This report describes what, to our knowledge, the first purification to near homogeneity of an enzyme involved in the biosynthesis of the teichuronic acid of *Micrococcus luteus* cell walls. The glucosyltransferase of *M. luteus*, which participates in the biosynthesis of teichuronic acid, was solubilized from cytoplasmic membrane fragments by extraction with buffer solutions containing the detergents Thesit (dodecyl alcohol polyoxyethylene ether; 1 mg/ml) and 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (0.5 mg/ml). The detergent-solubilized enzyme was purified 150-fold, with a recovery of 13% by adsorbtion column chromatography, ion-exchange chromatography, gel filtration, and preparative non-denaturing gradient polyacrylamide gel electrophoresis. On the basis of its mobility on native gradient gel, the glucosyltransferase was estimated to have a molecular mass of 440 kDa. The purified native enzyme was a multisubunit protein consisting of subunits of two sizes; their molecular masses were determined to be 52.5 and 54 kDa, respectively, by observation of the mobility of the protein bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The isoelectric point of the enzyme was 5.5.

Polysaccharides located on the bacterial surface or embedded in the outer envelope, including peptidoglycan, teichoic acid, lipoteichoic acids, teichuronic acids, cell wall polysaccharides, lipopolysaccharides, and capsular polysaccharides, play an important role in the integrity of the bacterial cell. The biosynthesis of these polysaccharides requires a special group of enzymes: the glycosyltransferases, which are usually located in the cytoplasmic membrane and are capable of adding specific sugar(s) onto a polysaccharide chain. The activity of such membrane-associated enzymes was initially reported many years ago (1, 2), but their purification has not been easy. Because of the importance of bacterial surface polysaccharides in virulence and immunity (3–9), much research has been directed in the importance of bacterial surface polysaccharides in virulence and immunity (3–9), much research has been directed in

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**EXPERIMENTAL PROCEDURES**

Isolation of Cytoplasmic Membrane Fragments (Particulate Enzyme Fraction)—Cultures of *M. luteus* (ATCC 4698) were grown to mid-logarithmic phase at 30 °C with vigorous shaking in a liquid medium containing peptone (10 g/liter) and NaCl (5 g/liter). The cells were harvested by centrifugation at 3,000 × g at 4 °C for 10 min. The pellet of wet packed cells was washed in TMM buffer (50 mM Tris-HCl buffer, pH 8.8, 0.1 mM magnesium acetate, and 2 mM 2-mercaptoethanol); sedimented again; and then suspended at a concentration of 1 g/ml in 10-fold diluted TMM buffer to which lysozyme (1 mg/g of cells), DNase (3 mg/g), and RNase (30 mg/g) had been added. After incubation at 25 °C for 120 min to effect lysis, the suspension was homogenized and fractionated three times by centrifugation at 4 °C for 10 min. The particulate enzyme fraction was defined as the fraction sedimenting at 3,000–12,000 × g and containing cytoplasmic membrane fragments. This fraction was resuspended in TMM buffer to a final protein concentration of 5–15 mg/ml and stored at −15 °C.

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The abbreviations used are: ManNAcU, N-acetyl-b-mannosaminuronic acid; CHAPS, 3-(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PAGE, polyacrylamide gel electrophoresis.
Preparation of Solubilized Enzyme Extracts—The cytoplasmic membrane fragments were solubilized by thorough mixing of 2 volumes of buffer D, which consisted of 40 mM Tris-HCl, pH 8.2, 1.6 mM glycerol, 0.5 mM EDTA, 0.5 mM magnesium acetate, 1 mM 2-mercaptoethanol, 0.3 mM diithiothreitol, 0.5 mg/ml CHAPS, and 1.0 mg/ml Thiop. The insoluble residue was removed by centrifugation at 20,000 × g for 15 min and discarded. The detergent-solubilized enzyme extract was stored at either 4 or −15 °C.

Adsortent Column Chromatography—The detergent-solubilized enzyme samples were applied to 10-ml adsorbent columns with Bio-Beads SM-2 (Bio-Rad) and were eluted at room temperature with buffer D, pH 7.3, at a flow rate of 0.3−0.5 ml/min. The nonadsorbed material that passed directly through the column was collected and assayed. Fractions containing glucosyltransferase activity were pooled.

DEAE-cellulose Column Chromatography—Pooled fractions containing enzyme activity from the adsorbent column were applied to a column of DEAE-cellulose (4.5 × 20 cm) and eluted first with 3 bed volumes of buffer D, pH 7.3, and then with a 1000-ml linear gradient of 0.13−0.55 mM NaCl in the same buffer at a flow rate of 1 ml/min. Fractions (4.3 ± 0.2 ml) were collected and assayed for enzyme activity and protein concentration. Dilute fractions were concentrated by loading of a 2-ml volume of sample on a Centricon 10 microconcentrator or larger volumes on a Centriflo CF25 membrane cone (Amicon, Inc., Beverly, Massachusetts) and centrifugation at 3,000 × g for 1 hr at room temperature. Aliquots of each fraction were applied to SDS-PAGE (26). Proteins were subjected to staining with Coomassie Brilliant Blue R-250 or double staining with silver/Coomassie Blue.

Quantitative Protein Analysis—Protein composition was determined spectrophotometrically by the bicinchoninic acid method at 562 nm (28). Bovine serum albumin served as the reference standard.

Glucosyltransferase Activity Assay—Glucosyltransferase activity (24) was assayed by incubation of 50 ml of enzyme sample in a reaction mixture (final volume, 100 ml) containing 0.7 mM UDP-[14C]glucose (2 mCi/mmol), 50 mM HEPES buffer, pH 8.2, 20 mM magnesium acetate, 5 mM 2-mercaptoethanol, and 30 mg/ml teichuronic acid (the glucose acceptor). After 60 min of incubation at 25 °C, the reaction was stopped by adding the reaction mixture to a solution of yeast RNA (30 mg/ml) and of 7.5% trichloroacetic acid. The residue was separated by centrifugation and was quantitated by liquid scintillation counting. This product at the origin had previously been analyzed by mass spectrometry, NMR imaging, and carbohydrate PAGE and had been confirmed to be teichuronic acid (18, 24).

Glucosyltransferase activity on native polyacrylamide gels or on detergent-solubilized enzyme was determined by cutting the gel into 2-mm slices with a razor blade, soaking each gel slice in 50 ml of buffer D, and incubating these gel slices in the glucosyltransferase assay reaction mixture (final volume, 100 ml) for 120 min at 25 °C. A standard portion of the reaction mixture was removed for paper chromatography and subsequent determination of the amount of glucose added to the acceptor. The sensitivity of glucosyltransferase was tested by incubation of different concentrations of antibiotics (novobiocin, tunicamycin, bacitracin, and tetracycline) with the enzyme 1 h prior to the standard assay for glucosyltransferase activity.

RESULTS

Cytoplasmic Membrane Fragments—The purification of glucosyltransferase was begun with 120 g (wet weight) of M. luteus.
cells harvested in the mid-logarithmic phase of growth, when the enzyme’s activity was maximal. A suspension of the cells in hypotonic medium was digested with lysozyme to solubilize peptidoglycan and to lyse resulting spheroplasts. The crude cell extract was treated with DNase and RNase to reduce viscosity and thereby facilitate subsequent purification procedures. These digestion steps were normally conducted at room temperature since it had previously been observed that incubation at or above 37°C caused the loss of glucosyltransferase activity.

Cytoplasmic membrane fragments were recovered from the crude extract by centrifugation between 3,000 and 12,000 × g. This fraction was resuspended and recovered twice more by centrifugation. Sedimentation and washing of the membrane fragments resulted in an increase in specific activity, but only with a considerable loss of total activity, much of which remained in the supernatant solutions. SDS-PAGE of the membrane fraction showed a substantial decrease in the diversity of contaminating proteins.

**Solubilization of Glucosyltransferase with Detergents**—Since glucosyltransferase is associated with cytoplasmic membrane fragments, a significant step in its isolation and purification is its release from the membrane with a minimal loss of activity and in a form amenable to subsequent purification. Detergents are routinely used for this purpose. Although some success has accompanied the use of Thesit (24, 25), electrophoresis of samples containing substantial amounts of Thesit has been problematic. Hence, other detergents, such as CHAPS, Triton X-100, and Tween 20, were evaluated as alternatives. Of these, CHAPS was the most promising. The effect of treatment of representative crude cell fractions with a combination of Thesit and CHAPS was examined by measurement of the glucosyltransferase activity released to the supernatant fraction. Table I shows that detergent treatment approximately doubled the glucosyltransferase specific activity detected in spent culture supernatant and in a suspension of whole cells, whereas it more than tripled that released from cytoplasmic membrane fragments. Presumably, the increase in specific activity reflected the solubilization or dispersal of the cytoplasmic membrane or similar lipid-based micelles, with the concomitant solubilization or release of enzyme previously sequestered inside vesicles and thus unavailable to the assay system. In light of these observations, membrane components were solubilized by treatment with both Thesit and CHAPS. Glycerol and magnesium ion were added to stabilize the solubilized enzyme.

**Purification of Glucosyltransferase**—Detergent-solubilized extract was passed through an adsorbent column (Bio-Beads SM-2) for the removal of nonpolar substances. Both protein and glucosyltransferase activity came directly through the column, with a slight enhancement of specific activity. The material that passed through the column was applied directly to a DEAE-cellulose column and then eluted with a gradient of NaCl (Fig. 2). About 25% of the protein and most of the carotenoids from the membrane were washed directly through the column. Several protein peaks were eluted with salt, but only those fractions that eluted at an NaCl concentration between 0.25 and 0.30 M contained glucosyltransferase. A faint yellow color was characteristic of the fractions containing the enzyme and could be used to predict which fractions would test positive for its activity.

Glucosyltransferase recovered from DEAE-cellulose was concentrated and applied to a Bio-Gel P-300 column, which was eluted with buffer D supplemented with 0.1 M NaCl. Glucosyltransferase eluted in the void volume (Fig. 3). The presence of salt in the developing buffer improved the recovery of enzyme activity. It was not determined whether the enzyme bound to the gel matrix or was dissociated in the absence of salt. In contrast, attempts to use gel-filtration matrices of dextran were unsuccessful: no activity was eluted from the column, nor was any matrix-bound enzyme detected. Dextran-based matrices were eliminated from further consideration after it was determined that the addition of dextran to any preparation containing glucosyltransferase caused a loss of all activity.

Glucosyltransferase recovered from Bio-Gel P-300 column was subjected to electrophoresis on a native gradient polyacrylamide gel (Fig. 4). Enzyme activity was determined from the sample in one lane (Fig. 4, right panel), and protein composition was revealed by staining of an adjacent duplicate lane (Fig. 4, left panel). Although several protein bands (migrating from 3 to 10.5 cm) were detected by native gradient PAGE, only the band migrating at 4.25 cm displayed glucosyltransferase activity. A duplicate gel lane containing glucosyltransferase was incubated with UDP-[14C]Glc in situ. Incubation was followed by exhaustive washing of the gel to remove unbound substrate. An autoradiogram of this gel displayed only one band, whose location corresponded to that of glucosyltransferase activity (data not shown). These results provide evidence that all other protein bands (~90% in mass) detected by native PAGE were either contaminants or the dissociated subunits of glucosyltransferase that were no longer active. Therefore, in a further study, native gradient PAGE was used as the final preparative step for purification of glucosyltransferase. The protein in the single band migrating at 4.25 cm was isolated either by excision or by electroelution and was ana-

| Fraction, detergent treatmenta | Protein conc b | Activityb | Specific activity |
|-------------------------------|---------------|-----------|------------------|
| Culture supernatant           | 2.13          | 8.0       | 3.76             |
| Cell suspension               | 1.90          | 15.1      | 7.95             |
| Membrane fragments            | 4.78          | 24.3      | 5.08             |
|                               | 5.10          | 58.9      | 11.5             |
| No                            | 2.69          | 53.2      | 19.8             |
| Yes                           | 2.31          | 168.0     | 72.7             |

a The fraction was suspended in buffer lacking detergents or in buffer containing Thesit (1 mg/ml) and CHAPS (0.5 mg/ml).

b One unit is equal to 1 nmol of [14C]glucose incorporated in 60 min in a standard assay.
Molecular mass standards of 545, 272, 132, and 66 kDa (top to bottom) were visualized by staining with Coomassie Blue. The migration of the column was developed with buffer D, pH 7.3, containing 0.1 M NaCl (to prevent the enzyme from binding to the gel). The migration of the molecular mass standard jack bean urease hexamer (545 kDa) was found in a band that migrated at 4.25 cm, only slightly faster than the molecular mass standard jack bean urease hexamer (545 kDa). A plot of distance migrated versus log molecular mass was constructed by computer. The curve in the plot is the best fit log regression line based on the migration of the molecular mass markers. According to the location of the band with enzyme activity, the molecular mass of glucosyltransferase was estimated to be 440 kDa.

**Molecular Mass of Native Glucosyltransferase—**In the native gradient PAGE shown in Fig. 4, glucosyltransferase activity was found in a band that migrated at 4.25 cm, only slightly faster than the molecular mass standard jack bean urease hexamer (545 kDa). A plot of distance migrated versus log molecular mass was constructed by computer. The curve in the plot is the best fit log regression line based on the migration of the molecular mass markers. According to the location of the band with enzyme activity, the molecular mass of glucosyltransferase was estimated to be 440 kDa.

**Stability—**Glucosyltransferase was stable for 3–6 months when stored at −15 °C in buffer containing 2 M glycerol, 20 mM magnesium acetate, 2 mM 2-mercaptoethanol, 1 mM diethyldithio-rotetol, and 0.1–3% Thesit and CHAPS. However, repeated freezing (to −15 °C) and thawing caused a gradual loss of activity. Activity was enhanced by 30% after 24 h of incubation at 25 °C. However, at higher temperatures, the enzyme was susceptible to inactivation. Incubation at 37 °C for 5 and 24 h resulted in the loss of 70 and 90% of the enzyme’s activity, respectively. Glucosyltransferase was stable when the pH of the above solution was maintained between 5.5 and 9.0 at 4 °C, but at its isoelectric point, it underwent inactivation.

**Partial Purification of Glucosyltransferase—**In our early attempt to purify glucosyltransferase, the active samples obtained by adsorbent column and DEAE-cellulose column chromatography were isoelectrically precipitated at pH 5.0 by centrifugation at 14,000 × g at 4 °C. The sedimented materials were resuspended in buffer D, pH 8.8, and centrifuged again to remove the insoluble precipitates. By this method, the specific activity of glucosyltransferase in the supernatant was significantly increased. The purification resulted in a >2-fold increase in specific activity (∼250 units/mg). Significantly fewer protein bands were obtained by SDS-PAGE compared with the previously used purification process (DEAE-cellulose column chromatography) (Fig. 5). SDS-PAGE also yielded two major bands (54 and 52.5 kDa), with many minor protein bands in the background indicative of only partial purification (Fig. 5, lane 5). Two problems were later found with regard to this purification step. First, purification resulted in low yield (20%), with more than half of glucosyltransferase irreversibly denatured. The solubilized fraction in the supernatant contained some protein aggregates that interfered with further purification. Second, similar problems arose when isoelectric focusing PAGE was used as the purification method. Since isoelectric precipitation is not practical for the final purification of glucosyltransferase, we eliminated this step in our later purification effort.

**Molecular Mass of Glucosyltransferase Subunits—**The protein band displaying glucosyltransferase activity was recovered from a duplicate native gradient gel and subjected to SDS-PAGE. Silver staining revealed two major bands, presumably subunits of glucosyltransferase (Fig. 6). The two bands took on a purplish brown color after double staining, a result suggesting the presence of a small amount of carbohydrate or lipid. The approximate molecular masses for these bands (52.5 and 54 kDa) were determined from another plot on the basis of molecular mass standards (Faint minor bands with mobilities corresponding to 23, 58, 70, and 75 kDa were occasionally observed; however, their quantity was insignificant). The results of SDS-PAGE of partially purified glucosyltransferase fractions are shown in Fig. 5. Although many minor bands can be detected in the last fraction (lane 5), it is obvious that the 54- and 52.5-kDa bands have changed from minor bands to major bands as a result of the purification process. These results prove that the increase in the specific activity of glucosyltransferase during purification is associated with the increase in the intensity of the bands of 52.5 and 54 kDa (Fig. 5).

In addition, we observed that the running condition of native gradient PAGE is critical. When the partially purified glucosyltransferase was exposed to prolonged electrophoresis, a waving band, which is an aggregate of dissociated glucosyltransferase components with similar 54- and 52.5-kDa subunits, was observed below the 4.25-cm glucosyltransferase band in native PAGE. It seems that the longer the period of electrophoresis, the farther the waving band migrated.

**Gel-fltration profile of M. luteus glucosyltransferase.** Glucosyltransferase concentrated from an elution peak obtained from a DEAE-cellulose column (Fig. 2) together with 1.4 mg of colorfull molecular mass standards were applied to a Bio-Gel P-300 column (1.5 × 50 cm). The column was developed with buffer D, pH 7.3, containing 0.1 M NaCl (to prevent the enzyme from binding to the gel). ○, glucosyltransferase specific activity; ●, protein concentration.

![Fig. 3. Gel-filtration profile of M. luteus glucosyltransferase.](http://www.jbc.org/)

**Native gradient PAGE and M. luteus glucosyltransferase activity.** Glucosyltransferase (GTF) from a Bio-Gel P-300 gel-filtration column (Fig. 3) was subjected to electrophoresis on 3–12% native gradient polyacrylamide gels (left panel, lane 2). Protein bands were visualized by staining with Coomassie Blue. The migration of molecular mass standards of 545, 272, 132, and 66 kDa (top to bottom) is labeled (left panel, lane 1). The results obtained in the glucosyltransferase activity assay on the same native gel are shown in the right panel. ○, glucosyltransferase activity; —, polyacrylamide gel concentration.

![Fig. 4. Native gradient PAGE and M. luteus glucosyltransferase activity.](http://www.jbc.org/)

**In vitro activity assay of partially purified glucosyltransferase.** The activity of partially purified glucosyltransferase was determined by the activity assay on the same native gel. A plot of distance migrated versus log molecular mass was constructed by computer. The curve in the plot is the best fit log regression line based on the migration of the molecular mass markers. According to the location of the band with enzyme activity, the molecular mass of glucosyltransferase was estimated to be 440 kDa.

**Molecular Mass of Glucosyltransferase Subunits—**The protein band displaying glucosyltransferase activity was recovered from a duplicate native gradient gel and subjected to SDS-PAGE. Silver staining revealed two major bands, presumably subunits of glucosyltransferase (Fig. 6). The two bands took on a purplish brown color after double staining, a result suggesting the presence of a small amount of carbohydrate or lipid. The approximate molecular masses for these bands (52.5 and 54 kDa) were determined from another plot on the basis of molecular mass standards (Faint minor bands with mobilities corresponding to 23, 58, 70, and 75 kDa were occasionally observed; however, their quantity was insignificant). The results of SDS-PAGE of partially purified glucosyltransferase fractions are shown in Fig. 5. Although many minor bands can be detected in the last fraction (lane 5), it is obvious that the 54- and 52.5-kDa bands have changed from minor bands to major bands as a result of the purification process. These results prove that the increase in the specific activity of glucosyltransferase during purification is associated with the increase in the intensity of the bands of 52.5 and 54 kDa (Fig. 5).

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The isoelectric point of the purified glucosyltransferase, as determined by isoelectric focusing on polyacrylamide gels, was between 4.5 and 4.9. Isoelectric precipitation of the enzyme yielded a pI value of 5.0 (Fig. 7). The diminished recovery of glucosyltransferase in the middle of the isoelectric precipitation plot is an indication of rapid loss of activity at the isoelectric point. The enzymatic reaction followed simple Michaelis-Menten kinetics. The apparent $K_m$ value of glucosyltransferase for its substrate, UDP-Glc, is $300 \mu M$.

Antibiotic Sensitivity—Purified glucosyltransferase was assayed for its sensitivity to inhibition by several antibiotics (novobiocin, tunicamycin, bacitracin, and tetracycline) at different concentrations. Of the antibiotics tested, only novobiocin substantially inhibited the enzyme. Specifically, $10 \mu M$ inhibited $60\%$ of glucosyltransferase activity, while a relatively high concentration of novobiocin ($100 \mu M$) was required for nearly complete inhibition. In contrast, $100 \mu M$ tunicamycin inhibited glucosyltransferase activity by only $50\%$, and bacitracin and tetracycline had no inhibitory effect.

**DISCUSSION**

The glucosyltransferase of *M. luteus* is one of a pair of enzymes that act together to effect the synthesis of teichuronic acid, in which the alternating residues of the polymer are glucose and ManNAcU (Fig. 1) (23, 24, 29). Glucosyltransferase catalyzes the transfer of a glucosyl residue from UDP-glucose to an acceptor teichuronic acid that has a nonreducing terminal ManNAcU residue. The other enzyme, ManNAcU-transferase, catalyzes the addition of a ManNAcU residue from UDP-ManNAcU to an acceptor teichuronic acid that has a nonreducing terminal glucose residue (the product of the glucosyltransferase reaction). As part of our ongoing investigation of teichuronic acid synthesis (20, 24), we sought to purify these enzymes so that we could study the mechanism of bacterial polysaccharide biosynthesis and examine why D-glucosyl residues are incorporated as $\alpha$-glycosides, whereas D-ManNAcU residues are incorporated as the $\beta$-anomer (18). We chose to purify glucosyltransferase first since UDP-[14C]glucose is more readily available than UDP-[14C]ManNAcU. Knowledge gained from the solubilization and purification of glucosyltransferase

**TABLE II**

| Purification step                  | Total protein mg | Total activity units | Yield % | Specific activity units/mg | Purification fold |
|------------------------------------|------------------|----------------------|---------|----------------------------|-------------------|
| Lysozyme digestion                | 3,000            | 19,800               | 100     | 6.6                        | 1.0               |
| Membrane fraction                 | 570              | 10,800               | 57      | 19                         | 2.9               |
| Detergent extraction              | 162              | 11,200               | 65      | 80                         | 12                |
| Adsorbent column                  | 160              | 12,800               | 65      | 80                         | 12                |
| DEAE-cellulose column             | 79               | 9,890                | 45      | 115                        | 17                |
| Gel-filtration column (P-300)     | 26               | 7,710                | 39      | 295                        | 45                |
| Protein band isolated at 4.25 cm  | 2.6              | 2,530                | 13      | 970                        | 150               |

*a* One unit is equal to 1 nmol of [14C]glucose incorporated in 60 min in a standard assay.
should be applicable to the purification of ManNAcU-transferase. The purified enzymes should prove useful in generating antibodies for studies of the enzymes’ cellular localization.

Enzymes involved in the biosynthesis of bacterial cell wall polymers have been detected mainly in cytoplasmic membrane fragments. Indeed, all prior investigations of teichuronic acid biosynthesis had made use of either cytoplasmic membrane fragments (also termed the particulate enzyme fraction) (19, 29) or a wall-plus-membrane complex (23, 30). Thus, the first major step toward purification of glucosyltransferase was the solubilization of protein components of the membrane fragments. Several detergents were tested for their ability to solubilize membranes while maintaining the activity of the glucosyltransferase. Thesit proved to be suitable for solubilization (24, 25), but later was found to interfere with subsequent purification steps as well as with PAGE analysis. For example, Thesit-solubilized enzyme fractions contain many other membrane proteins, such as ATPase (31), and membrane lipids, in particular, the typical membrane pigment patterns of M. luteus, carotenoids (32). Thesit did not fully solubilize the enzyme from the pieces of membrane fragment. CHAPS is a suitable detergent for solubilization, but is quite expensive. Because of the adverse effects of one and the cost of the other, the use of these two detergents in combination appeared to be superior to the use of either by itself.

The purification of glucosyltransferase was monitored routinely by nondenaturing PAGE and SDS-PAGE. Samples always displayed several protein bands, suggesting the presence of substantial amounts of contaminant protein. Furthermore, the use of numerous purification steps in various combinations consistently failed to increase the level of specific activity above 1000 units/mg. It is now apparent that glucosyltransferase is a multisubunit enzyme whose disaggregation upon prolonged electrophoresis or electroelution on polyacrylamide gel permits the detection of subunits. This finding is consistent with the observation that glucosyltransferase elutes in the exclusion volume of a Bio-Gel P-300 column (Fig. 3) and has a mobility in native gradient PAGE that is in line with a molecular mass of 440 kDa (Fig. 4). This interpretation was verified by elution of the glucosyltransferase band from native gradient PAGE and by the result of subsequent analysis by SDS-PAGE, in which the usual pattern of protein bands was generated. The bands at 52.5 and 54 kDa are always predominant and of equal intensity. The stoichiometry of the subunits has not been accurately determined; however, if the 54- and 52.5-kDa subunits are considered to be present with a stoichiometry of four copies each per aggregate, then the masses of the subunits add up to 426 kDa, a figure in reasonable agreement with the observed mass of 440 kDa for the aggregate. Glucosyltransferase strictly requires four pairs of subunits to maintain catalytic function. Since glucosyltransferase is a multisubunit protein, it is easy to understand why early attempts to purify a conventional-sized protein (≤200 kDa) led to a loss of enzyme activity. Furthermore, the glucosyltransferase subunits did not display the well defined mobilities and staining properties associated with many standard proteins. This difference may be a consequence of hydrophobicity or of special modifications of this membrane-derived protein or may be due to an unknown factor.

Reviewing the purification processes, we found that the most critical step in the final purification of this “fragile” membrane enzyme was the application of native gradient PAGE. Like SDS-PAGE, this procedure separates native proteins according to their molecular sizes; unlike SDS-PAGE, it causes no enzyme denaturations. The availability of a commercial gradient gel made this purification possible. The advantages offered by such gels are that they are easy to use and the reproducibility of results obtained with them (especially for migration distance) and the multiple subsequent uses of the purified protein band for enzyme assays, subunit analysis, and preparative purifications. The disadvantage of this method is the difficulty of scaling up purification.

The glucosyltransferase of M. luteus differs substantially from other glycosyltransferases that have been described. Unlike the glycosyltransferases involved in the biosynthesis of cell envelope polysaccharides of other bacteria, the glucosyltransferase of M. luteus is capable of elongating the teichuronic acid polysaccharide chain without a lipid carrier (such as glycosyl-polysisoprenyl phosphate) as the glycosyl donor. The glycosyltransferases involved in the synthesis of membrane-derived oligosaccharides of E. coli (33) and the cyclic glucans of Bradyrhizobium japonicum (34) both use UDP-glucose as a glycosyl donor. However, membrane-derived oligosaccharide synthesis also requires both polysisoprenyl phosphate (33) and acyl carrier protein (35). Several other properties of the M. luteus glucosyltransferase were also characterized in this study, such as its thermostability, its isoelectric point, its $K_{m}$, and its sensitivity to antibiotics. Further studies involving molecular cloning as well as structural and functional characterization will help to elucidate the catalytic mechanism of this molecule and will eventually establish the precise relationship between the two enzymes (glucosyltransferase and ManNAcU-transferase) that are responsible for the synthesis of the entire cell wall polymer teichuronic acid.

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REFERENCES

1. Osborn, M. J., Rosen, S. M., Rothfield, L., and Horecker, B. L. (1962) Proc. Natl. Acad. Sci. U. S. A. 48, 1831–1838
2. Robbins, P. W., Bray, D., Dankert, M., and Wright, A. (1967) Science 158, 1536–1542
3. Delph, P. J., Majerczak, D. R., and Coplin, D. L. (1988) J. Bacteriol. 170, 865–871
4. Wessels, M. R., Rubens, C. E., Benedi, V.-J., and Kasper, D. L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 8983–8987
5. Wessels, M. R., Moses, A. E., Goldberg, J. B., and DiCesare, T. J. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 8317–8321
6. Shiro, H., Muller, E., Gutierrez, N., Reist, S., Grout, M., Tosteson, T. D., Goldman, D., and Pier G. B. (1994) J. Infect. Dis. 169, 1042–1049
7. Melaugh, W., Phillips, N. J., Campagnari, A. A., Karalus, R., and Gibson, B. W. (1992) J. Biol. Chem. 267, 13434–13439
8. Martin, T. R., Ruzinski, J. T., Rubens, C. E., Chi, E. Y., and Wilson, C. B. (1992) J. Infect. Dis. 165, 306–314
9. DeKimpe, S. J., Kengatharan, M., Thienermann, C., and Vane, J. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 10359–10366
10. Rubens, C. E., Heggen, L. M., Haft, R. F., and Wessels, M. R. (1993) Mol. Microbiol. 8, 843–855
11. DeAngelis, P. L., Papaconstantinou, J., and Weigel, P. H. (1993) J. Biol. Chem. 268, 19181–19184
12. Lin, W. S., Cunneen, T., and Lee, C. Y. (1994) J. Bacteriol. 176, 7045–7051
13. Shiro, H., Muller, E., Gutierrez, N., Reist, S., Grout, M., Tosteson, T. D., Goldman, D., and Pier G. B. (1994) J. Biol. Chem. 269, 13434–13439
14. Kao, C. C., and Sequeira, L. (1991) J. Bacteriol. 173, 306–314
15. Page, R. L., and Anderson, J. S. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 681–685
16. Hase, S., and Matsushima, Y. (1972) J. Biochem. (Tokyo) 71, 1117–1128
17. Johnson, S. D., Lacher, K. P., and Anderson, J. S. (1981) J. Bacteriol. 148, 1015–1022
26. Laemmli, U. K. (1970) *Nature* **227**, 680–685
27. Dzandu, J. K., Deh, M. E., Barratt, D. L., and Wise, G. E. (1984) *Proc. Natl. Acad. Sci. U. S. A.* **81**, 1733–1737
28. Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, R. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., and Klenk, D. C. (1985) *Anal. Biochem.* **150**, 76–85
29. Stark, N. J., Levy, G. N., Rohr, T. E., and Anderson, J. S. (1977) *J. Biol. Chem.* **252**, 3466–3472
30. Weston, A., and Perkins, H. R. (1977) *FEBS Lett.* **76**, 195–198
31. Huberman, M., and Salton, M. R. J. (1979) *Biochim. Biophys. Acta* **547**, 230–240
32. Rothblat, G. H., Ellis D. S., and Kritchevski, D. (1964) *Biochim. Biophys. Acta* **84**, 340–347
33. Weissborn, A. C., Rumley, M. K., and Kennedy, E. P. (1991) *J. Biol. Chem.* **266**, 8062–8067
34. Cohen, J. L., and Miller, K. J. (1991) *J. Bacteriol.* **173**, 4271–4276
35. Therisod, H., Weissborn, A. C., and Kennedy, E. P. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83**, 7236–7240
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