JOURNAL OF BACTERIOLOGY, Oct. 1981, p. 257-264
0021-9193/81/100257-08$02.00/0

Vol. 148, No. 1

Isolation of III^{Glc} of the Phosphoenolpyruvate-Dependent Glucose Phosphotransferase System of *Salmonella typhimurium*

B. J. SCHOLTE, A. R. SCHUITEMA, AND P. W. POSTMA*

Laboratory of Biochemistry, B. C. P. Jansen Institute, University of Amsterdam, 1018 TV Amsterdam, The Netherlands

Received 7 April 1981/Accepted 15 June 1981

We report a procedure for the isolation of III^{Glc} of *Salmonella typhimurium*, a protein component of the phosphoenolpyruvate-dependent sugar phosphotransferase system. III^{Glc} is a soluble protein with a molecular weight of 21,000, as determined by gel filtration and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified protein is involved in the phosphoenolpyruvate-dependent phosphorylation of methyl α-glucoside in vitro. Its affinity for octyl-Sepharose may be an indication of the partial hydrophobic nature of III^{Glc}. A specific antiserum against purified III^{Glc} was prepared. Growth on different carbon sources did not affect the synthesis of III^{Glc}, as determined by quantitative immunoelectrophoresis. Mutations which lower the adenosine 3',5'-phosphate level, such as *cya* and *pts*, do not alter the III^{Glc} level. The closely related enteric bacteria *Escherichia coli* and *Klebsiella aerogenes* contain a protein factor which is closely related to III^{Glc} of *S. typhimurium*, whereas *Staphylococcus aureus* does not.

The enteric bacteria *Escherichia coli*, *Salmonella typhimurium*, and *Klebsiella aerogenes* contain a phosphoenolpyruvate-dependent sugar phosphotransferase system (PTS) which is active in the translocation and concomitant phosphorylation of several hexoses (5, 9, 22). A schematic representation of the PTS is given in Fig. 1. Transport of all substrates of the PTS is dependent upon the activity of the general PTS components enzyme I and HPr. Sugar specificity resides in the different membrane-bound enzymes II, some of which are inducible, whereas others are constitutive (24, 31). The glucose-specific membrane-bound enzyme IIB^{Glc} requires the presence of an additional soluble protein factor, designated III^{Glc}, for activity (4, 14, 16).

The PTS is thought to be involved in the regulation of cell metabolism through its capability to modulate the activity of adenylate cyclase (2, 3, 7, 8, 21, 26) and several nonrelated transport systems (12, 13, 28, 29). A mechanism has been proposed in which changes in the degree of phosphorylation of a PTS component directly affect the enzymatic activities mentioned above (Fig. 1). Changes in the metabolic state of the cell, i.e., the addition of a PTS substrate or manipulation of the phosphoenolpyruvate-to-pyruvate ratio, tend to affect the degree of phosphorylation of PTS components (27, 30). This, in turn, is proposed to affect the interaction of a hypothetical regulatory protein with adenylate cyclase and several transport proteins. The regulatory protein is thought to be a phosphoprotein in equilibrium with the PTS, and its regulatory activity would reflect changes in the degree of phosphorylation of the PTS. A good candidate for the role of regulatory protein is III^{Glc}, as *crr* mutations which abolish PTS-mediated regulation also affect the activity of III^{Glc} (4, 11, 13, 23, 28). However, this correlation cannot be considered proof for the proposed mechanism. Further study on the nature of *crr* mutations and on the interaction of III^{Glc} with other proteins is required, and more information about the properties of III^{Glc} in vitro and in vivo is needed. Furthermore, a specific antibody against III^{Glc} will be very helpful both in the characterization of mutants with altered regulation and as a probe for III^{Glc} in binding studies. We report here the isolation of III^{Glc} of *S. typhimurium* and the preparation of a specific antiserum against III^{Glc}.

MATERIALS AND METHODS

Isolation of factor III^{Glc}. Wild-type cells strain SB3507 (*trpB223*) were grown on minimal salts medium (30), supplied with 20 μg of tryptophan per ml and 0.4% (wt/vol) glucose. The cells were grown in 10-liter batches in a fermentor (Microferm; New Brunswick Scientific Co., New Brunswick, N.J.) at 37°C.

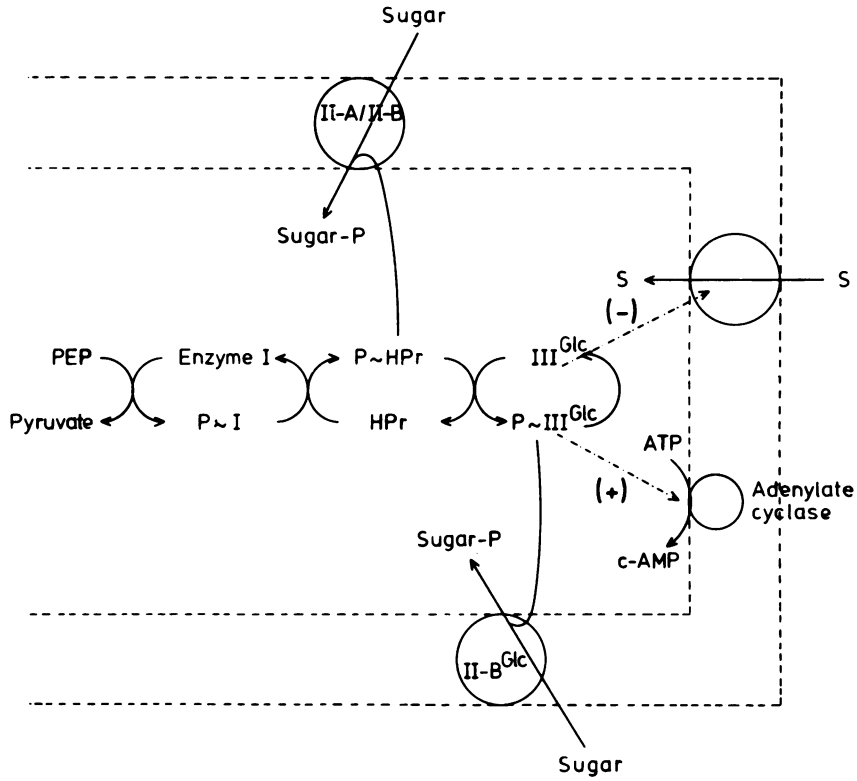

FIG. 1. *Phosphenolpyruvate (PEP)-dependent sugar phosphotransferase system. Inducer exclusion is indicated by the inhibition (−) of non-PTS transport systems (S represents maltose, melibiose, or glycerol) by non-phosphorylated III^{Glc}. Activation (+) of adenylate cyclose by phosphorylated III^{Glc} is also shown.*

Aeration was 10 to 12 liters/min, and agitation was 300 rpm. Cells were harvested by centrifugation during the late-logarithmic phase (optical density at 600 nm = 2) and stored at −70°C. All further steps were performed at 4°C unless stated otherwise. Cells obtained from a 10-liter culture (approximately 50 g [wet weight]) were suspended in 250 ml of a buffer containing 10 mM Tris-hydrochloride, 1 mM EDTA, 1 mM sodium azide, and 1 mM dithiothreitol, at a final pH of 7.5 (buffer B). Phenylmethylsulfonyl fluoride was added to a final concentration of 0.5 mM. The cells were broken in a French pressure cell at 9,000 lbs/in^2. Whole cells and debris were removed by centrifugation for 10 min at 10,000 × *g*. The resultant crude extract was centrifuged at 100,000 × *g* for 90 min. The membrane pellet was washed twice with buffer B and suspended in 25 ml of a buffer containing 50 mM potassium phosphate, 1 mM EDTA, and 1 mM dithiothreitol, pH 7.5. This membrane preparation was used as a source of IIB^{Glc}. The combined 100,000 × *g* supernatants were stirred for 30 min with 500 g of DEAE-cellulose (DE-52; Whatman Inc., Clifton, N.J.), equilibrated with buffer B. For large-scale isolations, it was convenient to elute the ion-exchange resin batchwise in a large Büchner funnel with 2 liters of buffer B, 2 liters of buffer B plus 50 mM KCl, 2 liters of buffer B plus 100 mM KCl, and 3 liters of buffer B plus 200 mM KCl. III^{Glc} was found mainly in the 200 mM KCl fraction and generally also in the 100 mM KCl fraction. HPr was found in the 50 mM KCl fraction. The active fractions were concentrated to 50 ml by ultrafiltration (PM10 filter; Amicon Corp., Lexington, Mass.) and applied to a 700-ml Sephadex G-75 column with a diameter of 2.5 cm, equilibrated with buffer B. After elution, the active fractions were pooled and concentrated by ultrafiltration to approximately 20 ml, after which ammonium sulfate was added to 200 mM. The sample was added to an octyl-Sepharose column equilibrated with buffer B plus 200 mM $(NH_4)_2SO_4$. No more than about 1 mg of protein per ml of Sepharose slurry should be used, because overloading results in bad separation. The flow rate was high enough to prevent peak broadening and loss of activity on the column (we used routinely a 125-ml column with a 3-cm diameter which was eluted at a flow rate of 0.6 ml·min^{-1}·cm^{-2}). The column was eluted with a linear 200 to 0 mM ammonium sulfate gradient in buffer B of at least 10 column volumes. The active fractions were pooled and lyophilized, suspended in water, and dialyzed against buffer B. At this stage, the protein was more than 90% pure as judged from sodium dodecyl sulfate (SDS)-polyacrylamide slab gel electrophoresis, which was performed essentially by the method of Laemmli (17). The samples were boiled for 10 min in the presence of 2% SDS and 2% mercaptoethanol at pH 6.8.

Measurement of III^{Glc} activity. III^{Glc} activity was determined by the method of Waygood et al. (31) by measuring the rate of methyl α-[^{14}C]glucoside phosphorylation. The membrane preparation obtained after the 100,000 × *g* centrifugation step as described above was used as a source of IIB^{Glc}. HPr was partially purified from the 50 mM KCl fraction of the DE-52 column described above by gel filtration on a 700-ml Sephadex G-75 column. Enzyme I was partially purified on octyl-Sepharose by the method of Robillard et al. (25) from *E. coli* (strain K235). The HPr and the enzyme I preparations contained 0.3 and 0.5 U/ml (1 U = 1 μmol·min^{-1}), respectively, with mannose used as a substrate. Routinely, the incubations (100 μl, 37°C, 30 min) contained 10 μl of enzyme I, 10 μl of HPr, and 10 μl of the membrane suspension, 1 mM methyl α-[^{14}C]glucoside (200 cpm·nmol^{-1}) and various amounts of sample. III^{Glc} activity is defined as the difference between incubations with and without added sample and expressed as micromoles per milligram of protein per minute. Care was taken to ensure that the activity increased linearly with the amount of added sample. Protein was determined by the method of Lowry et al. (20).

Preparation of a rabbit anti-III^{Glc} serum. To prepare a specific antiserum, III^{Glc} was further purified after the octyl-Sepharose column by preparative SDS-polyacrylamide gel electrophoresis. The gel system used was described by Laemmli (17). The gel strip containing III^{Glc} was cut and eluted with water at room temperature. After lyophilization, the sample was suspended in and dialyzed against water. Antiserum against pure III^{Glc} was obtained by injecting rabbits with 100 μg of protein in Freund complete adjuvant (Difco Laboratories, Detroit, Mich.). A second injection in Freund incomplete adjuvant was given 6 weeks after the first injection. The rabbits were bled 5 and 9 days after the second injection, and the blood was left to clot for 1 h at room temperature. After storage at 4°C overnight, aggregated material was removed by centrifugation, and the resultant supernatant was used as the antiserum.

Immunochemical procedures. To determine III^{Glc} in intact cells, the cells were treated with toluene and chloroform as described by Lengeler et al. (19).

Rocket immunoelectrophoresis was performed as described by Axelsen et al. (1), using pure III^{Glc} as a standard. Electrophoresis was performed overnight at 16°C; the voltage applied was 4 V/cm. Crossed immunoelectrophoresis was performed as described by Laurell (18). Voltage applied in the first dimension was 5 V/cm for 2 h; voltage applied in the second dimension was 4 V/cm overnight.

Double-diffusion immunoprecipitation was performed as described by Lengeler et al. (19). The gel system routinely used consisted of 1.5-mm agarose plates containing 38 mM Tris, 100 mM glycine (pH 8.7), and 1% Triton X-100. For rocket electrophoresis and crossed immunoelectrophoresis, 1% antiserum was added. The precipitates were pressed and stained as described by Axelsen et al. (1).

Chemicals. Methyl α-[^{14}C]glucoside (184 Ci/mol) was obtained from Amersham Corp. (Arlington Heights, Ill.). Dithiothreitol was obtained from Sigma Chemical Co. (St. Louis, Mo.). Octyl-Sepharose CL-4B was obtained from Pharmacia Fine Chemicals, Inc. (Piscataway, N.J.). Agarose was from Merck Sharp & Dohme (West Point, Pa.) (for electrophoresis).

Bacterial strains. The following strains of *S. typhimurium* were used: SB3507, *trpB223*; TT2104, *cya-961 argI539 proAB47 amtA1 Δtrp-130 zid-62*::Tn*10* (obtained from J. Roth, University of Utah); PP642, Δ(*cysK-ptsHI*)*41*; SB2950, Δ (*cysK-ptsHI-crr*)*49* (4); *E. coli* L146 (obtained from J. Lengeler, University of Regensburg,); *K. aerogenes* NCTC 418 and *S. aureus* were obtained from D. Tempest (University of Amsterdam).

RESULTS

Isolation of factor III^{Glc}. Wild-type cells were grown to the late exponential phase on minimal salts medium containing glucose. After the cells were harvested, they were ruptured in a French pressure cell, and the membranes were removed by centrifugation as described above. The membrane pellet contained about 15% of the total III^{Glc} activity after washing (Table 1). III^{Glc} could be isolated from the 100,000 × *g* supernatant by use of DEAE-cellulose (Whatman DE-52). When a sample of supernatant was eluted from a DEAE cellulose column with a KCl gradient, the III^{Glc} activity eluted as one peak at 170 mM KCl. For handling large amounts of supernatants, it was more convenient to elute the DE-52 batchwise. Activity was usually found in two fractions with this procedure (Table 1). The ultrafiltration step can cause considerable loss of activity. This will result in variable specific activities of partially purified preparations. The next step was gel filtration of the concentrated active fraction on Sephadex G-75. III^{Glc} eluted as one peak corresponding to a molecular weight of 21,000 (column volume, 700 ml; elution volume of III^{Glc}, 300 ml). At this point, the protein was purified about 30-fold, and the recovery was about 50% of the total activity.

For further purification, we found that chromatography on an octyl-Sepharose column was

TABLE 1. *Isolation of III^{Glc}* [a]

Fraction	Protein (mg)	Activity[b] (U)	Sp act (U/mg^{-1})
Crude extract	3,250	22	0.007
DE-52, 100 mM KCl	120	5	0.042
DE-52, 200 mM KCl	400	11	0.023
Sephadex peak[c]	50	10	0.200

[a] Purification of III^{Glc} and phosphorylation of methyl α-[^{14}C]glucoside was measured as described in the text. The 100,000 × *g* pellet contained 3 to 4 U of activity after washing.

[b] Activities were determined after ultrafiltration.

[c] The Sephadex peak fraction was obtained after elution of the 200 mM KCl fraction as described in the text.

successful. III^{Glc} bound to octyl-Sepharose with an affinity that was increased by increasing salt concentrations (either KCl or ammonium sulfate can be used). This is a property that III^{Glc} has in common with enzyme I (25) and may reflect the fact that III^{Glc} and possibly enzyme I are active on the inner surface of the cytoplasmic membrane. Figure 2 shows the elution pattern of a sample obtained after gel filtration of a 200 mM KCl fraction, which contained 120 mg of protein and 3.5 U of III^{Glc} activity, on a 100-ml octyl-Sepharose column. The column was eluted with a negative gradient of ammonium sulfate. The active fraction contained about 4 mg of protein and 3.6 U of activity. This step resulted in a 30- to 40-fold purification, depending on the purity of the applied sample. The recovery of activity was high, generally 80 to 100%. The specific activity obtained was consistently about 0.9 U/mg of protein. The protein was over 90% pure as judged by SDS-polyacrylamide gel electrophoresis (Fig. 3); note that bovine serum albumin was added to the purified protein to minimize nonspecific binding to glassware and dialysis tubing. The mobility of III^{Glc} in the gel indicates a molecular weight of about 21,000. Because gel filtration in buffer B gave the same result, we concluded that III^{Glc} is a monomer with a molecular weight of 21,000. Phosphoenolpyruvate-dependent phosphorylation of methyl α-glucoside by purified III^{Glc} could only be measured in the presence of enzyme I, HPr, and IIB^{Glc} (membranes) (Table 2). The activity of the purified III^{Glc} can be calculated after correcting for the activity of the membranes. The membranes used in this experiment were prepared from strain PP1237 (*ptsM* Δ[*ptsHI-crr*]*49*) which contained

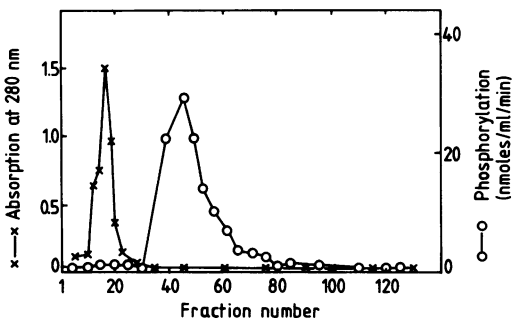

FIG. 2. *Elution of III^{Glc} from an octyl-Sepharose column. A column of octyl-Sepharose was loaded with a III^{Glc} preparation obtained from gel filtration as described in the text. The sample (18 ml, 120 mg of protein) contained 3.5 U as measured by methyl α-[^{14}C]glucoside phosphorylation. Fractions (7 ml) were collected and assayed for absorbance at 280 nm (×) and methyl α-[^{14}C]glucoside phosphorylation* (○).

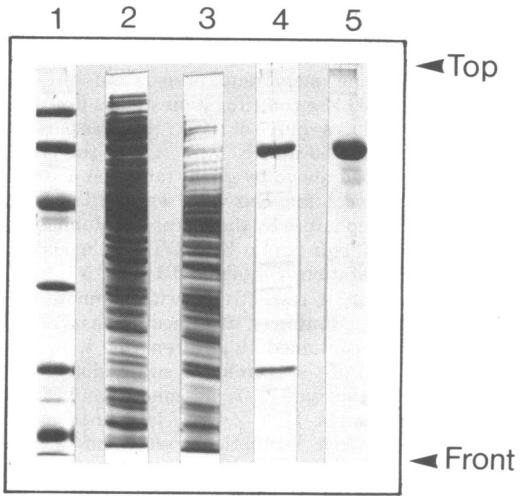

FIG. 3. *SDS-polyacrylamide gel electrophoresis of III^{Glc}. Electrophoresis was performed in a 12% polyacrylamide slab gel as described in the text. The fractions used were those described in Table 1. Lane 1, marker proteins with molecular weights of 94,000, 67,000, 43,000, 30,000, 20,100, and 14,400, respectively; lane 2, 200 mM KCl fraction, 100 μg of protein; lane 3, III^{Glc} peak fraction after gel filtration of 200 mM KCl fraction, 50 μg of protein; lane 4, III^{Glc} fraction after octyl-Sepharose chromatography, 5 μg of protein (note that bovine serum albumin was added to reduce nonspecific binding of III^{Glc}); lane 5, bovine serum albumin.*

TABLE 2. *Methyl α-[^{14}C]glucoside phosphorylation by a III^{Glc} preparation*[a]

Omission	III^{Glc} added (μl)	Phosphorylation (nmol·min^{-1})
PEP[b]	5	0
Enzyme I	5	0
HPr	5	0
Membranes	5	0
None	0	1.3
None	1	1.6
None	3	2.2
None	5	2.4

[a] Phosphorylation of methyl α-[^{14}C]glucoside was measured as described in the text. The complete reaction mixture contained 10 mM phosphoenolpyruvate, 10 μl of enzyme I, 10 μl of HPr, membranes from PP1237 (*ptsM* Δ [*ptsHI-crr*]*49*; 100 μg), and various amounts of III^{Glc} obtained after chromatography on octyl-Sepharose.

[b] PEP, Phosphoenolpyruvate.

neither precipitable III^{Glc} (Table 3) nor IIA-IIB activity (as measured with mannose). The phosphorylation activity found in these membranes appears to be due to a membrane-bound factor which is immunochemically related to III^{Glc} (unpublished data).

TABLE 3. *III^{Glc} in cells grown on different carbon sources*

Strain	Relevant genotype	Carbon source[a]	Relative peak ht[b] (%)
SB3507	Wild type	Glucose	100
		Galactose	85
		Glycerol	95
		Maltose	88
		Melibiose	76
		Lactate	80
		Nutrient broth	110
TT2104	*cya*	Nutrient broth	105
PP642	Δ(*ptsHI*)*41*	Nutrient broth	95
SB2950	Δ(*ptsHI-crr*)*49*	Nutrient broth	0

[a] Cells were grown overnight on the growth medium indicated.

[b] After cells were harvested and treated with toluene and chloroform, III^{Glc} content was measured by rocket electrophoresis as described in the text. III^{Glc} content is expressed as relative peak height after correction for differences in protein content of the samples. Cells that were grown on glucose were set at 100%.

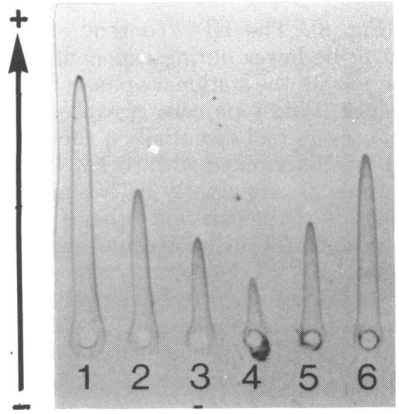

FIG. 4. *Rocket electrophoresis of III^{Glc}. Rocket electrophoresis was performed as described in the text. The gel contained 1% anti-III^{Glc} serum. Lanes 1, 2, and 3: pure III^{Glc} from octyl-Sepharose column at 200, 100, and 50 ng, respectively. Lanes 4, 5, and 6: chloroform-toluene-treated wild-type cells 1, 2, and 5 μl, respectively. The content of soluble III^{Glc} of wild-type cells as determined with this method is 2.7 μg of III^{Glc} per mg (dry weight).*

Characterization of a rabbit anti-III^{Glc} serum. With a preparation further purified by preparative SDS-polyacrylamide gel electrophoresis, we prepared an antiserum as described above. This serum was used to quantify antigenic determinants of III^{Glc}. In rocket electrophoresis and in crossed immunoelectrophoresis, both purified III^{Glc} and ruptured wild-type cells produced one continuous precipitation line. Figure 4 shows a quantitative determination of purified III^{Glc} and III^{Glc} in ruptured wild-type cells, using rocket electrophoresis. In crossed immunoelectrophoresis, a sample of the 100,000 × *g* supernatant used for the isolation of III^{Glc} showed two peaks in one continuous precipitation line (Fig. 5A). Purified III^{Glc} essentially gave the same result, though the ratios of the peaks differed (Fig. 5B). This indicates that III^{Glc} exists in multiple forms in our preparation. This is probably due to aggregation of III^{Glc} monomers, resulting in trimeric and hexameric structures (unpublished data). The fact that a crude soluble protein preparation produced only one continuous precipitation line shows that the serum is specific for III^{Glc}.

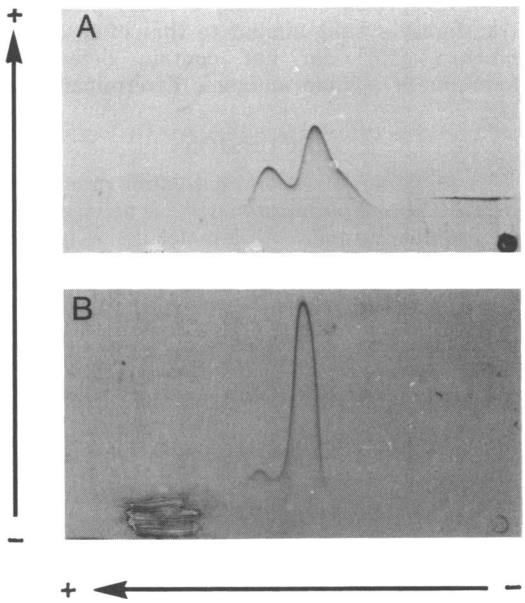

FIG. 5. *Crossed immunoelectrophoresis of III^{Glc}. Crossed immunoelectrophoresis was performed as described in the text. A, Protein (100 μg) from the 100,000 × g supernatant of wild-type cells; B, purified III^{Glc} (0.5 μg) (octyl-Sepharose preparation). The second-dimension gel contained 1% anti-III^{Glc} serum.*

Regulation of the synthesis of III^{Glc}. In connection with the possible role of III^{Glc} as a regulatory protein, it was relevant to measure the absolute amount of III^{Glc} under different growth conditions.

It has been reported (15) that in the late-logarithmic phase of growth of *E. coli* on glucose minimal medium a transient fourfold increase of III^{Glc} activity occurs. We were not able to confirm this result in *S. typhimurium*. The amount of III^{Glc} present in wild-type cells during different stages of growth on glucose minimal salts medium was determined with rocket electropho-

resis (Fig. 6). The III^{Glc} content of the cells tended to be lower during exponential growth compared with the stationary phase, but did not vary much. Wild-type cells grown on different carbon sources had essentially a constant III^{Glc} content, as determined with rocket electrophoresis (Table 3). Apparently III^{Glc} is not induced by glucose. Strains carrying either a *cya* mutation or a *ptsHI* deletion contained normal amounts of III^{Glc}. Consequently, the production of III^{Glc} does not seem to be dependent on the presence of intracellular cyclic AMP. Strain SB2950 (Δ[*ptsHI-crr*]*49*), which appears to contain a deletion of the *crr* gene (4), did not contain detectable amounts of III^{Glc} (Table 3). Strains of *E. coli*, *K. aerogenes*, and *S. aureus* were tested for the presence of antigenic determinants of III^{Glc} with a double-immunodiffusion technique (Fig. 7). It was found that both closely related strains of *E. coli* and *K. aerogenes* contained a component that cross-reacted with III^{Glc}. In fact, with our serum, no immunological difference between *S. typhimurium* and either *E. coli* or *K. aerogenes* could be detected. *S. aureus*, a gram-positive species containing a sugar phosphotransferase system similar to that of *S. typhimurium* (10), did not contain detectable amounts of soluble antigenic determinants of III^{Glc}.

DISCUSSION

In this paper, we report an isolation procedure for III^{Glc} of *S. typhimurium*. III^{Glc} is defined here as a soluble protein with a molecular weight of approximately 21,000 which is required for the phosphoenolpyruvate-dependent phosphorylation of methyl α-glucoside in vitro. The procedure makes use of the fact that III^{Glc} binds to octyl-Sepharose, which indicates that III^{Glc} has a hydrophobic site. This is a property which III^{Glc} has in common with enzyme I of the PTS and indicates a possible affinity for membranes. In view of the role of III^{Glc} in sugar transport and possibly in the regulation of several membrane proteins, this observation is not surprising. Under the isolation conditions used here, III^{Glc} acts as a soluble protein not associated with other proteins. This is indicated by the fact that (i) III^{Glc} eluted as a single peak from DE-52, Sephadex G-75, and octyl-Sepharose and (ii) the molecular weight as determined in gel filtration and SDS-polyacrylamide gel electrophoresis was approximately 21,000.

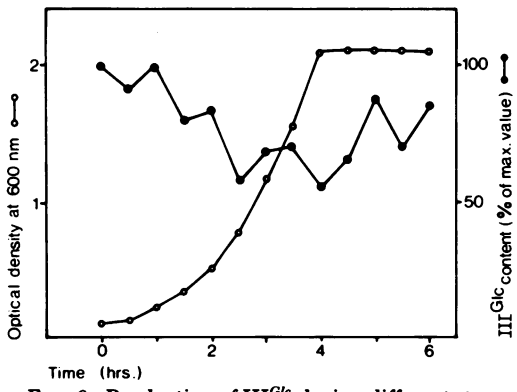

FIG. 6. *Production of III^{Glc} during different stages of growth. Wild-type cells were grown in a minimal salts medium containing 0.2% glucose as a carbon source. At different times, samples were taken and assayed for optical density (○) and III^{Glc} content of chloroform-toluene-treated samples as measured by rocket electrophoresis (●). Growth stopped at 4 h because of lack of glucose.*

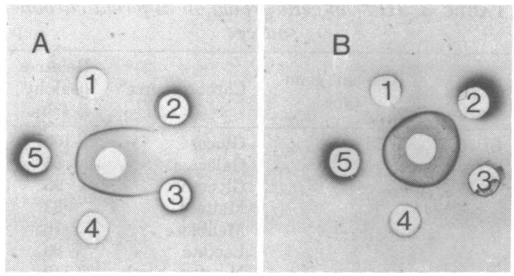

FIG. 7. *Double-diffusion immunoprecipitation. Cells of different strains were grown overnight on nutrient broth, harvested, and treated with toluene and chloroform as described in the text. Precipitation was performed during 24 h. A: 1, E. coli; 2 and 3, S. aureus; 4, E. coli; and 5, S. typhimurium. B: 1, E. coli; 2, S. typhimurium; 3, K. aerogenes; 4, E. coli; and 5, S. typhimurium. In the Center well, 5 μl of 30% anti-III^{Glc} serum.*

A previous report on the purification of III^{Glc} of *E. coli* (14, 15), claimed that III^{Glc} is a trimeric complex of subunits with a molecular weight of 7,000. In contrast to these results, the protein that we isolated did not dissociate after it was boiled for 20 min in sample buffer containing 2% SDS and 2% mercaptoethanol and had a molecular weight of 21,000. Another difference is that the preparation described by Kundig contained sugar phosphatase activity which was claimed to be associated with III^{Glc} (15). Our preparation did not show significant phosphatase activity with glucose 6-phosphate as a substrate. Phosphatase activity of a preparation obtained after octyl-Sepharose chromatography was less than 30 $nmol \cdot min^{-1} \cdot mg^{-1}$, whereas its methyl α-[^{14}C]glucoside phosphorylation activity was 850 $nmol \cdot min^{-1} \cdot mg^{-1}$. The phosphatase activity of the preparation of Kundig, however, was twice as high as its phosphorylation activity (15). In

our opinion, this is because of a contamination with a phosphatase. Our immunochemical data indicate that *E. coli* and *K. aerogenes* contain a protein component which is immunochemically closely related to III^{Glc} of *S. typhimurium* (Fig. 7). From this, we conclude that no great structural differences exist among these species with respect to III^{Glc}. Starting with highly purified III^{Glc}, we prepared a rabbit anti-III^{Glc} serum. In crossed immunoelectrophoresis, both a crude extract of wild-type cells and purified III^{Glc} produced one continuous precipitation line showing two peaks (Fig. 4). This means that (i) the serum used is specific to III^{Glc} and (ii) III^{Glc} exists in electrophoretically different forms. This electrophoretic inhomogeneity can be explained by a tendency to form multimeric strictures (unpublished data).

III^{Glc} is a constitutive protein, (Table 3, Fig. 6). Transport activity via the III^{Glc}-IIB^{Glc} pathway of the PTS can vary considerably, however, depending on the growth conditions. This can be explained by the fact that IIB^{Glc} is inducible (24, 30). The synthesis of PTS components other than III^{Glc} has been shown to be dependent on both an extracellular inducer and the level of cyclic AMP inside the cell (24). Apparently the synthesis of III^{Glc} is not regulated in the same way as that of the other PTS components, in particular, its complement in transport function, IIB^{Glc}. The reason for this may be that III^{Glc} has functions in the cell in addition to glucose transport.

All experiments concerning PTS-mediated regulation can be nicely explained by a model in which III^{Glc} acts as a regulatory protein (5, 22), although other mechanisms for the regulation of carbohydrate metabolism are not excluded (6, 32). However, no direct evidence for the regulatory function of III^{Glc} has been brought forward yet. The isolation of III^{Glc} and the preparation of a specific antiserum as reported here are the first steps toward the solution of this problem. It will make the study of the interactions of III^{Glc} with other proteins possible and facilitate the characterization of *crr* mutants, which appear to be defective in III^{Glc}.

ACKNOWLEDGMENTS

This work was supported in part by a grant from The Netherlands Foundation for Chemical Research (S.O.N.) with financial aid from the Netherlands Organization for the Advancement of Pure Research (Z.W.O.)

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