within the cytoplasmic membrane (26) and, presumably, in proximity to the peptidoglycan layer (16-18). Further experiments will be required, however, to prove that any of the PBPs and the 35S-labeled detergent-phase proteins of similar molecular masses are identical proteins.

The data in this study provide the first structural and biochemical evidence for the presence of peptidoglycan in *T. pallidum*. This was necessary in view of the fact that Barbour et al. (2) demonstrated that it is possible for an organism (*Chlamydia trachomatis*) to possess PBPs but not detectable peptidoglycan. In the present study, after extensive detergent extraction, the residual cell bodies had a coarse, ghostlike appearance resulting from exposure of the underlying peptidoglycan sacculus. Because this material contained the insertion sites for the endoflagella and also withstand rather harsh extraction conditions, it seems likely that, as in other spirochetes (16, 18), the peptidoglycan sacculus is associated with the cytoplasmic membrane. By maintaining the characteristic configuration of the cell, this structure appears to be analogous to the sacculus of conventional gram-negative bacteria (5). The presence of ornithine, rather than diaminopimelic acid, in the peptidoglycan was not surprising since this is a distinctive component of mureins in other spirochetes (16, 18, 28). Glycine has not, to our knowledge, been detected in other spirochete peptidoglycans. Glutamine and alanine, on the other hand, are components of peptidoglycan in a wide variety of bacteria (28).

Elucidation of the complex interactions between beta-lactam antimicrobial agents, PBPs, and peptidoglycan in *Escherichia coli* and other microorganisms has provided important insights into bacterial physiology and cell wall morphogenesis. Characterization of the interactions between these agents and the analogous components of *T. pallidum* may similarly enhance our limited understanding of treponemal ultrastructure and metabolism. Such investigations also have clinical implications. In other bacterial species it has been possible to correlate the relative potencies and the morphological effects of different antibiotics with their affinities for specific PBPs (10, 14, 30). Similar correlations in *T. pallidum* may facilitate selection of antimicrobial agents for evaluation in experimentally produced and human syphilis.

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**LITERATURE CITED**