Molecular model for elongation of the murein sacculus of *Escherichia coli*

(muropeptide dimer turnover/directed movement of membrane proteins/labeling with ^{14}C -labeled-*meso*-2,6-diaminopimelic acid/acceptor/donor radioactivity ratio/assembly of murein in helical form)

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ABSTRACT Labeling experiments are presented that suggest that new (radioactive) strands of murein are initially inserted adjacent to old strands. After 8 min, new strands start to be inserted adjacent to the previously inserted radioactive strands. Analysis of these data suggests that, for *Escherichia coli* to double the length of the sacculus in each generation, about 90 separate membrane-bound enzyme complexes travel unidirectionally around the circumference of the cell. They travel at a constant rate, six times each generation, synthesizing, inserting, and crosslinking two strands of murein at a time, thereby doubling the length of the sacculus.

The murein sacculus of Gram-negative bacteria (1) consists of a single continuous layer of murein completely surrounding the cell between the cytoplasmic membrane and a separate outer membrane. The murein of Gram-negative bacteria is a unique polysaccharide whose repeating unit, the muropeptide, consists of alternating units of *N*-acetylglucosamine and *N*-acetylmuramic acid to which a short peptide composed of L-alanyl-D-glutamyl-*meso*-2,6-diaminopimelic acid and D-alanine is attached. Many strands of murein with an average chain length of 30–60 muropeptides (2–5), aligned perpendicularly to the axis of the cell (6), are crosslinked to each other to form a net-like structure that serves as a rigid exoskeleton for the cell (Fig. 1). The crosslinks are formed by a transpeptidation reaction in which the penultimate D-alanine of a donor muropeptide (one with a pentapeptide whose carboxyl terminus is D-alanyl-D-alanine) links to the free amino group of the diaminopimelic acid (A_2pm) of an acceptor muropeptide of another strand.

Although many enzymatic components involved in murein synthesis have been studied *in vitro* (7–10), the *in vivo* mode of growth of *Escherichia coli* murein remains poorly understood. Autoradiography has been the principal method to study growth of the sacculus. An early autoradiography experiment indicating that incorporation of new murein takes place at a single zone (11) contrasts with more recent autoradiographic (12–14), morphologic (15), and biochemical (15) data that support a multisite process for elongation of the murein sacculus in *E. coli*.

We have devised a new approach for the study of the elongation process, which depends on chemically distinguishing newly synthesized strands of murein from preexisting strands in the sacculus. It is based on the assumption that only new murein strands serve as donors for the crosslinking transpeptidation reaction. This is reasonable because the newly synthesized strands contain the pentapeptide side chains terminating in D-alanyl-D-alanine, which is required for the transpeptidation reaction (16–19), and over 97% of the muropeptides inserted into the sacculus during a 3-min

FIG. 1. Dimer formed by crosslinking a muropeptide of a newly synthesized glycan strand containing radioactive A_2pm to an existing unlabeled strand in the murein sacculus. The dimer is released from the sacculus by lysozyme, which degrades the glycan chains to disaccharide-peptide units (muropeptides). Note that only the new, or donor, A_2pm in the dimer has a free amino group.

pulse lose their terminal D-alanine and, thus, their ability to serve as donors within that brief period (3). The crosslinked muropeptides from adjacent strands can be recovered as bis-disaccharide peptide dimers, referred to simply as dimers. The two halves of the dimer and, hence, the strands from which they come can be measured separately because the A_2pm of the donor strand has a free amino group and that from the acceptor does not. Thus, by measuring the amount of radioactive A_2pm in each half of the dimers from *E. coli* pulse-labeled with ^{14}C A_2pm , we could follow the fate of new murein strands inserted in the sacculus.

MATERIALS AND METHODS

Organisms, Growth, and Labeling Conditions. *E. coli* W7 (*dap*, *lysA*) (12) was grown and pulse-labeled with ^{14}C A_2pm essentially as described (14). Exponentially growing cells were washed by filtration, resuspended in minimal glucose medium without A_2pm , and incubated for the 25 min required to deplete *E. coli* W7 of its intracellular pool of A_2pm (14, 15). After depletion of A_2pm , about 0.13 μg of ^{14}C A_2pm per ml was added to pulse-label the murein for 5 min, or 1 μg or more of ^{14}C A_2pm was added per ml when labeling was continued for periods of 40 min or longer.

BUG 6, a temperature-sensitive division mutant of *E. coli* strain K-12 (20) was labeled with ^{14}C A_2pm by the addition

Abbreviations: A_2pm , diaminopimelic acid; ADRR, acceptor/donor radioactivity ratio; C lengths, circumference lengths; DNP, dinitrophenol.

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of about 0.1 μg of [^{14}C]A₂pm per ml to cells growing exponentially in L broth as described (5).

Measurement of the [^{14}C]A₂pm Content of the Acceptor and Donor Halves of Muropeptide Dimers. Dimers released by lysozyme (5) were isolated by paper chromatography (15) and treated with fluorodinitrobenzene (21). After hydrolysis with HCl and separation by paper chromatography, the ^{14}C content of the dinitrophenol (DNP)-conjugated A₂pm from the donor muropeptide and the A₂pm from the acceptor muropeptide was measured (15).

Enzymes and Chemicals. Lysozyme (EC 3.2.1.17) was obtained from Sigma. Uniformly ^{14}C -labeled 2,6-diaminopimelic acid (*meso* and some LL, 315 mCi/mmol) was purchased from Research Products International (Mt. Prospect, IL).

RESULTS

In Fig. 2 are shown the results of a typical experiment in which *E. coli* W7 cells, whose intracellular pools of A₂pm had been depleted, were fed [^{14}C]A₂pm and the fate of the radioactive muropeptides in dimers was determined. The results are expressed as the ratio of the radioactivity in the acceptor muropeptides to the radioactivity in the donor muropeptides (i.e., the acceptor/donor radioactivity ratio, or ADRR). The ADRR was low and fairly constant for the first 8 min, and then the ADRR rose rapidly during the next 10 min and more slowly thereafter.[‡] The low initial ADRR indicates that at first about 77% of the [^{14}C]A₂pm was incorporated into the donor position of the dimers and only 23% into the acceptor position, which demonstrates that most new strands are crosslinked to old strands during the initial minutes.

It should be noted that when uniformly labeled cells were analyzed by this technique, an ADRR of 1.4 was obtained (15) instead of the theoretical value of 1.0. The data in the figures have been corrected for this constant error, which is possibly caused by partial loss of DNP-A₂pm during acid hydrolysis or by quenching of the DNP-A₂pm sample by DNP.

A similar labeling experiment performed with BUG 6, which is temperature sensitive for division (20), is shown in Fig. 3. In both cells growing normally in L broth at 32°C and in cells growing at the restrictive temperature, the ADRR was initially low and relatively constant and then rose as it did in experiments with *E. coli* W7. The fact that similar curves were obtained (cf. Figs. 2 and 3) suggests that the curve is an expression of the elongation process and is not

FIG. 2. *E. coli* W7 was labeled with [^{14}C]A₂pm starting at zero time, and the changes with time of the ADRR of muropeptide dimers were plotted.

[‡]After preparation of this paper, a paper appeared by Goodell and Schwarz (22) that provides independent support for our observation that the ADRR remains constant for 8 or 10 min.

FIG. 3. *E. coli* K-12 strain BUG 6 (temperature conditional for cell division) was grown in L broth at 32°C (A) or at 42°C (B). At zero time [^{14}C]A₂pm was added, and the ADRR in muropeptide dimers was determined at intervals.

affected by the starvation period or the growth conditions.

During the chase in a pulse-chase experiment (Fig. 4), there was an initial rise in the ADRR followed by a brief plateau, and then the ratio increased rapidly to a value of about 1.3. This dramatic change in the ratio indicates that about half of the radioactive muropeptides initially present in the donor strands were shifted into the acceptor position of dimers during the chase. Obviously the murein sacculus is in a dynamic state during the elongation process, even though the overall composition and structure of the murein changes relatively little as it ages (5).

One change observed by de Pedro and Schwarz (3) and which we have confirmed (5) is a significant increase in the percentage of the radioactivity of pulse-labeled murein found in dimers beginning after a lag of about 10 min. The changes that occur during the chase are shown more clearly in Fig. 5, where we have plotted the amount of radioactivity in the do-

FIG. 4. *E. coli* W7 was pulse-labeled with [^{14}C]A₂pm (final concentration, 0.10–0.13 $\mu\text{g}/\text{ml}$) at zero time and chased with 10 μg of unlabeled A₂pm per ml at 5 min. The ADRR of muropeptide dimers was determined at intervals.

FIG. 5. *E. coli* W7 was pulse-chased as in Fig. 4. The fraction of the total radioactivity in murein that was present in the acceptor and donor mucopeptides of the mucopeptide dimers was determined.

nor and acceptor mucopeptides from the experiment shown in Fig. 4, taking into account the rise in dimers. The results suggest that, between 6 and 15 min after the chase was started, for each donor mucopeptide lost (presumably as a result of opening crosslinks in dimers containing radioactive donors) about two new dimers were formed in which new, nonradioactive strands are crosslinked to radioactive acceptors. Thereafter, the label in donor mucopeptides continued to decrease, but that in acceptor mucopeptides in dimers increased in a reciprocal fashion.

A Molecular Model for Elongation of the Murein Sacculus. These results led us to the proposed molecular model.[§]

Interpretation of the data and development of the model rests on three assumptions. One is that the glycan strands are arranged perpendicularly to the length of the axis of the cell as hypothesized by Pelzer (23). This is now supported by an electron microscopic study of sacculi partially digested with murein endopeptidase (6). The second assumption is that the glycan strands are simultaneously linked to the existing murein as they are being polymerized. This assumption is supported by *in vivo* labeling experiments (5) and the findings of Matsuhashi *et al.* (7), which indicate that both penicillin-binding proteins PBP-1A and PBP-1B, involved in elongation of the sacculus (24), can polymerize the glycan strands and also crosslink them by transpeptidation. It seems likely that such two-headed enzymes would use both catalytic activities on the same mucopeptide as it is being processed into the growing murein strand. The third assumption is that only new strands possess the pentapeptide substrate required for transpeptidation. This follows from the intuitive argument just made. It is supported by results of de Pedro and Schwarz (3), who showed that mucopeptides incorporated into new murein lose the terminal D-alanine from their pentapeptides very rapidly. This implies that a new strand can provide donor mucopeptides only during the first minute or two of its life and, thereafter, can only serve as an acceptor.

There are three experimental observations that any model must explain: (i) the relatively constant ADRR observed during the first 7–9 min of labeling, (ii) the significant rise in the ratio that follows, and (iii) the actual value of the ADRR during the initial period and subsequently.

To appreciate the meaning of these observations, it is necessary to consider the dimensions of the murein sacculus in molecular terms. The sacculus of *E. coli* growing in minimal

glucose medium contains about 2.7×10^6 mucopeptides (25). The distance around the circumference of the cell, which is slightly more than 2500 nm, will be referred to as one C length. Because the length of one disaccharide unit is 1.03 nm (25), one C length contains nearly 2500 mucopeptides.

For purposes of discussion, let us assume a generation time of 48 min for *E. coli* W7 growing in minimal glucose medium at 37°C and assume 8 min for the initial period during which the ADRR remains constant. Because 8 min is one-sixth of a generation, it follows that 450,000 mucopeptides ($2.7 \times 10^6 \times 1/6 = 450,000$) were inserted during the 8-min period. Because one C length contains only 2500 mucopeptides, the equivalent of 180 C lengths of murein must have been inserted in 8 min. If the new strands were incorporated in a single growth zone, the ADRR would rapidly approach a value of 1.0. However, the ratio remained nearly constant for 8 min at a value of much less than 1.0, thus indicating multiple growth sites. Because 180 C lengths of murein were actually added during the first 8 min, it follows that (i) either 180 strands were added at separate growth sites or (ii) pairs of C-length strands were added to 90 independent sites or (iii) 3 strands of C length were added simultaneously at 60 sites, etc.

Regardless of whether one, two, or more strands were inserted concurrently at a given growth site, the constant ratio indicates that it requires about 8 min to add one C length of the insert at each growth site. It seems likely that the enzymes involved move in one direction around the cell's circumference once in 8 min, using the strands in the sacculus as the substrate to which the new strands must be crosslinked. In a sense, this is similar to the synthesis of a new strand of DNA by enzymes traveling along a DNA template. Murein synthesis proceeds at a rate of about 5 mucopeptides per second compared to about 1500 nucleotides per second for DNA synthesis. The enzymes that polymerize glycan strands and form crosslinks are membrane-bound (7, 18, 19, 26), but the indicated rate of movement is well within the range reported for mobility of membrane proteins (27).

In addition to identifying the time required for insertion of one C length of murein, the fact that the ADRR rises after the first 8 min suggests that the next C length of murein is frequently inserted adjacent to the first. A second round of strand insertion, adjacent to the first, would cause the ratio to rise because: (i) the crosslinks between the first round of radioactive strands and their nonradioactive neighbors are opened on one side to allow insertion of the second round of strands, thus causing a loss of radioactive donor mucopeptides, and (ii) the strands inserted during the second round become crosslinked to the first round of radioactive strands, leading to an increase in radioactive acceptor mucopeptides in dimers. Thus, the enzymes appear to travel in one direction along a roughly continuous helical path such that new murein is crosslinked to strands that were inserted 8 min earlier.

In the pulse-chase experiments (Fig. 4), the ratio increased for about 4 min, then held steady for a few minutes before again increasing markedly. Because the amount of [^{14}C]A₂pm available and incorporated during the pulse only allowed for synthesis of one-half of a C length of radioactive murein (data not shown), a rise in the ADRR during a 4-min period (from the 6th to 10th min in Fig. 4) followed by a constant ratio for the next 4 min is exactly as predicted by the model. This is because, during the second half of the first round of the chase, the enzyme "complexes" would contact only nonradioactive strands to use as acceptors.

The ADRR varied between 0.21 and 0.29 during the first 8 min in all experiments (Figs. 2–4). If only one strand at a time were inserted at each growing point, the ratio would approach zero because only the new donor strands would be radioactive and all acceptor mucopeptides would be nonra-

[§]A preliminary description was presented at the International FEMS Symposium: Burman, L. G. & Park, J. T., The Murein Sacculus of Bacterial Cell Walls: Architecture and Growth, March 13–18, 1983, Berlin, Federal Republic of Germany.

FIG. 6. Structure of the murein in a growth site for elongation of the murein sacculus as it may exist during a pulse-labeling experiment. The thick lines represent the new pair of radioactive strands of murein inserted between preexisting strands (thin vertical lines) during the first few minutes of the pulse (A) and 8 min later when radioactive strands begin to contact other radioactive strands as a result of continued insertion along a spiral path (B). The arrows between strands indicate the direction of crosslinks in dimers from donor to acceptor strands. $\bullet$, Radioactive donor; $\blacktriangleright$, radioactive acceptor; $\triangleright$, nonradioactive acceptor. The regions in A and B with arrows indicating the direction of the crosslinks represents about 1/300th of the circumference of the sacculus. Dimers from A contain one radioactive acceptor and four radioactive donors, $ADRR = 0.25$. After insertion of two complete C lengths of pairs of radioactive strands adjacent to each other, the situation shown by the "arrow-linked" dimers in B should exist. Dimers from B contain four radioactive acceptors for every seven radioactive donors, $ADRR = 0.57$.

dioactive. Therefore, it appears that two or more strands are introduced together at each site. Our data agrees best with a model in which two strands are added at a time (see *Discussion*). The observed increase in the radioactive dimer content of sacculi relative to monomers beginning 8 or 10 min after labeling with [^{14}C]A₂pm has started (3, 5) apparently is a reflection of the observation that, during the round of insertion after labeling, more label is gained in acceptors than is lost from donor muropeptides (Fig. 5). This suggests that about twice as many crosslinks are formed on the permanent or trailing side of the newly inserted strand pair than on the temporary or leading edge.

Fig. 6A illustrates the situation at one growth site during the initial minutes of labeling, and Fig. 6B illustrates the situation after the enzyme complex has moved around the circumference of the cell once and has begun a second round, extending the strand pair into the leading groove between the radioactive and nonradioactive strands in a continuing spiral path.

The lines and arrows between strands represent the crosslinks that form dimers. By examining the arrow-linked dimers in Fig. 6A, one can see that when a pair of strands are inserted, four radioactive donor muropeptides are present for every radioactive acceptor muropeptide in the dimers, which gives an ADRR of 0.25. After two complete C lengths (i.e., four strands) of pulse-labeled murein have been inserted, the situation seen in the arrow-linked zone in Fig. 6B should exist. At this point there are seven radioactive donor muropeptides for every four acceptor muropeptides, so that the ratio has increased to 0.57. These are the ratios to be expected if, as illustrated in the figure, two strands are inserted at a time and there are twice as many crosslinks on the trailing side of the spiral insert (to the left in the figure) as there are between new strands or between the leading side of the growing spiral insert and the nonradioactive edge of the sacculus.

DISCUSSION

Our data indicate that two strands are inserted into the sacculus at each growth site with a strand polymerization rate of about five muropeptides per second at 37°C. We propose that the enzymes associated with each site move at this rate along a spiral path around the circumference of the cell, following the acceptor strands of the preexisting sacculus to which the new strands become crosslinked, and they move in the direction dictated by extension and insertion of the new strands. Helical fibers composed of the cells of certain chain-forming mutants of *Bacillus subtilis* (28) may be an in-

direct expression of the insertion of murein strands as described by the model. Because the length of a newborn cell of *E. coli* W7 grown in minimal glucose medium at 37°C is about 2200 nm (13) of which the cylinder's length would be 1400 nm, the cylinder would contain about 1100 C-length strands of murein running parallel to each other if, as has been estimated (25), the strands are 1.25 nm apart. Therefore, in order to achieve a doubling of the length of the murein cylinder in a generation time of 48 min, our model requires about 90 active enzyme complexes, each inserting two C lengths of murein every 8 min ($90 \times 2 \times 6 = 1080$ C lengths).

We have used the term enzyme complex to indicate that a number of enzymes must be present at each growth site, although there is no evidence that the enzymes are associated as a complex. Thus, what we mean by an enzyme complex is a group of enzymes that cooperate to open crosslinks between existing strands and form, insert, and crosslink two new strands to the existing network in an ordered fashion. Candidates for the enzymes involved include the penicillin-binding proteins, PBP-1A and PBP-1B, shown by Matsuhashi *et al.* (7) to each have glycan polymerizing and crosslinking activity. These two enzymes would form the two strands and crosslink them to the adjacent existing strands and possibly to each other. PBP-4, an endopeptidase (29), would act at the proper time to open crosslinks between existing strands in the sacculus to allow insertion of the new pair of strands. An endopeptidase activity of *E. coli* W7, uncoupled by A₂pm starvation, cleaves murein crosslinks at the rate of about 100 C lengths per generation (15). It would have to work about five times more efficiently when performing the coordinated function envisioned for elongation of the sacculus. D,D-Carboxypeptidases (e.g., PBP-5 and/or PBP-6) (8) may be considered part of the complex whose function is to convert the unused pentapeptides of the two new strands into tetrapeptides before the next round of insertion when one of the strands assumes an acceptor role. Spratt (24) has estimated that an *E. coli* cell contains about 230 molecules of PBP-1A and PBP-1B combined and 110 molecules of PBP-4, which is sufficient for the estimated 90 growth sites.

The strands of murein in the sacculus are linear polymers that should be viewed as substrates for the enzymes involved in elongation. Therefore, one would expect that enzymes, once associated with a particular strand in the sacculus, would proceed along that strand in the direction dictated by extension and insertion of the new strands. This directed movement causes the enzymes to travel around the circumference of the cell in an eternal helical path. However, be-

cause the strands in the sacculus being used as acceptors average only 30 muropeptides in length (2–5), the complexes must seek other strands many times during synthesis of each C length of murein and apparently show a preference for the strands inserted 8 min earlier, which still contain two low crosslinked grooves (Fig. 6).

The crosslinking of one strand of a new strand pair to the acceptor strand on the left may be seen as stereochemically different from crosslinking the other new strand to an acceptor strand on the right. This may explain why two polymerase–transpeptidase enzymes (i.e., PBP-1A and PBP-1B) are normally involved in elongation. The endopeptidase that opens the crosslinks to allow insertion of new pairs of strands on the leading side of the growing helix would have specificity for “right-handed” crosslinks (as shown in Fig. 6). Further analysis of our data indicates that reciprocal donor-to-acceptor conversion of label detectable 15 min after incorporation (Figs. 4 and 5) is due to new enzyme complexes initiating insertion of pairs of strands between the pulse-labeled strand pairs (unpublished data). Because the same endopeptidase may be involved in insertion of this pair of strands, it is likely that crosslinks between pairs (i.e., in the middle groove) are “right-handed.” If this proves to be correct, the sacculus would contain grooves with “right-handed” crosslinks subject to reopening during elongation and “left-handed” crosslinks that are conserved and are more highly crosslinked.

Formation of the crosslinks between the strands of a new pair may be catalyzed by PBP-2, another penicillin-binding protein associated with elongation (30) that has crosslinking activity (9), although only 20 molecules of PBP-2 are reported to be present in an *E. coli* cell (24). Alternatively, PBP-1A may form these crosslinks by wobbling between grooves because purified PBP-1A can form trimers (10), which suggests that it may be able to interact with muropeptides from three adjacent strands.

The discussion with regard to the role of individual PBPs is obviously speculative. However, with this model as a guide, it may now be possible to examine the *in vivo* role of the various penicillin-binding proteins thought to be involved in the elongation process. It remains to be determined whether this model applies to Gram-positive rod-shaped bacteria. We predict that all prokaryotes that use a multisite mechanism for elongation of the cell use a mechanism similar to the one proposed here.

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1. Weidel, W. & Pelzer, H. (1964) *Adv. Enzymol.* **26**, 193–232.
2. Schindler, M., Mirelman, D. & Schwarz, U. (1976) *Eur. J. Biochem.* **71**, 131–134.

3. de Pedro, M. A. & Schwarz, U. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 5856–5860.
4. Gmeiner, J., Essig, P. & Martin, H. H. (1982) *FEBS Lett.* **138**, 109–112.
5. Burman, L. G. & Park, J. T. (1983) *J. Bacteriol.* **155**, 447–453.
6. Verwer, R. W. H., Nanninga, N., Keck, W. & Schwarz, U. (1978) *J. Bacteriol.* **136**, 723–729.
7. Matsuhashi, M., Ishino, F., Nakagawa, J., Mitsui, K., Nakajima-Iijima, S. & Tamaki, S. (1981) in *β -Lactam Antibiotics: Mode of Action New Developments and Future Prospects*, ed. Salton, M. R. J. (Academic, New York), pp. 297–310.
8. Blumberg, P. M. & Strominger, J. L. (1974) *Bacteriol. Rev.* **38**, 291–335.
9. Ishino, F., Tamaki, S., Spratt, B. G. & Matsuhashi, M. (1982) *Biochem. Biophys. Res. Commun.* **109**, 689–696.
10. Tomioka, S., Ishino, F., Tamaki, S. & Matsuhashi, M. (1982) *Biochem. Biophys. Res. Commun.* **106**, 1175–1182.
11. Ryter, A., Hirota, Y. & Schwarz, U. (1973) *J. Mol. Biol.* **78**, 185–195.
12. Schwarz, U., Ryter, A., Rambach, A., Hellio, R. & Hirota, Y. (1975) *J. Mol. Biol.* **98**, 749–759.
13. Verwer, R. W. H. & Nanninga, N. (1980) *J. Bacteriol.* **144**, 327–336.
14. Burman, L. G., Reichler, J. & Park, J. T. (1983) *J. Bacteriol.* **155**, 983–988.
15. Burman, L. G., Reichler, J. & Park, J. T. (1983) *J. Bacteriol.* **156**, 386–392.
16. Wise, E. M. & Park, J. T. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 75–81.
17. Tipper, D. J. & Strominger, J. L. (1965) *Proc. Natl. Acad. Sci. USA* **54**, 1133–1141.
18. Araki, Y., Shirai, A., Shimada, A., Ishimoto, N. & Ito, E. (1966) *Biochem. Biophys. Res. Commun.* **23**, 466–472.
19. Izaki, K., Matsuhashi, M. & Strominger, J. L. (1966) *Proc. Natl. Acad. Sci. USA* **55**, 656–663.
20. Reeve, J. N., Groves, D. J. & Clark, D. J. (1970) *J. Bacteriol.* **104**, 1052–1064.
21. Ghuysen, J.-M., Tipper, D. J. & Strominger, J. L. (1966) in *Methods in Enzymology, Complex Carbohydrates*, eds. Neufeld, E. F., Ginsburg, V., Colowick, S. P. & Kaplan, N. O. (Academic, New York), Vol. 8, pp. 694–696.
22. Goodell, E. W. & Schwarz, U. (1983) *J. Bacteriol.* **156**, 136–140.
23. Pelzer, H. (1967) in *Molekularbiologie, Bausteine des Lebendigen*, eds. Wieland, T. & Pfeleiderer, G. (Umschau, Frankfurt am Main), pp. 147–160.
24. Spratt, B. G. (1977) *Eur. J. Biochem.* **72**, 341–352.
25. Braun, V., Gnirke, H., Henning, U. & Rehn, K. (1973) *J. Bacteriol.* **114**, 1264–1270.
26. Mirelman, D. (1979) in *Bacterial Outer Membranes*, ed. Inouye, M. (Wiley, New York), pp. 115–166.
27. Sim, E. (1982) *Membrane Biochemistry* (Chapman and Hall, London), pp. 18–25.
28. Mendelson, N. H. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2478–2482.
29. Matsuhashi, M., Takagaki, Y., Maruyama, I. N., Tamaki, S., Nishimura, Y., Suzuki, H., Ogino, U. & Hirota, Y. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2976–2979.
30. Spratt, B. G. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 2999–3003.