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Mechanism of Inducer Expulsion in *Streptococcus pyogenes*: a Two-Step Process Activated by ATP

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The mechanism of methyl-β-D-thiogalactoside-phosphate (TMG-P) expulsion from *Streptococcus pyogenes* was studied. The expulsion elicited by glucose was not due to exchange vectorial transphosphorylation between the expelled TMG and the incoming glucose since more β-galactoside was displaced than glucose taken up, and the stoichiometry between TMG and glucose transport was inconstant. Instead, two distinct and sequential reactions, intracellular dephosphorylation of TMG-P followed by efflux of free TMG, mediated the expulsion. This was shown by temporary accumulation of free TMG effected by competitive inhibition of its efflux and by the aid of arsenate, which arrested dephosphorylation of TMG-P but did not affect efflux of free TMG formed intracellularly before arsenate addition. The competitive inhibition of TMG efflux by its structural analogs suggests that a transport protein facilitates the expulsion. Iodoacetate or fluoride prevented TMG-P dephosphorylation and its expulsion. However, provision of ATP via the arginine deiminase pathway restored these activities in the presence of the glycolytic inhibitors and stimulated expulsion in their absence. Other amino acids tested did not promote this restoration, and canavanine or norvaline severely inhibited it. Arginine without glucose neither elicited the dephosphorylation nor evoked the expulsion of TMG-P. Ionophores or ATPase inhibitors did not prevent the expulsion as elicited by glucose or its restoration by arginine. The results suggest that activation of the dephosphorylation-expulsion mechanism occurs independently of a functional glycolytic pathway, requires ATP provision, and is possibly due to protein phosphorylation controlled by a yet unknown metabolite. The in vivo phosphorylation of a protein (approximate molecular weight = 10,000) under the conditions of expulsion was demonstrated.

Transport of solutes across cellular membranes allows for nutrient entry as well as exit of deleterious and nonessential metabolic products (15). The extent of solute accumulation and the direction of translocation may change with the physiological state and metabolic requirements of the cell. Such changes in carbohydrate transport are governed by several regulatory mechanisms which satisfy the requirements of efficiently growing bacteria to sense and accumulate selectively a preferable energy-carbon source and to avoid futile energy consumption (4, 18).

In previous reports we described a novel vectorial mechanism which effectively serves these requirements and regulates the accumulation of β-galactoside phosphate in species of streptococci (12, 22). These bacteria accumulate cytoplasmic methyl-β-D-thiogalactopyranoside (TMG) as the phosphate ester due to a phosphotransferase system (PTS) which catalyzes vectorial sugar phosphorylation. Addition of glucose to cells previously exposed to TMG promptly elicits rapid expulsion of the free analog from the intracellular TMG-phosphate (TMG-P) pool. Expulsion of preaccumulated TMG-P is similarly elicited by other energy sources, but non-metabolizable sugar analogs are essentially without effect.

Since internal galactoside-phosphate may induce the lactose regulon (2, 10), the expulsion of intracellular TMG-P from streptococci can be regarded as an inducer expulsion mechanism. Inducer expulsion is distinct from mechanisms of inducer exclusion which decrease the intracellular inducer concentration by inhibition of uptake (4). The expulsion process has the physiological advantage of depleting the intracellular inducer content, thereby effectively arresting inducible enzyme synthesis. Lack of a gratuitous inducer for the *lac* genes in *Streptococcus pyogenes* precluded the demonstration of expul-

sion of an actual inducer. Nevertheless, the induction by TMG-P of both β-D-phosphogalactoside galactohydrolase and lactose PTS activities in *Lactobacillus casei* and the expulsion elicited by glucose of this non-metabolizable inducer (1a) validate the physiological significance of the expulsion mechanism (12).

The results presented here confirm and extend our previous findings (12, 22). They provide definitive evidence that the expulsion process in *S. pyogenes* consists of two distinct and sequential reactions: intracellular dephosphorylation of TMG-P and a subsequent efflux of the free analog mediated by a transport protein, possibly enzyme II^{lac} (see accompanying paper [14]). They also show that TMG-P dephosphorylation depends on both energy and a glycolytic intermediate, possibly due to ATP-dependent activation or phosphorylation of a sugar-phosphate phosphatase. The phosphorylation of a small protein under the specific conditions of expulsion is demonstrated in vivo.

MATERIALS AND METHODS

Organism and growth. Cultures of *S. pyogenes* type 12 were grown at 37°C without shaking in a complex medium containing (grams per liter): NaCl, 5; tryptone (Difco Laboratories, Detroit, Mich.), 15; Difco Proteose Peptone, 5; yeast extract (Difco), 2; and lactose (autoclaved separately), 5. Growth of cells was monitored by following the optical density at 660 nm with a Hitachi 100-40 spectrophotometer.

Transport studies. Cells from a 300- to 400-ml culture in the midlogarithmic phase of growth were harvested by centrifugation (12,000 × *g* for 5 min) at 4°C, washed twice with 0.05 M sodium phosphate buffer (pH 7.0) containing 10 mM $MgCl_2$, and suspended in the same buffer at a concentration of 1.5 to 2.5 mg (dry weight) per ml. In later experiments the phosphate buffer was replaced with 0.05 M Tris-maleate buffer (pH 7.2) which contained 5 mM $MgCl_2$. Cells were prewarmed at 37°C for 10 min and then incubated (5 to 10 min) with [^{14}C]TMG (5×10^{-4} M; specific activity, 0.5 or 1 μCi/μmol) in the absence or presence of arginine (2 to 10 mM) or peptone (1%). Expulsion was initiated by rapid injection of 10 or 20 μl of 0.5 M glucose into the cell suspension (1 ml). Alternatively, expulsion of preaccumulated TMG-P was initiated in cell suspensions devoid of external [^{14}C]TMG. In these experiments preloaded cells were collected by centrifugation at 12,000 × *g* for 2 min at 4°C; the supernatant fluid was removed by aspiration, and the interior walls of the test tubes were carefully wiped. The preloaded cell pellet was suspended by rapid vortexing with buffered medium containing the appropriate additions, and after temperature equilibration (5 min at 37°C) expulsion of TMG-P was elicited by glucose addition as described above. The transport reaction was terminated by collecting cells at intervals on membrane filters. Radioactivity was determined as described previously (13). An intracellular volume of 2.0 ml/g (dry weight) was used to calculate the intracellular concentrations of substrates accumulated (13).

Determination of intracellular [^{14}C]TMG and [^{14}C]TMG-P. Intracellular free [^{14}C]TMG, [^{14}C]TMG-P, and total ^{14}C-labeled galactoside were determined as follows. Duplicate samples were withdrawn from the transport reaction, and cells were collected by filtration through membrane filters (0.45 μm; Millipore Corp., Bedford, Mass.). One filter was used for determination of total intracellular label ([^{14}C]TMG plus [^{14}C]TMG-P), whereas cells retained on the other filter were immediately resuspended in 3 ml of boiling water and extracted at 100°C for 10 min. Cell debris was removed by centrifugation (12,000 × *g* for 10 min), and free as well as phosphorylated galactosides were determined in the clarified extract by ion-exchange chromatography (AG1-X2 resin, 50 to 100 mesh; Bio-Rad Laboratories, Richmond, Calif.) as previously described (9).

In vivo protein phosphorylation. Samples (3 ml) of cultures in the late logarithmic growth phase were pulse-labeled for 5 min at 37°C with 100 to 150 μCi of $^{32}P_i$. The labeled cells were harvested by centrifugation at 12,000 × *g* for 2 min at 4°C, and the external label was removed by repeated washing with 0.05 M Tris-maleate buffer (pH 7.2) containing 5 mM $MgCl_2$. The cells from each 3-ml sample were suspended in 0.1 ml of Tris-maleate–$MgCl_2$ buffer and incubated for 10 min at 37°C with 0.5 mM unlabeled TMG. The cells were then exposed for 3 min at 37°C to glucose or the appropriate additions as described in the text, and the expulsion was terminated by lysing the cells with vigorous vortexing in 2% sodium dodecyl sulfate (SDS). In later experiments loading with unlabeled TMG was omitted. The samples were heated at 95°C for 5 min in sample buffer which contained the following (fivefold concentrated): 120 mM Tris-hydrochloride (pH 6.8), 2% β-mercaptoethanol, 6% SDS, 50% glycerol, and 0.02% bromophenol blue. The SDS-insoluble debris was removed by centrifugation, and the soluble fractions were subjected to SDS-polyacrylamide slab gel electrophoresis, using 15% polyacrylamide gels (1). To examine RNase and pronase sensitivity, the solubilized samples were incubated for 12 h at 30°C with pancreatic RNase I (100 μg/ml), and a part of the RNase-treated extract was further incubated with pronase (100 μg/ml) for 6 h at 30°C. After electrophoresis the gels were stained with Coomassie brilliant blue (0.25% in a mixture of 50% methanol and 10% acetic acid), destained, and dried. The positions of phosphoproteins on the gels were located by means of autoradiography.

Glucose determination. Samples (1 ml) were withdrawn at intervals from cultures during an expulsion experiment and filtered, and the filtrates were immediately frozen. The glucose contents of the filtrates were determined colorimetrically, using the glucose oxidase reagent kit (Sigma Chemical Co., St. Louis, Mo.).

Chemicals. Radioactive sugars and $^{32}P_i$ were obtained from New England Nuclear Corp., Boston, Mass. Nonradioactive amino acids and sugars were purchased from Nutritional Biochemicals Corp., Cleveland, Ohio, or from Sigma Chemical Corp. All chemicals used were of analytical grade from standard sources.

RESULTS

Effect of a nonglycolytic energy source on TMG expulsion. Although the addition of glucose to *S.*

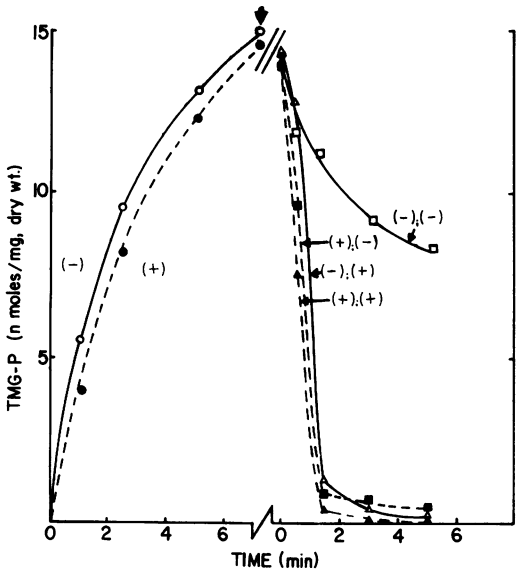

FIG. 1. Stimulation by arginine of TMG expulsion from *S. pyogenes*. Cells were allowed to take up [^{14}C]TMG in the absence (○; solid line) or presence (●; dashed line) of 2 mM arginine. At 7 min (arrow) the preloaded cultures were chilled rapidly by shaking in an ice-water bath, and each culture was divided in half. The cells were collected by centrifugation in the cold, the supernatant fluid was removed, and the cell pellets were suspended in the original volume of prewarmed (37°C) buffered medium without (squares) or with (triangles) 2 mM arginine. The expulsion was elicited by addition of 5 mM glucose. The presence or absence of arginine during preloading or efflux is denoted by the sign (+) or (−).

pyogenes cells preloaded with TMG elicited expulsion of the sugar, a prominent stimulation of the expulsion was observed when the preloaded cells were also provided with a nonglycolytic source of ATP such as arginine (Fig. 1). Arginine stimulated the expulsion when it was present during the preloading period or when it was added together with glucose to cells previously loaded with TMG. Stimulation of expulsion was similarly observed when peptone (1%) replaced arginine (data not shown). The requirement for energy expenditure during expulsion was further indicated by the fact that glucose did not elicit TMG expulsion in the presence of NaF (15 mM) or iodoacetate (5 mM), but addition of arginine (2 mM) instantaneously restored the expulsion (Fig. 2). Arginine similarly restored the expulsion from cultures of *S. lactis* ML_3 preloaded with TMG-P and poisoned with fluoride (data not shown). Restoration of the expulsion by arginine was also observed with cultures exposed to higher fluoride concentrations (30 to 50 mM) and suspended at a pH ≥ 5.5. An acidic pH has been reported to promote fluoride accumulation (5, 24). Replacement of arginine with the non-metabolizable amino acid α-aminoisobutyric acid or with glutamate, alanine, phenylalanine, citrulline, or ornithine did not promote release of preaccumulated TMG-P. Similarly, arginine alone, in the absence of glucose, did not evoke expulsion of this β-galactoside (data not shown). The efficacy of arginine in restoring expulsion in fluoride-treated cells was severely inhibited by structural analogs of arginine and ornithine: only 50% of the intracellular TMG-P was expelled in 90 s in the presence of canavanine or norvaline (10 mM) as compared with approximately 90% in their absence. Canavanine and norvaline inhibited restoration of the expulsion rather than the expulsion per se, since their addition to cultures preloaded with arginine and unexposed to fluoride did not significantly affect TMG release.

Since intracellular dephosphorylation of TMG-P precedes the release of free TMG (see below) and because arginine restores this fluoride-inhibited reaction, we suggest that energy is required for activation of the dephosphorylation mechanism. These findings also suggest that

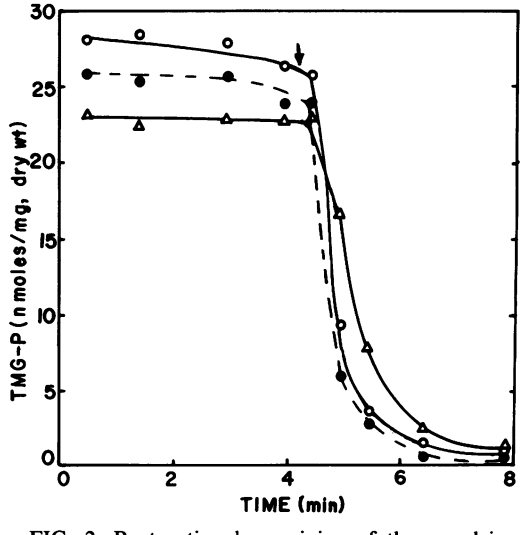

FIG. 2. Restoration by arginine of the expulsion from NaF- or iodoacetate-treated *S. pyogenes*. Cells preloaded with [^{14}C]TMG-P were collected by centrifugation, suspended in buffered medium containing 15 mM NaF (○) or 5 mM iodoacetate (dashed line; ●), and preincubated for 10 min at 37°C before glucose (5 mM) addition at time zero. At 4.25 min (arrow) arginine (2 mM) was added to the poisoned cells. A control culture (△) not treated with the glycolytic inhibitors or arginine during expulsion was exposed only to glucose (5 mM) at 4.25 min.

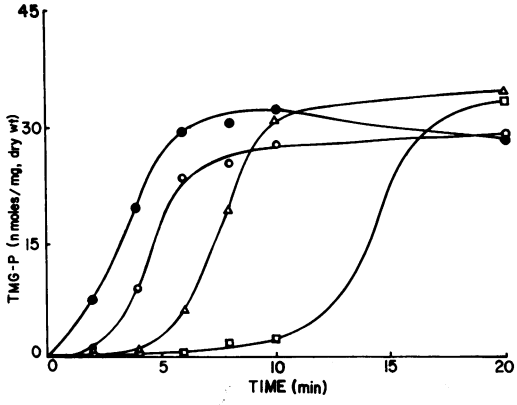

FIG. 3. Inhibition of TMG uptake by glucose. Uptake of [^{14}C]TMG by *S. pyogenes* was determined in the absence (●) or presence of 1 (○), 2 (△), or 4 (□) mM glucose.

fluoride prevents the expulsion by inhibiting energy production rather than the dephosphorylation reaction per se.

Inhibition of TMG uptake by glucose and NaF. Uptake of TMG by *S. pyogenes* was inhibited by glucose (Fig. 3). Inhibition possibly results from both competition for phospho-HPr and inducer expulsion (4, 11, 12, 22; S. S. Dills, personal communication). The duration of inhibition was directly proportional to the initial concentration of glucose in the medium: about 2.5 min per 1 mM glucose added. After exhaustion of glucose from the medium, TMG accumulation occurred at a rate similar to that observed before glucose addition. Expelled TMG was similarly reaccumulated when the expulsion was promoted by glucose (Fig. 4), mannose, or glucosamine (data not shown). However, this reaccumulation of TMG was completely prevented, despite the presence of arginine, when glucose utilization was inhibited by fluoride (see Fig. 4). These observations suggest that the expulsion process in *S. pyogenes* does not require completion of glycolytic metabolism if an independent ATP source is available. They imply that ATP rather than phosphoenolpyruvate provides the energy required for expulsion. Thus, inhibition of enolase by fluoride prevents phosphoenolpyruvate synthesis (as is evident from the inhibition of glucose utilization and TMG reaccumulation) but does not affect the expulsion when nonglycolytic ATP is provided via the arginine deiminase pathway (3, 19).

Stoichiometry of TMG expulsion to glucose uptake. Our previous results indirectly suggested that expulsion in *S. pyogenes* is not due to an exchange vectorial transphosphorylation mechanism (12). If a mechanism similar to exchange vectorial transphosphorylation (16, 17) is responsible for the expulsion of TMG-P, a stoichiometry of 1:1 would be expected between the TMG expelled and the sugar taken up. Quantitation revealed that about fivefold more [^{14}C]TMG was displaced from the cells than [^{3}H]glucose taken up (data not shown, but see also Fig. 4). Moreover, the ratio of TMG released to glucose accumulated was inconstant during expulsion of the β-galactoside. These results show that vectorial transphosphorylation is not primarily responsible for TMG expulsion in *S. pyogenes*.

Evidence for intracellular dephosphorylation of TMG-P during expulsion. Two distinct mechanisms may account for the expulsion of TMG-P from *S. pyogenes*: (i) a two-step mechanism consisting of intracellular TMG-P dephosphorylation followed by efflux of the free β-galactoside; or (ii) a single membranous reaction in which dephosphorylation of TMG-P occurs simultaneously with efflux of the free analog. To distinguish between these possibilities, we elicited the expulsion of [^{14}C]TMG-P after preincubation of the cells with NaF (10 mM) together with arginine (5 mM) and unlabeled TMG or isopropyl-1-thio-β-D-galactopyranoside (IPTG). *S. pyogenes* takes up IPTG via the lactose-PTS, and addition of glucose to preloaded cells elicits rapid expulsion of IPTG from the preaccumulated IPTG-P pool, as was observed for TMG (J. Reizer and M. H. Saier Jr., unpublished data). If mechanism i is responsible for expulsion, unla-

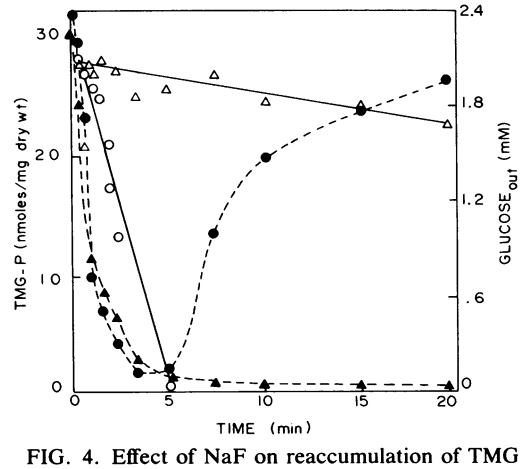

FIG. 4. Effect of NaF on reaccumulation of TMG after its expulsion from preloaded *S. pyogenes*. Expulsion of preaccumulated [^{14}C]TMG-P from *S. pyogenes* was elicited by 2 mM glucose alone (circles) or by addition of glucose (2 mM) to cells preincubated at 37°C for 5 min with 15 mM NaF and 4 mM arginine (triangles). The intracellular content of [^{14}C]TMG-P (dashed lines) and the extracellular concentration of glucose (solid lines) were determined as described in the text.

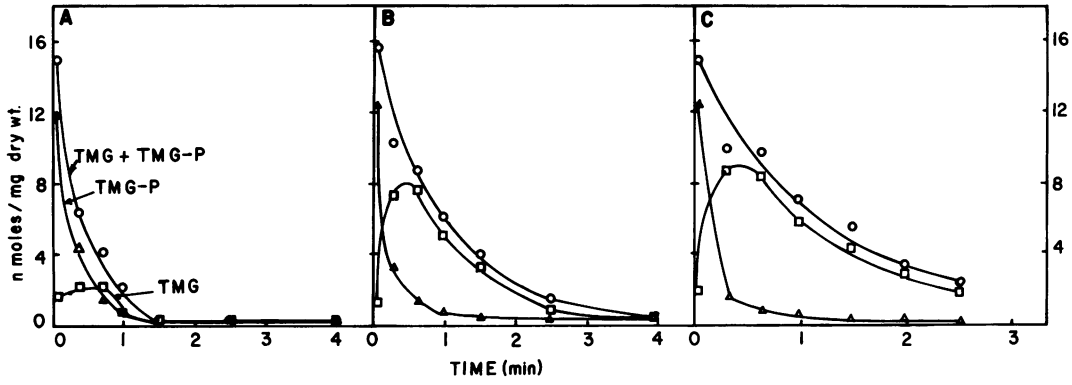

FIG. 5. Intracellular content of TMG, TMG-P, and TMG plus TMG-P during expulsion. *S. pyogenes* cells were preloaded with [^{14}C]TMG-P for 7.5 min at 37°C and collected by centrifugation. The cell pellets were suspended in buffered medium containing (A) NaF (10 mM) and arginine (5 mM); (B) NaF (10 mM), arginine (5 mM), and unlabeled TMG (100 mM); or (C) NaF (10 mM), arginine (5 mM), and IPTG (100 mM). After preincubation (5 min) at 37°C expulsion was elicited by addition of 10 mM glucose. Duplicate samples were removed at the indicated intervals for determination of (○) total intracellular radioactivity, (□) free TMG, and (△) TMG-P (see text).

beled intracellular galactosides should competitively inhibit the efflux of free intracellular [^{14}C]TMG, resulting in the transient accumulation of this labeled analog in the cells. On the other hand, if mechanism ii is responsible for expulsion, little or no inhibition by the unlabeled analog would be expected. Expulsion of [^{14}C]TMG was strongly inhibited, and virtually all of the labeled intracellular galactoside was free TMG (Fig. 5B and 5C). Of particular significance was the observation that only the efflux of [^{14}C]TMG was inhibited by the unlabeled free analogs whereas the dephosphorylation of intracellular [^{14}C]TMG-P was unaffected (cf. Fig. 5A with 5B and C). These results are consistent with the two-step mechanism. Since glucose alone, without arginine, did not elicit dephosphorylation of TMG-P in fluoride-poisoned cultures, these findings also suggest that arginine-promoted expulsion in the presence of fluoride is due to restoration of intracellular ATP levels and that ATP activates a sugar-phosphate phosphatase. Furthermore, the inhibition of [^{14}C]TMG efflux exerted by its structural analogs suggests that a transport protein, possibly enzyme II^{lac} (see reference 14), facilitates the efflux of TMG after its intracellular dephosphorylation.

Effect of arsenate on expulsion of TMG-P. Our previous results demonstrated that arsenate severely inhibits the expulsion of TMG-P (12). This observation was extended in the present study (Fig. 6). Addition of arsenate during expulsion arrested the intracellular dephosphorylation of TMG-P, in both the presence and the absence of arginine. By contrast, arsenate did not significantly affect the efflux of free TMG already present in the cells due to the dephosphorylation of TMG-P before arsenate addition. Similar results were obtained when arsenate was added to the cells at different times during the expulsion process. These findings support the conclusion that expulsion occurs in two steps which can be manipulated independently and

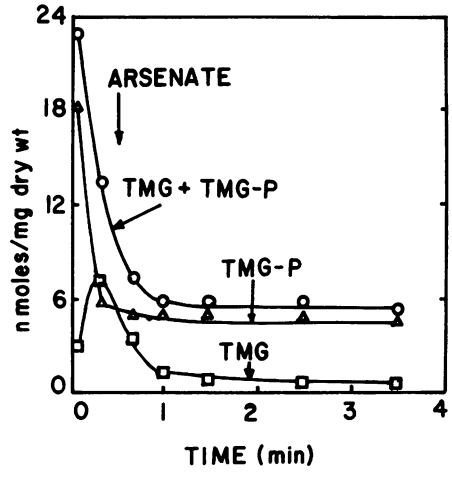

FIG. 6. Effect of arsenate on expulsion of TMG from TMG-P-loaded cells of *S. pyogenes*. *S. pyogenes* cells, preloaded with [^{14}C]TMG-P, were collected by centrifugation, and the cells were suspended in prewarmed (37°C) buffered medium. Expulsion was elicited by addition of glucose (5 mM), and at 30 s (arrow) arsenate (10 mM) was added. Duplicate samples were taken for determination (see text) of (○) total content of intracellular label, (□) free TMG, and (△) TMG-P.

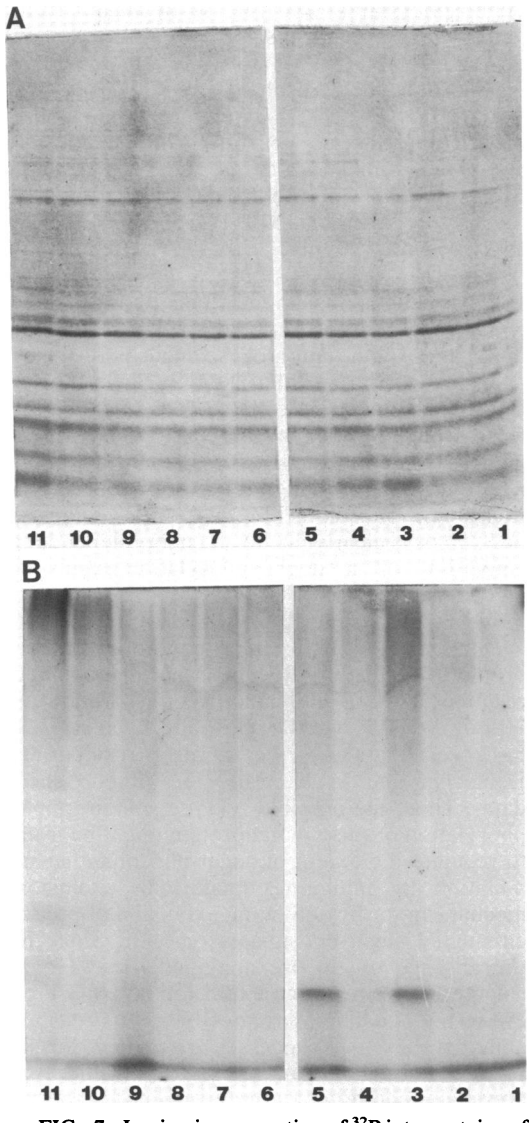

FIG. 7. In vivo incorporation of ^{32}P into proteins of *S. pyogenes*. An *S. pyogenes* culture was labeled with ^{32}P, and samples were exposed to the indicated additions for 3 min. Proteins from the soluble fraction were analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography as described in the text. (A) Coomassie blue-stained cell extracts. (B) Autoradiogram of (A). Lane 1, No addition; lane 2, arginine (2 mM); lane 3, glucose (10 mM); lane 4, glucose (10 mM) plus NaF (15 mM); lane 5, glucose (10 mM) plus NaF (15 mM) plus arginine (2 mM); lane 6, galactose (10 mM); lane 7, galactose (10 mM) plus arginine (2 mM); lane 8, 2-deoxyglucose (10 mM); lane 9, 2-deoxyglucose (10 mM) plus arginine (2 mM); lane 10, arsenate (10 mM) added 1.5 min after exposure of the cells to glucose (10 mM); lane 11, reaction terminated 45 min after glucose (5 mM) addition. The approximate molecular weight of the labeled 10,000-dalton protein was estimated by comparison with the mobility of the indicated molecular-weight standards: insulin (3,200), bovine trypsin inhibitor (6,200), cytochrome *c* (12,300), lysozyme (14,300), β-lactoglobulin (18,400), α-chymotrypsinogen (25,700), and ovalbumin (43,000). The variable labeling in the high-molecular-weight region of the gel is not understood, but does not correlate with the conditions of expulsion and is therefore presumed to be unrelated.

suggest that energy is required for dephosphorylation. The possibility that arsenate directly inhibits the dephosphorylation per se rather than its energy-dependent activation cannot be eliminated.

Protein phosphorylation during expulsion. The apparent ATP requirement for the activation of TMG-P hydrolysis prompted us to compare the profile of ^{32}P-labeled proteins in cells performing the expulsion with the labeled protein profile of a control culture. For this purpose cells were exposed to $^{32}P_i$ with or without unlabeled TMG, the designated additions were then made to elicit expulsion, and the pattern of protein phosphorylation in extracts of these cultures was examined by SDS-polyacrylamide gel electrophoresis and autoradiography. A prominent labeled protein with approximate molecular weight of 10,000 distinguished those cultures which performed the expulsion from those which were unable to expel TMG-P (Fig. 7). Thus, incorporation of ^{32}P into this pronase-sensitive and RNase-resistant band occurred only in the presence of glucose (lane 3) or in its presence together with arginine in fluoride-poisoned cells (lane 5). In contrast, the phosphorylation of this protein was not observed in cultures unexposed to glucose (lane 1) or treated with glucose together with fluoride (lane 4). Moreover, arginine alone (lane 2) or 2-deoxyglucose plus arginine (lane 9) did not induce phosphorylation of the 10,000-dalton protein in the absence of glucose. Similar results were obtained when methyl-α-glucoside or 5-thioglucose replaced 2-deoxyglucose (data not shown). The correlation between expulsion and phosphorylation of the 10,000-dalton protein was further strengthened by the observation that galactose with or without arginine did not elicit expulsion of TMG-P (unpublished data) or phosphorylation of this protein (lanes 6 and 7). Phosphorylation of the protein was independent of de novo protein synthesis since it occurred 30 to 60 s after addition of glucose or in cells treated with chloramphenicol (150 μg/ml; 30 min at 37°C) before exposure to glucose (data not shown). These observations suggest that in vivo phosphorylation of the 10,000-dalton protein occurs independently of an intact glycolytic pathway but is modulated by the concentration of a fluctuating glycolytic intermediate.

Since arsenate inhibits dephosphorylation of TMG-P it was interesting to examine the effect

of this inhibitor on the in vivo phosphorylation of the 10,000-dalton protein. Addition of arsenate (10 mM) to ^{32}P-labeled cells previously exposed to glucose resulted in dephosphorylation of this protein (Fig. 7, lane 10). This result suggests that phosphorylation of the 10,000-dalton protein is a reversible process possibly due to the action of a protein-phosphate phosphatase. Similarly, exhaustion of glucose from the medium correlated with dephosphorylation of the 10,000-dalton phosphoprotein (lane 11).

Effect of ionophores and ATPase inhibitors on expulsion. The restoration by arginine of expulsion from fluoride-poisoned cells and the expulsion activity in the absence of fluoride were examined in the presence of various ion conductors and ATPase inhibitors. Neither the expulsion nor the restoration of efflux was blocked by *N,N*-dicyclohexylcarbodiimide (0.5 mM), an inhibitor of the proton-translocating ATPase, or by sodium azide (20 mM). Similarly, the proton-conducting uncouplers tetrachlorosalicylanilide (25 μM), carbonyl cyanide *m*-chlorophenylhydrazone (25 μM), and 2,4-dinitrophenol (0.5 mM) did not inhibit the expulsion from cells treated with fluoride and arginine. Even the combination tetrachlorosalicylanilide–*N,N*-dicyclohexylcarbodiimide or carbonyl cyanide *m*-chlorophenylhydrazone–*N,N*-dicyclohexylcarbodiimide did not prevent TMG expulsion. Gramicidin (3 μg/ml), which renders the cytoplasmic membrane permeable to K^+, Na^+, and H^+, and nigericin (5 μM), which catalyzes an electroneutral exchange of protons for alkali cations, also did not inhibit expulsion. Similarly, exposure of preloaded cells to these ionophores at an acidic pH (6.5) did not significantly affect the extent of TMG displacement. Control experiments demonstrated that these ionophores and ATPase inhibitors severely inhibited the accumulation of α-aminoisobutyric acid in *S. pyogenes* (13). These findings suggest that an electrochemical gradient of protons is not required for dephosphorylation of TMG-P or extrusion of intracellular TMG.

DISCUSSION

The ability of bacteria to survive and to maintain their selective advantage in a frequently changing environment depends on their ability to react rapidly to these environmental changes. Such reactions may govern the rates of intracellular metabolism by modulating the intracellular levels of nutrients and end products of metabolism (15). The exclusion of external β-galactosides and the expulsion of intracellular β-galactoside phosphates represent two control mechanisms by which streptococci regulate sugar accumulation and metabolism. Intracellular dephosphorylation and release of sugars from various microorganisms have been previously described (6–8, 20, 21). However, the expulsion of β-galactoside phosphate from *S. pyogenes* and *S. lactis* (12, 22) is unique because the process responds to structurally unrelated sugars (glucose, mannose, glucosamine, and maltose) and because of its extremely rapid rate. The release of 2-deoxyglucose-P from *S. lactis* is approximately 10-fold slower (21). Accumulation of 2-deoxyglucose-P by *S. lactis* represents a dynamic equilibrium between phosphoenolpyruvate-dependent uptake, energy-independent dephosphorylation of the sugar phosphate, and leak of intracellular 2-deoxyglucose (21). Thus, the stimulation of [^{14}C]-deoxyglucose release from *S. lactis* by addition of unlabeled glucose, 2-deoxyglucose, or iodoacetate may result largely from inhibition of uptake. In contrast, the expulsion of TMG-P from *S. pyogenes* or *S. lactis* is clearly due to an energy-dependent activation of a dephosphorylation-expulsion mechanism.

Our results demonstrate phosphorylation of a single major protein under conditions which promote expulsion. Recent experiments have resulted in the identification of this protein as HPr of the PTS, and the phosphorylated residue has been shown to be a seryl residue (J. Deutscher and M. H. Saier, Jr., unpublished data). Thus, the possible ATP-dependent phosphorylation of this pivotal protein might be used to regulate the extent of sugar-phosphate accumulation by inhibiting uptake (an exclusion mechanism) or by activating expulsion of preaccumulated sugar-phosphates or both. Since in the absence of glucose nonglycolytic ATP or galactose does not elicit expulsion of TMG-P or evoke the phosphorylation of HPr, it is reasonable that these two processes are related. Possibly a glycolytic intermediate is responsible. Accordingly, addition of a preferred energy source such as glucose may alter the intracellular concentration of a critical metabolite which provides a signal for ATP-dependent phosphorylation of pivotal proteins, and the latter phosphoproteins may affect the dephosphorylation of TMG-P. Such a control mechanism, in accordance with that suggested for protein phosphorylation in *Salmonella typhimurium* (23), allows the rapid response required for efficient inducer expulsion or exclusion and may prevent futile energy consumption.

ACKNOWLEDGMENTS

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