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Glucosylation of Teichoic Acid: Solubilization and Partial Characterization of the Uridine Diphosphoglucose: Polyglycerolteichoic Acid Glucosyl Transferase from Membranes of *Bacillus subtilis*[1]

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Polyglycerolteichoic acid:glucosyl transferase (TAG transferase), one of the three enzymes involved in the pathway leading to the glucosylation of teichoic acid in *Bacillus subtilis* 168, was investigated. During the early stages of the growth of *B. subtilis*, TAG transferase is predominantly a soluble enzyme found in the cytoplasm. As growth proceeds, the amount of soluble enzyme decreases and the proportion of insoluble, membrane-bound TAG transferase increases, reaching a maximal value at the close of the logarithmic phase. Data are presented which suggest that these are two forms of the same enzyme, or have some common component. The effects of chaotropic agents, such as sodium trichloroacetate and sodium perchlorate, on the cytoplasmic membrane were also studied. These data show that such compounds can effectively remove the TAG transferase from the membrane in a water-soluble form. A study of some of the physical properties of this solubilized enzyme suggests that there is little difference between the two forms of the enzyme. Experiments are described which indicate that the glucosyl transfer by both the membrane-bound and soluble enzymes is not mediated by lipids.

The question of how enzymes are intercalated in structural components to ensure orderly growth and division of cells has been approached in many model microbial and mammalian systems in recent years (15). Studies with *Salmonella typhimurium* have clearly demonstrated the importance of utilizing phage-resistant mutants in investigations of membrane-associated enzymes (17), despite the disadvantages of the complex nature of the cell wall of the gram-negative organism and the difficulties involved in obtaining good preparations of cytoplasmic membranes. The observation that glucosylation of the wall teichoic acid is essential for the adsorption of viruses which infect *Bacillus subtilis* provides a simple model system in a gram-positive organism (20). Thus, it is possible to obtain mutants with defects in the soluble and particulate enzymes in this pathway by selecting for phage resistance (20, 21).

Most of the enzymes involved in the biosynthesis of the cell wall are in the particulate fraction of the cell, presumably associated with the cytoplasmic membrane. Solubilization of these enzymes has been a persistent problem. The recent discovery that chaotropic agents promote the release of enzymes from membranes (12) has enabled us to solubilize uridine diphosphate glucose:polyglycerolteichoic acid glucosyl transferase (TAG transferase) from membranes. This paper includes data describing the association of the enzyme with the membrane, the solubilization of the enzyme, a comparison of some of the properties of the soluble and membrane-associated enzymes, and an evaluation of the requirement for a lipid intermediate.

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MATERIALS AND METHODS

Strains. *B. subtilis* 168 carrying *trpC2* and *B. subtilis* strains BY51 carrying *trpC2 gtaC51* and BY12 carrying *trpC2 gtaA12* (21) were used in this study.

Growth of bacteria. Cells were grown at 37 C in a 150-liter fermentor (Fermentation Design, Inc.) in Spizizen's minimal medium (22) supplemented with 22 mM glucose, 0.02% acid hydrolyzed casein (Nutritional Biochemicals Corp.), 50 mM $MgSO_4$, and 0.25 mM L-tryptophan. The medium was inoculated from an overnight culture of the organisms to give a cell density of 10^7 cells/ml. In growth experiments, samples were removed periodically. For isolation of the enzyme, the cells were grown to approximately mid-log phase (2×10^8 cells/ml), harvested by use of a refrigerated Sharples centrifuge, washed once with Spizizen's minimal medium, and stored at −20 C until required.

Preparation of membranes. Cells were thawed at room temperature and suspended in 50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, *p*H 8, containing 10 mM $MgCl_2$ and 1 mM ethylenediaminetetraacetate at a concentration of 1 g of cells (wet weight) per 5 ml of buffer. To this was added lysozyme (0.2 mg/ml), ribonuclease (10 μg/ml), and deoxyribonuclease (10 μg/ml). The mixture was incubated at 37 C until complete lysis of the cells occurred (as judged by phase-contrast microscopy). The membrane and supernatant fractions were separated by centrifugation at 27,000 × *g* for 15 min in a refrigerated Sorvall RC-2B centrifuge. The pellet was washed three times with the Tris-hydrochloride buffer and stored at −20 C.

To investigate the distribution of TAG transferase, lysates were centrifuged for 2 hr at 113,000 × *g* at 4 C in a Beckman L-2 ultracentrifuge. The pellets were again washed three times with the buffer. Each sedimentation was done at 113,000 × *g* for 2 hr.

Extraction of membranes. Frozen membranes were thawed at room temperature and suspended in the appropriate buffer at a concentration of approximately 10 mg of protein/ml. This suspension was homogenized thoroughly before a predetermined volume of a solution of the chaotrope was added. This was then incubated under the conditions described for each experiment. At the end of the incubation period, the samples were chilled rapidly to 0 C and centrifuged at 59,000 × *g* for 30 min at 4 C.

The excess chaotrope was removed from the membranes by washing twice with buffer. Each sedimentation was done at 27,000 × *g* for 15 min at 0 to 4 C. Removal of the chaotrope from the supernatant fraction was accomplished by passing the extract through a column of Sephadex G-25 (41.5 cm by 2.5 cm) equilibrated with 10 mM Tris-hydrochloride, *p*H 8 (at 4 C). The enzymatic activity was eluted in the void volume and concentrated with a Diaflow membrane (μM-2, Amicon Corp.). Also at this point, the buffer, in which the enzyme was dissolved, could be changed conveniently. The membranes were stored at −20 C and the soluble extracts at 0 to 4 C.

Preglucosylation of membranes. Because the membranes contained endogenous glucose acceptor sites, it was necessary first to incubate them with unlabeled uridine diphosphate glucose (UDP-glucose). This procedure effectively increased the dependence of the subsequent reaction on the amount of exogenous glucose acceptor added. In early experiments, membranes were incubated with unlabeled UDP-glucose for 2 hr as described by Glaser and Burger (10), but it was found that considerable denaturation occurred under these conditions (Fig. 1). Because incubation periods of 40 min resulted in enzyme preparations in which most of the endogenous acceptor sites had been filled and no enzyme activity had been lost, these conditions were used in subsequent experiments (Fig. 1).

A typical preglucosylation mixture contained: membranes suspended at 10 mg of protein/ml in 50 mM Tris-hydrochloride, *p*H 8, 0.1 ml; Tris-hydrochloride, *p*H 8, 16 μmoles; $MgCl_2$, 4 μmoles; UDP-glucose, 1.1 μmoles; and water to a final volume of 670 μliters. This mixture was incubated at 37 C for 40 min before the reaction was stopped by cooling in ice water. The excess UDP-glucose was removed from the membranes by washing twice with the Tris-hydrochloride and sedimenting in the usual fashion. This preglucosylation step was mandatory for all experiments involving the measurement of membrane-bound activity but was omitted in assays of extracts and supernatant fractions.

Preparation of the glucose acceptor. Previous studies have demonstrated that most phage-resistant mutants lack glucose on their teichoic acid (20, 21). Cell walls were isolated from one such phage-resistant mutant, BY51, by mechanical disintegration and autolyzed as described previously (3). The autolysate was subsequently dialyzed, and the nondialyzable fraction was chromatographed on diethylaminoethyl cellulose (21). The teichoic acid enriched fraction was dialyzed, lyophilized, and chromatographed sequentially on Biogel P-100 and hydroxylapatite. The second phosphorus-containing peak eluted from the hydroxylapatite was used as the acceptor; analysis showed it to contain phosphorus, glycerol, glucosamine, and muramic acid in the molar ratio 4:4:1.6:1 (F. E. Young, A. P. Jackson, and L. Arias, Fed. Proc. **27**:437, 1968).

Assay of TAG transferase. The enzymatic activity in the intracellular supernatant fractions was measured by the procedure of Burger and Glaser (4) as modified by Young (20). In the majority of the assays, a modification of "Assay B" of Chin et al. (5) was used. In this procedure, the enzyme reaction was stopped by the addition of 2 ml of a solvent containing ethanol-1 M ammonium acetate, *p*H 3.6 (2:1, v/v; solvent A). This mixture was added to a funnel containing a filter (Whatman no. 1 paper, 2.3 cm in diameter) covered with 2 ml of the same solvent. The assay tube was then washed out three times with 1-ml portions of solvent A. The mixture was filtered, and the filter paper was washed four times with 4 ml of the solvent. The paper was dried at 50 C, and the adsorbed radioactivity was measured in the usual fashion. For reproducible results with this assay, it was essential to use filters which had been presoaked and then washed by filtration with solvent A.

A typical reaction mixture contained: *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid-NaOH (TES-NaOH) buffer, *p*H 7, 1.25 μmoles; $MgCl_2$, 5 μmoles; UDP-glucose, 0.11 μmole; UDP-[^{14}C]-glucose, 0.05 μCi; acceptor, 0.025 mg; enzyme in 10 mM TES-NaOH, *p*H 7, 25 μliters; and glass-distilled

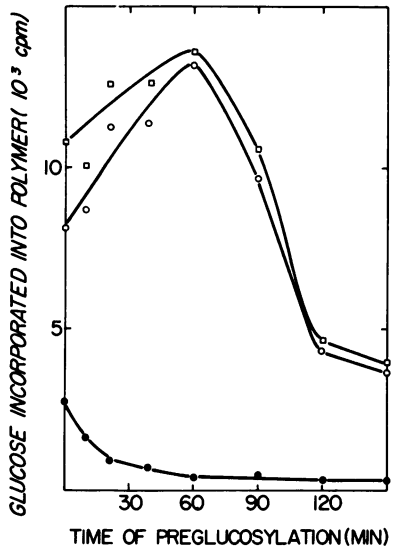

FIG. 1. *Membrane enzyme was preglucosylated with nonradioactive glucose for various lengths of time, as described in Materials and Methods. The excess glucose was then washed out, and the TAG transferase activity of the membranes was measured in the absence (●) and presence (□) of exogenous acceptor. Subtraction of the two results gave the net incorporation into the acceptor* (○).

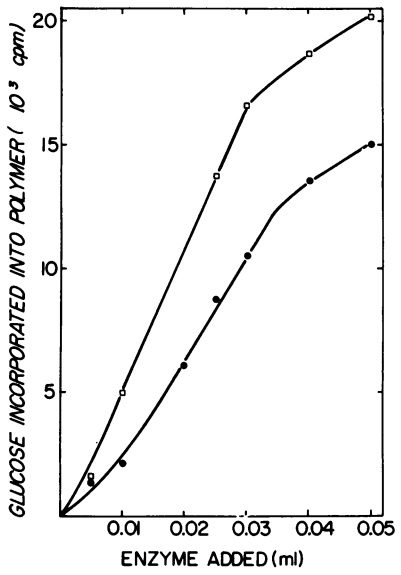

FIG. 2. *Glucose incorporated into the exogenous acceptor as a function of enzyme concentration. Membrane-bound enzyme was suspended in 10 mM TES-NaOH, pH 7, at a concentration of 10 mg of protein/ml* (□). *The soluble enzyme was dissolved in the same buffer at a concentration of 1.5 mg of protein/ml* (●).

water to a final volume of 150 μliters. This mixture was normally incubated at 37 C for 8 or 10 min (see Table 4). Two such reactions were routinely carried out. The exogenous acceptor was omitted from one. The net amount of glucose incorporated into the exogenous acceptor was calculated by subtracting the incorporation in the absence of acceptor from the total amount of glucose incorporated in the presence of the exogenous acceptor.

All results were determined in the linear portions of the curves obtained by correlating glucose incorporations with time, enzyme concentration (Fig. 2), and UDP-glucose and acceptor concentrations. One unit of enzyme is defined as the amount necessary to catalyze the transfer of 1 μmole of glucose from UDP-glucose to acceptor per minute at 37 C. The product of these reactions is glucosylated acceptor (teichoic acid); its characterization has previously been published (4, 20).

Lipid extractions. Two types of experiments were performed to determine whether a lipid intermediate was involved in the glucosylation of the acceptor. In the first experiment, complete reaction mixtures were incubated at 37 C for 10 min and then subjected to descending paper chromatography on Whatman no. 1 paper in solvent A (20). The material remaining at the origin was extracted with a mixture of chloroform-methanol (2:1, v/v). The radioactivity in this extract was determined. Alternatively, after the incubation period the mixture was extracted with butan-1-ol (2), and the radioactivity of the butanol-soluble fraction was determined.

In a second series of experiments, solubilized enzyme which had been partially purified by reverse-flow chromatography on a column of Sephadex G-200 (30 cm by 2.5 cm) at 4 C in 50 mM Tris-hydrochloride, *p*H 8, was extracted with a mixture of chloroform-methanol (1:1, v/v) at −20 C, according to the method of Dietrich et al. (7). The aqueous phase was assayed for activity with and without the readdition of the lipid extract. Controls were also carried out to show that the processes of lyophilization and solvent extraction did not adversely affect the enzymes.

Measurement of radioactivity. Radioactivity adsorbed on paper chromatograms was located with a Nuclear-Chicago Radiochromatogram Scanner. The areas were cut out and counted in a Packard Tri-Carb scintillation counter, model 527, by immersing them in a scintillation fluid of the following composition: toluene, 1.0 liter; 2,5-diphenyloxazole, 4.0 g; 1,4-bis-(4-methyl-5-phenyloxazole-2-yl) benzene, 0.1 g (2). The radioactivity on the assay filters was measured in a similar manner. The radioactivity dissolved in the organic solvents was determined by first evaporating off the solvent in a counting vial and then dissolving the residue in the above scintillant.

Chemical analysis. Protein was measured by the biuret reaction in the presence of sodium deoxycholate to solubilize the membranes (11). Phosphorus, amino acids, and amino sugars were determined as described previously (22).

Materials. UDP-[$^{14}C_6$]-glucose (237 mCi/mmole) was purchased from New England Nuclear Corp.; UDP-glucose was obtained from Sigma Chemical Co.; Sephadex G-25, from Pharmacia Ltd.; *N*-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) and crystalline egg white lysozyme, from Calbiochem, Los

Angeles, Calif.; and ribonuclease and deoxyribonuclease, from Worthington Biochemical Corp., Freehold, N.J.

RESULTS

Distribution of TAG transferase activity during growth. The distribution of enzyme activity between the membrane and supernatant fractions was measured at several stages during the growth of the bacteria. Activity was detected in both fractions (Fig. 3 and 4); however, the proportions of the enzyme in each varied considerably. For instance, only 36% of the activity was associated with the membrane during early logarithmic growth, whereas at the end of this phase 90% of the activity was bound to the membrane (Fig. 3). Also, the specific activity of the membrane-associated enzyme increased during growth (Fig. 4), reaching a maximum near the transition from the logarithmic to the stationary phase of the growth cycle. The activity in the membrane fraction decreased markedly in the stationary phase.

To determine whether the presence of the soluble and particulate activities was due to one or multiple enzymes, a mutant, BY12, which lacks the membrane-bound TAG transferase, was examined. The soluble and particulate activities were absent during all phases of growth.

Requirement for a lipid intermediate. Two series of experiments were carried out to determine whether a lipid was required for transferase activity. The first type of experiment involved the extraction, with lipid-solvents, of the base-line material obtained after paper chromatography of the reaction mixtures and the direct extraction of reaction mixtures with butan-1-ol. The butanolic extractions were carried out because there was the possibility that a very acid-labile intermediate might have been degraded by the chromatography solvent. Such experiments were done with samples of both the solubilized and membrane-bound enzymes. In all experiments, the amount of radioactivity in the lipid solvents was small, varying from 0 to 2% of the total radioactivity incorporated into the acceptor.

In a second type of experiment, a portion of the solubilized TAG transferase was lyophilized and extracted at low temperatures with chloroform-methanol (1:1, v/v). After removal of the excess solvent from the extracted enzyme, it was reconstituted with buffer and assayed for activity with and without the readdition of the lipid extract. As shown in Table 1, the incorporation of glucose into the acceptor was not affected by the extraction procedure (extracted enzyme I), nor did the readdition of the lipid material particularly enhance the reaction (extracted enzyme II). The addition of the 3.3% sodium deoxycholate did not increase the incorporation; it had rather the reverse effect (extracted enzyme III).

Solubilization of the membrane-bound enzyme. The studies of Davis and Hatefi (6) using various chaotropic agents have demonstrated that enzymes can be released, and thus solubilized, from mitochondrial membranes and complexes. Similar experiments were carried out to investigate the suitability of these agents for solubilizing the TAG transferase. In initial experiments, the

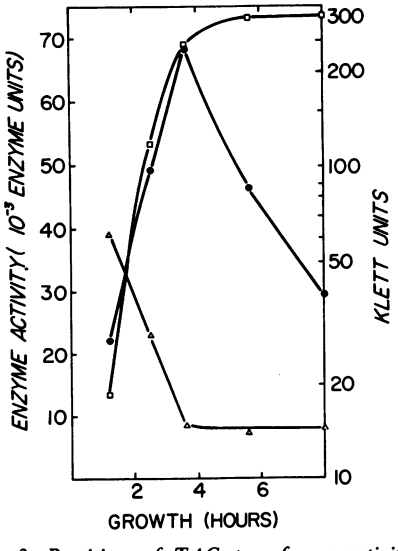

FIG. 3. *Partition of TAG transferase activity between the membrane* (●) *and supernatant fluid* (△) *as a function of growth* (□).

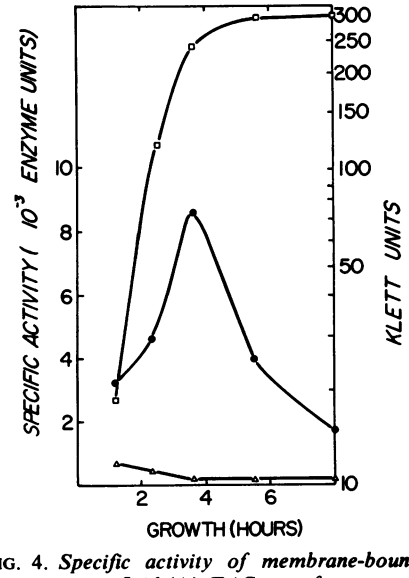

FIG. 4. *Specific activity of membrane-bound* (●) *and supernatant fluid* (△) *TAG transferase as a function of growth* (□).

TABLE 1. *Absence of a lipid requirement for TAG transferase*

Incubation time (min)	Glucose incorporated into acceptor (nmoles)			
	Control enzyme (lyophilized only)	Extracted enzyme		
		I[a]	II[b]	III[c]
10	20	21	21	18
30		21	19	16
60	22	24	14	15

[a] Incorporation in the presence of acceptor only.
[b] Incorporation in the presence of acceptor plus lipid extract.
[c] Incorporation in the presence of acceptor, lipid extract, and 3.3% sodium deoxycholate.

chaotrope sodium perchlorate (ClO_4^-) was used at a concentration of 0.50 M. These conditions released the enzyme quite effectively (Table 2, experiment 3). A disadvantage of this procedure was the inactivation of the enzyme, presumably by the ClO_4^-. By decreasing the ClO_4^- concentration, however, it was found that less inactivation took place and, in fact, the yield of soluble enzyme was considerably increased (Table 2, experiment 4). Optimum conditions for the solubilization of the TAG transferase were therefore taken to be 0.25 M $NaClO_4$ (*p*H 7) for 40 min at 15 C.

More recently, Hatefi and Hanstein (12) have shown that sodium trichloroacetate is more effective as a chaotropic agent than ClO_4^- (W. G. Hanstein and Y. Hatefi, Fed. Proc. **29**:3630, 1970). But when this was substituted, at comparable concentrations, in the solubilization procedure, the yield of soluble enzyme was lower than with the ClO_4^- and in one case the inactivation was more extensive (see Table 2, experiments 5, 6, and 7).

An interesting observation was that a small amount of enzyme was solubilized by incubation in the buffer alone (Table 2, experiment 2). This was not an artifact but was regularly observed. Whether the solubilization was due to the buffer or to the freezing and thawing encountered in the manipulation of the membranes remains to be investigated (1).

If the solubilization process were an equilibrium between membrane-attached enzyme and the soluble enzyme plus membrane, then a second extraction of the membrane pellet should also release a significant amount of enzyme. This was tried with the two chaotropic agents at their most effective concentrations (Table 3). It was found that, although the first extraction was satisfactory, the second resulted in only a small degree of solubilization and extensive inactivation with ClO_4^-.

TABLE 2. *Chaotropic extractions of membranes*

Expt	Conditions of extractions[a]	Activity (units)		Per cent total recovery (A+B)
		Membrane fraction (A)	Supernatant fraction (B)	
1	None	62.8×10^{-3}	0	100
2	TES buffer	59.3×10^{-3}	5.1×10^{-3}	105
3	+0.50 M ClO_4^-	8.5×10^{-3}	12.4×10^{-3}	33
4	+0.25 M ClO_4^-	31.8×10^{-3}	25.2×10^{-3}	91
5	+0.50 M TCA^-	2.7×10^{-3}	6.3×10^{-3}	14
6	+0.25 M TCA^-	36.5×10^{-3}	13.3×10^{-3}	80
7	+0.125 M TCA^-	51.0×10^{-3}	1.8×10^{-3}	83

[a] Membranes were suspended at a concentration of 10 mg of protein/ml in 50 mM TES-OH, *p*H 7, and incubated at 15 C for 40 min. ClO_4^-, sodium perchlorate; TCA^-, sodium trichloroacetate.

TABLE 3. *Repeated chaotropic extractions*

Expt	Extraction	Conditions[a]	Activity in soluble form (%)	Total activity recovered (%)
1	1	0.125 M TCA^-	14	94
	2	0.125 M TCA^-	5	109
2	1	0.25 M ClO_4^-	26	96
	2	0.25 M ClO_4^-	6	57

[a] Membrane suspended at a concentration of 10 mg of protein/ml, in TES-OH, *p*H 7, and incubated at 15 C for 40 min. TCA^-, sodium trichloroacetate; ClO_4^-, sodium perchlorate.

Recombination of the membrane and soluble enzyme. To investigate the reversibility of the solubilization process, a sample of the membranes was extracted in 50 mM TES-NaOH, *p*H 7, with 0.25 M ClO_4^- as usual. The extract and membranes were separated. The excess ClO_4^- was washed out of the membranes and removed from the soluble extract by Diaflow filtration. Samples were removed for assay. The ClO_4^--free membranes and extract were then homogenized together and allowed to remain at room temperature for 5 min with further intermittent homogenization. At the end of this period, the two portions of the mixture were separated and assayed for TAG transferase activity. Only about 9% of the soluble activity had been lost during the remixing procedure. This suggests that the solubilization is not a readily reversible step.

Physical properties of the enzymes. Extraction of the membranes with the chaotropic agents results in the appearance of a soluble TAG transferase. To ascertain whether the extraction had

TABLE 4. *Comparison of the soluble and insoluble enzymes*

Property	Sample	
	Membrane[a]	Extracted enzyme
Rate of glucose incorporation	linear 8 min	linear 10 min
Optimal *p*H	7.5	6.8
Optimal temperature	37 C	37 C
Optimal Mg^{2+} concentration	33 mM	33 mM
K_m (UDP-glucose)[b]	1.3×10^{-5} M	1.7×10^{-5} M
K_m (acceptor)[b]	0.5 mg/ml	0.1 mg/ml

[a] Refers to membrane-bound enzyme.
[b] Derived from standard Lineweaver-Burk plot.

altered the enzyme, a survey of some of its properties was undertaken. As shown in Table 4, the initial rate and the optimal *p*H, temperature, and magnesium concentration for the two forms of the enzyme were the same. The Lineweaver-Burk constants for the two substrates (the UDP-glucose and exogenous acceptor) of the reaction were also determined. The K_m values for the UDP-glucose were the same, whereas the K_m for the acceptor with the particulate enzyme was five times greater than that for the soluble enzyme (Table 4).

DISCUSSION

Glucosylation of the teicholic acid of *B. subtilis* 168 is necessary for the adsorption of several phages (20). The enzyme required for this glucosyl transfer (TAG transferase) is present to a small extent in the cytoplasm but is largely found in association with the membrane. Furthermore, the partition of this enzyme between the two phases varies with the growth of the organism, in that the amount of membrane-associated activity rises from 36% during early logarithmic growth to a value of 90% at the end of logarithmic growth. The specific activity of the membrane-bound enzyme also reaches a maximum at the same stage of growth. The total activity and specific activity of the soluble enzyme decreases during this time and then continues at a low, approximately constant level. The decrease in membrane-bound TAG transferase may be related to the lack of teichoic acid in spores of *B. subtilis* (18). Because mutant BY12 lacks both the soluble and particulate activity, it is likely that these enzymes have at least some common component.

These data might suggest that the TAG transferase is initially produced in the cytoplasm, from which it migrates, in an active form and via some unknown mechanism, to the membrane where it becomes firmly attached. Alternatively, the ribosomes during the early stages of growth might be producing the enzyme at a faster rate than it can be adsorbed onto the membrane; thus the excess enzyme would appear in the supernatant fluid. Then, as growth accelerates and the capacity of the membranes to bind the enzyme increases, a different distribution of the enzyme between the membranes and supernatant fractions could occur. At the end of this rapid phase of growth during the stationary period and sporulation, teichoic acid synthesis and glucosylation decline.

A somewhat similar situation occurs in *S. typhimurium* in the biosynthesis of its O antigen. The two enzymes glucosyl and galactosyl lipopolysaccharide transferase occur in the cytoplasm but are predominantly membrane-bound. In an analysis of mutants lacking these enzymes, Osborn (16) observed that the soluble transferases were also missing. Endo and Rothfield (9) were thus able to avoid the problem of solubilizing the enzyme from the membrane by purifying the soluble UDP-galactose:lipopolysaccharide-α-3-galactosyl transferase. However, owing to the small amounts of soluble TAG transferase in *B. subtilis*, this approach was not possible.

A convenient method for attacking the problem of solubilization was suggested by the demonstration that chaotropic agents would solubilize membrane-associated proteins by altering the structure and lipophilicity of the surrounding water (6, 12). An examination of some of the parameters studied by Davis and Hatefi (6) revealed that approximately 40% of the membrane-bound TAG transferase could be solubilized without the concomitant denaturation as observed with detergents, alkali, or urea (13). The extent of solubilization of the enzyme (without denaturation) is dependent upon the concentration of the chaotrope, *p*H, temperature, and period of incubation. It is not possible to increase the amount of enzyme solubilized by re-extraction of the membrane, nor does the extracted enzyme readily reassociate with the membrane when the chaotrope is removed. Thus it is reasonable to assume that there does not exist during the extraction an equilibrium between the soluble and insoluble forms of the enzyme, but rather that as the enzyme becomes attached to the membrane it goes through an intermediary stage where it is only loosely bound and it is at this point that it is available for solubilization by the chaotrope.

Removal of the TAG transferase from the membrane alters its environment and solubility. A comparison of some of its physical properties with those of the membrane-attached enzyme would suggest that the extraction procedure had not greatly altered it in any other way. The major difference between the two enzymes is their K_m for acceptor, that for the membrane-

bound enzyme being five times greater than that for the soluble enzyme. This, however, may only be a reflection of the greater accessibility of the enzyme for the exogenous acceptor now that it is in a soluble form. It is interesting to note that although all the curves plotted to determine the optimal conditions for the glucosyl transfer had points of inflection in the same area, the curves for the soluble enzymes were generally steeper than those for the membrane-bound enzyme. Both forms of the enzyme were unstable towards heating at 50 C (*unpublished observations*). A difference in stability is observed in the storage conditions of the enzymes, i.e., the membrane-bound enzyme is stable through repeated freezing (at −20 C) and thawing, whereas the soluble enzyme is not.

Investigations into the biosynthetic pathways leading to the formation of a number of cell wall components have shown the involvement of a C_{55} polyisoprenoid lipid in at least one step (14, 19). Lipids of unknown structure have also been reported as taking part in the biosynthesis of a teichoic acid (8) and of a cell wall phosphorylated polysaccharide (2). Experiments carried out with both the soluble and insoluble forms of the TAG transferase have so far failed to provide any evidence that this glucosyl transfer is in any way mediated by a lipid. The evidence, although strong, is not conclusive; it might be that the amount of lipid utilized in the reaction is so small and its turnover so rapid that the methods of detection used were not sensitive enough.

A simple system in *B. subtilis* is now available for the study of the interactions between the cell membrane and its associated enzymes. The ease of selection of conditional TAG transferase mutants should enable investigation of the biochemical genetics and regulation of this enzyme.

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LITERATURE CITED

1. Baginsky, M. L., and Y. Hatefi. 1969. Reconstitution of succinate-coenzyme Q reductase (complex II) and succinate oxidase activities by a highly purified, reactivated succinate dehydrogenase. J. Biol. Chem. **244**:5313-5319.
2. Brooks, D., and J. Baddiley. 1969. A lipid intermediate in the synthesis of a poly-(*N*-acetylglucosamine 1-phosphate) from the wall of *Staphylococcus lactis* N.C.T.C. 2102. Biochem. J. **115**:307-314.
3. Brown, W. C., D. K. Fraser, and F. E. Young. 1970. Problems in purification of a *Bacillus subtilis* autolytic enzyme caused by association with teichoic acid. Biochim. Biophys. Acta **198**:308-315.
4. Burger, M. M., and L. Glaser. 1966. UDP-glucose: polyglycerolteichoic acid glucosyl transferase from *Bacillus subtilis*, p. 436-441. *In* E. F. Neufeld and T. Ginsberg (ed.), Methods in enzymology, vol. 8. Academic Press Inc., New York.
5. Chin, T., M. M. Burger, and L. Glaser. 1966. Synthesis of teichoic acids. VI. The formation of multiple wall polymers in *Bacillus subtilis* W-23. Arch. Biochem. Biophys. **116**:358-367.
6. Davis, K. A., and Y. Hatefi. 1969. Kinetics of the resolution of complex I (reduced diphosphopyridine nucleotide-coenzyme Q reductase) of the mitochondrial electron transport system by chaotropic agents. Biochemistry **8**:3355-3361.
7. Dietrich, C. P., A. V. Colucci, and J. L. Strominger. 1967. Biosynthesis of the peptidoglycan of bacterial cell walls. V. Separation of protein and lipid components of the particulate enzyme from *Micrococcus lysodeikticus* and purification of the endogenous lipid acceptors. J. Biol. Chem. **242**:3218-3225.
8. Douglas, L. J., and J. Baddiley. 1968. A lipid intermediate in the biosynthesis of a teichoic acid. FEBS Lett. **1**:114-116.
9. Endo, A., and L. Rothfield. 1969. Studies of a phospholipid-requiring bacterial enzyme. I. Purification and properties of uridine diphosphate galactose lipopolysaccharide α-3-galactosyl transferase. Biochemistry **8**:3500-3507.
10. Glaser, L., and M. M. Burger. 1964. The synthesis of teichoic acids. III. Glucosylation of polyglycerolphosphate. J. Biol. Chem. **239**:3187-3191.
11. Gornall, A. G., C. J. Bardawill, and M. M. David. 1964. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751-766.
12. Hatefi, Y., and W. G. Hanstein. 1969. Solubilization of particulate proteins and nonelectrolytes by chaotropic agents. Proc. Nat. Acad. Sci. U.S.A. **62**:1129-1136.
13. Heydanek, M. G., and F. C. Neuhaus. 1969. The initial stage in peptidoglycan synthesis. IV. Solubilization of phospho-*N*-acetylmuramyl-pentapeptide translocase. Biochemistry **8**:1474-1481.
14. Higashi, Y., J. L. Strominger, and C. C. Sweeley. 1967. Structure of a lipid intermediate in cell wall peptidoglycan synthesis: a derivative of a C_{55} isoprenoid alcohol. Proc. Nat. Acad. Sci. U.S.A. **57**:1878-1884.
15. Korn, E. D. 1969. Cell membranes: structure and synthesis. Annu. Rev. Biochem. **38**:263-288.
16. Osborn, M. J. 1968. Biochemical characterization of mutants of *Salmonella typhimurium* lacking glucosyl or galactosyl lipopolysaccharide transferases. Nature (London) **217**:957-960.
17. Osborn, M. J. 1969. Structure and biosynthesis of the bacterial cell wall. Annu. Rev. Biochem. **38**:501-538.
18. Salton, M. R. J., and B. J. Marshall. 1959. The composition of the spore wall and the wall of vegetative cells of *Bacillus subtilis*. J. Gen. Microbiol. **21**:415-420.
19. Wright, A., M. Dankert, P. Fennessey, and P. W. Robbins. 1967. Characterization of a polyisoprenoid compound functional in O-antigen biosynthesis. Proc. Nat. Acad. Sci. U.S.A. **57**:1798-1803.
20. Young, F. E. 1967. Requirement of glucosylated teichoic acid for adsorption of phage in *Bacillus subtilis* 168. Proc. Nat. Acad. Sci. U.S.A. **58**:2377-2384.
21. Young, F. E., C. Smith, and B. E. Reilley. 1969. Chromosomal location of genes regulating resistance to bacteriophage in *Bacillus subtilis*. J. Bacteriol. **98**:1087-1097.
22. Young, F. E., J. Spizizen, and I. P. Crawford. 1963. Biochemical aspects of competence in the *Bacillus subtilis* transformation system. I. Chemical composition of cell walls. J. Biol. Chem. **238**:3119-3130.
23. Young, F. E., D. J. Tipper, and J. L. Strominger. 1964. Autolysis of cell walls of *Bacillus subtilis*. Mechanism and possible relationship to competence. J. Biol. Chem. **239**:PC3600-PC3602.