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Phosphoenolpyruvate:Glycose Phosphotransferase System in Species of *Vibrio*, a Widely Distributed Marine Bacterial Genus†

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The genus *Vibrio* is one of the most common and widely distributed groups of marine bacteria. Studies on the physiology of marine *Vibrio* species were initiated by examining 15 species for the bacterial phosphoenolpyruvate:glycose phosphotransferase system (PTS). All species tested contained a PTS analogous to the glucose-specific (II^{Glc}) system in enteric bacteria. Crude extracts of the cells showed immunological cross-reactivity with antibodies to enzyme I, HPr, and III^{Glc} from *Salmonella typhimurium* when assayed by the rocket-line method. Toluene-permeabilized cells of 11 species were tested and were active in phosphorylating methyl α-D-glucoside with phosphoenolpyruvate but not ATP as the phosphoryl donor. Membranes from 10 species were assayed, and they phosphorylated methyl α-D-glucoside when supplemented with a phospho-III^{Glc}-generating system composed of homogeneous proteins from enteric bacteria. Toluene-permeabilized cells and membranes of seven species were assayed, as were phosphorylated fructose and 2-deoxyglucose. III^{Glc} was isolated from *Vibrio fluvialis* and was active in phosphorylating methyl α-D-glucoside when supplemented with a phospho-HPr-generating system composed of homogeneous proteins from *Escherichia coli* and membranes from either *E. coli* or *V. fluvialis*. These results show that the bacterial PTS is widely distributed in the marine environment and that it is likely to have a significant role in marine bacterial physiology and in the marine ecosystem.

The phosphoenolpyruvate:glycose phosphotransferase system (PTS) is a complex system of interacting cytoplasmic and membrane proteins with diverse functions in the bacterial cell (for recent reviews, see references 29 and 36). For example, the PTS concomitantly phosphorylates and translocates its sugar substrates across the cytoplasmic membrane, is involved in chemotaxis toward these sugars, regulates the uptake of at least some non-PTS sugars, regulates adenylate cyclase, and controls the transcription of operons required for the utilization of non-PTS sugars. Through these regulatory functions, the PTS is largely responsible for the phenomenon called diauxic growth in enteric bacteria.

While the PTS is widely distributed among species of terrestrial, pathogenic, and freshwater bacteria (4, 38), there have been few studies on its distribution in marine bacteria (10, 19). Since the latter are of great importance in the marine ecosystem, and since marine bacteria offer considerable potential in elucidating both the evolutionary significance and the mechanisms of action of the PTS, we surveyed the gram-negative genus *Vibrio* for the PTS. This genus is one of the most common and widely distributed of marine bacterial genera (2, 6, 41) and is in the family *Vibrionaceae*, closely related (6) to the family *Enterobacteriaceae* which contains *Escherichia coli* and *Salmonella typhimurium* (8), the organisms that have been most extensively studied in this laboratory.

Surveys for the PTS such as those conducted in the present experiments are limited by the number of sugars used for the assays. Whether a sugar is or is not a PTS substrate (i.e., a PTS-sugar) depends on the organism. For example, almost all sugars are PTS-sugars in gram-positive organisms such as *Staphylococcus aureus*, whereas a much more limited group are PTS-sugars in the members of the family *Enterobacteriaceae* (36). Since the latter include glucose and fructose, and since these two monosaccharides are ubiquitously distributed in the marine environment (17, 34), we assayed for glucose and fructose PTS activities in *Vibrio* species.

The uptake of glucose and fructose by the enteric bacteria is effected by phosphotransferase systems that are among the most complex of those yet described. Glucose is a substrate of two phosphotransferase systems, the II^{Glc} and II^{Man} systems, which are schematically illustrated in Fig. 1. One component of the II^{Glc} system, III^{Glc}, is encoded by the *crr* gene (31, 32; D. W. Saffen, K. A. Presper, T. L. Doering, and S. Roseman, J. Biol. Chem., in press) and is critical for many of the regulatory phenomena summarized above. The II^{Man} system is the only one yet described in which the sugar-specific membrane enzyme complex consists of three proteins (47). The II^{Glc} and II^{Man} systems are assayed with the analogs methyl α-glucoside and 2-deoxyglucose, respectively (42).

Fructose is also the substrate of two PT systems, one being the II^{Man} system described above and in Fig. 1 and the other being an inducible PTS (14). The inducible fructose PT includes a protein called FPr (14, 39, 40) which substitutes for HPr (11, 43). Thus, enzyme I is the only component that the inducible fructose PTS has in common with other PT systems.

In earlier work, Kubota et al. (19) presented clear evidence for the presence of the PTS, particularly of the II^{Glc} system, in *Vibrio parahaemolyticus*, and furthermore, they made the important observation that PTS proteins from this organism complemented the corresponding proteins from *E. coli* in in vitro sugar phosphorylation assays. These workers

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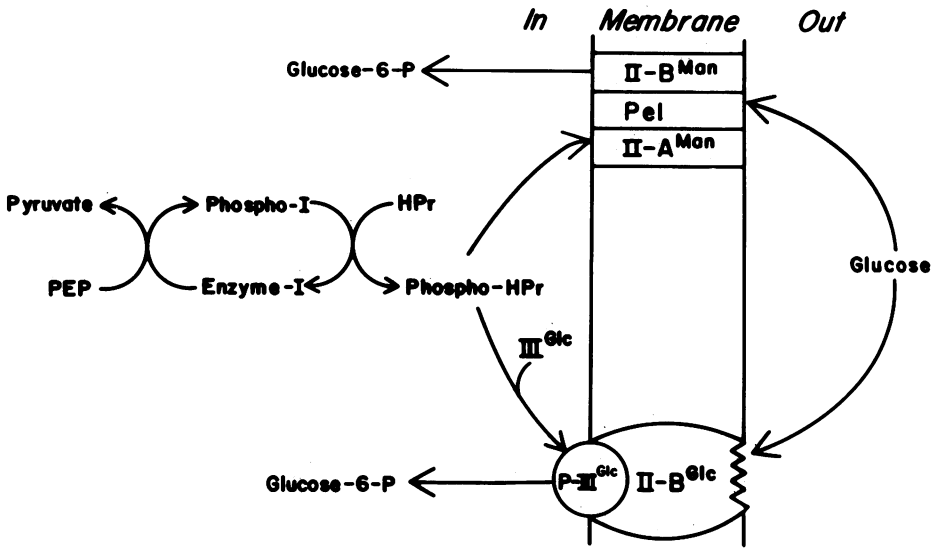

FIG. 1. Schematic representation of the bacterial phosphotransferase system. The overall reaction is the transfer of the phosphoryl group from intracellular PEP through the illustrated sequences of PTS proteins to external sugar with the concomitant translocation of the sugar to give internal sugar-phosphate (P) and pyruvate. The general proteins of the PTS are enzyme I and HPr (except FPr in the inducible fructose PTS), while the sugar-specific protein complexes are designated enzymes II. The proteins of a given enzyme II complex are either integral membrane proteins, such as II-A and II-B, or one is primarily located in the cytoplasm, and called III, while the other is in the membrane, II-B. The figure shows the two systems in *E. coli* and *S. typhimurium* which take up glucose. The III^{Glc}–$II\text{-}B^{Glc}$ complex is specific for glucose and methyl α-glucoside. III^{Glc} is critical for the regulatory functions of the PTS in enteric bacteria. The $II\text{-}A\text{–}II\text{-}B^{Man}$ complex functions with mannose, glucose, 2-deoxyglucose, *N*-acetylglucosamine (and mannosamine), and fructose at high concentrations. A third protein, Pel, has recently been shown to be part of this complex (47); its function has not yet been defined.

also showed that *pts* mutants behaved similarly to those from enteric bacteria in their phenotypic properties, with both PTS and non-PTS substrates. Gee et al. (10) showed that fructose is phosphorylated by a number of species of *Vibrio* by an inducible system which may be related to the fructose PTS of enteric bacteria.

The species that compose the genus *Vibrio* are phenotypically diverse with respect to the utilization of certain sugars such as lactose and melibiose (46). Therefore, valid generalizations concerning the distribution of a given set of proteins within the genus can be derived only by surveying a broad range of representative species. In the present study, we show that the PTS occurred in all 15 species of *Vibrio* that were assayed and that the PTS proteins from these species cross-reacted both immunologically and functionally with the corresponding proteins from the enteric bacteria. Our data suggest that the members of the genus *Vibrio* possess a diverse array of phosphotransferase systems that are closely related to the systems in enteric bacteria but that also may differ in some important respects, particularly in the specificities of their sugar-specific receptor enzymes. The implications of these results are considered in the Discussion.

MATERIALS AND METHODS

Materials. Methyl α-D-[U-^{14}C]glucopyranoside (methyl α-glucoside) was synthesized from D-[U-^{14}C]glucose (NEC-042X; 258 mCi/mmol; New England Nuclear Corp., Boston, Mass.) as previously described (25). D-[U-^{14}C]fructose was from ICN Radiochemicals (11028; 267 mCi/mmol), and 2-[^{3}H(G)]deoxy-D-glucose was from New England Nuclear Corp. (NET-328; 5 Ci/mmol). Marine broth was either prepared from the formula of ZoBell (48) or purchased from Difco Laboratories, Detroit, Mich. (2216). Gel Bond film (53440) and low electroendo-osmosis agarose (Sea-Kem LE) for the preparation of immunoelectrophoresis plates were from FMC Corp., Bioproducts, Rockland, Maine. Homogeneous preparations of enzyme I (45) and HPr (3) were isolated from an overproducing strain of *E. coli* DS703 as previously described (Saffen et al., in press). Homogeneous III^{Glc} prepared by procedure B (antibody affinity column) as previously described (30) was also obtained from an overproducing strain of *E. coli* DS336 (D. W. Saffen, Ph.D. dissertation, The Johns Hopkins University, Baltimore, Maryland, 1985).

Bacterial strains and growth of cells. A list of the strains used in this study is shown in Table 1. All *Vibrio* species were grown in marine broth (48) with the salts at either

TABLE 1. Bacterial species[a]

Vibrio species	Source
V. aestuarianus 35048	ATCC[b]
V. alginolyticus 17749	ATCC
V. anguillarum 19264	ATCC
V. campbellii 25920	ATCC
V. carchariae 35084	ATCC
V. cholerae V-69	7
V. cholerae 599717	This study
V. cincinnatiensis 35912	ATCC
V. damsela 33539	ATCC
V. diazotrophicus 33466	ATCC
V. fluvialis DJVP 7225	46
V. mediterranei 50^{T}	37
V. metschnikovii 7708	ATCC
V. parahaemolyticus 27969	ATCC
V. vulnificus 27562	ATCC

[a] Strains of *E. coli* and *S. typhimurium* used for the preparation of PTS components are given in the text where relevant.
[b] American Type Culture Collection, Rockville, Md.

one-half or full strength. Similar results were obtained in each case, and the data presented here were obtained from cells grown in full-strength salts. Cultures grown in a shaker incubator at 25°C were started by inoculation from agar stabs into 100 ml of medium in 500-ml Erlenmeyer flasks. After overnight growth, this culture was used to inoculate 600 ml of medium in a 2-liter flask, giving an initial A_{500} of approximately 0.1. The 600-ml culture was allowed to grow to the mid-exponential phase (A_{500} of approximately 1).

Harvesting of cells and preparation of extracts. The 600-ml cultures were harvested by centrifugation at 5,400 × *g* for 15 min at 4°C in a Beckman JA-20 rotor. They were then suspended in 300 ml of 50 mM Tris chloride (pH 7.5 at 25°C)–10 mM $MgCl_2$–1.5% NaCl and centrifuged as above. The washing procedure was repeated once more with 30 ml of wash solution. The cell pellet was then weighed and resuspended in 1 ml of the wash solution for each 0.2 g of cells.

Toluene permeabilization of portions of the cell suspension was performed as described by Gachelin (9). To each milliliter of cell suspension, a 10-μl portion of 10% (vol/vol) toluene in ethanol was added, the suspension was gently agitated for 1 min and incubated at room temperature for 20 min, and the cells were used for sugar phosphorylation assays within 30 min, while the remaining cells were frozen.

For immunological and sugar phosphorylation assays, the frozen cells were thawed and then homogenized by ultrasonication (Branson Sonifier with stepped micro-tip at a power setting of 7), using three 20-s bursts each followed by 30 s of cooling on ice. The homogenate was centrifuged at 6,000 × *g* for 15 min, and the supernatant was decanted and centrifuged at 170,000 × *g* for 1.5 h. The high-speed supernatant was dialyzed against 25 mM Tris chloride (pH 7.9 at 4°C)–1 mM EDTA and frozen until used for immunoelectrophoresis. The high-speed pellet (membrane fraction) was suspended in 0.5 to 1 ml of 25 mM Tris chloride (pH 7.9 at 4°C)–1 mM EDTA–0.2 mM dithiothreitol, centrifuged, and resuspended in the same solution.

Protein was assayed by the method of Markwell et al. (27).

Sugar phosphorylation by toluene-permeabilized cells. Each incubation mixture contained, in a volume of 50 μl, the following components: phosphoenolpyruvate (PEP) (10 mM) or ATP (10 mM); $MgCl_2$ (10 mM); Tris chloride (pH 8.0 at 25°C) (50 mM); KF (10 mM); dithiothreitol (0.5 mM); methyl-α-D-[U-^{14}C]glucopyranoside (4 mM, 170 cpm/nmol), or [^{3}H]2-deoxyglucose, (2 mM, 280 cpm/nmol), or [U-^{14}C]fructose (0.4 mM, 200 cpm/nmol); and toluene-permeabilized cells (5 or 10 μl) (each in duplicate). Incubations were conducted at 30°C for 10 and 20 min, and the mixtures were boiled for 10 min to stop the reactions. For assay of sugar phosphate, the mixtures were treated with 0.1 ml of a solution containing 1 μg each of DNase and RNase and 10 μmol of glucose-6-phosphate (as a carrier) for 5 min at room temperature. The radiolabeled sugar phosphate was separated from unreacted substrate on anion-exchange columns (45), and its radioactivity was counted.

Assay for PTS enzymes II in washed membranes. The incubation mixtures (0.1 ml) were prepared as described previously (30, 42, 44), with 2 mM methyl α-glucoside or 1 mM 2-deoxyglucose or 1 mM fructose, each at the same specific activity given above. The following PTS components were used: homogeneous *E. coli* enzyme I, 2 U; homogeneous *E. coli* HPr, 3 or 25 μM as indicated; and homogeneous *E. coli* or *S. typhimurium* III^{Glc}, 25 μM. The membranes from each species were tested at two levels in the assay (ranging from 20 to 150 μg of membrane protein for all species tested). Duplicate incubations were conducted at 30°C for 30 min and were stopped by chilling on ice, followed by measurement of sugar phosphate as described above.

Assay for partial proteolysis of III^{Glc}. Assays for partial proteolysis of III^{Glc} were conducted as described previously with outer membrane fraction from *E. coli* W3110 (28) or washed membranes from *Vibrio fluvialis* (60 μg) prepared as described above.

Immunological assays for presence of soluble PTS proteins. Rocket immunoelectrophoresis (24) was used for detecting homologs of enzyme I (21) and III^{Glc} (30), using partially purified immunoglobulin G (IgG) preparations from goat sera, with the concentration of IgG increased to 0.8 and 0.4 mg/ml of buffered agarose gel, respectively. Control plates were prepared from partially purified preimmune IgG from the same animal used for the immune IgG. HPr is a poor antigen, and the formation of optimal immunoprecipitates is very sensitive to conditions in the gel. For this reason, the pH of the Tris-Tricine (Sigma Chemical Co., St. Louis, Mo.) buffer of Monthony et al. (33) was modified to adjust the balance between electrophoresis and electroendo-osmosis for each new sample of agarose. The adjustments required were of the order of 0.3 pH unit.

The rocket-line method of Krøll (18) with pure antigen in the intermediate gel (1) was used with concentrations of antigens and antibodies as described in the figure legends. Plates for enzyme I and III^{Glc} employed the buffer described previously (21, 30); the buffer used for HPr plates was adjusted as indicated.

The optimal thickness of the agarose gels for all experiments was found to be 1.0 mm. All samples were 3 μl and contained from 12 to 30 μg of dialyzed crude extract high-speed supernatant protein; the quantities of homogeneous standards from *E. coli* and *S. typhimurium* are given in the figure legends.

Growth of *V. fluvialis* and purification of III^{Glc}. *V. fluvialis* was grown at room temperature in marine broth; three 600-ml inocula were grown overnight, and each was transferred to 12 liters of broth in 20-liter carboys aerated by compressed air bubbled through spargers. The cultures were grown to an optical density of approximately 1 (500 nm) and harvested by centrifugation. The packed cells (30 g) were suspended to 100 ml in 25 mM Tris chloride buffer (pH 7.5)–1 mM EDTA–0.2 mM dithiothreitol and homogenized by two passages through a French pressure cell at 23,000 lb/in^2. The homogenate was centrifuged at 20,000 × *g* for 15 min in a Beckman JA-20 rotor, and the supernatant was transferred to an anti-III^{Glc} antibody column (volume, 50 ml). After the column was washed exhaustively with buffer, the putative III^{Glc} was eluted with 0.5 N acetic acid and neutralized. The neutralized eluate was concentrated and chromatographed on a Sephadex G-75 column (30). III^{Glc} was detected by rocket immunoelectrophoresis.

RESULTS

Immunological evidence for presence of PTS proteins. Initially, PTS proteins were detected by rocket immunoelectrophoresis (24), with agarose gels containing preimmune IgGs as controls. These experiments gave clear, positive results for the presence of homologs of III^{Glc} in a number of *Vibrio* species. Figure 2 shows a representative sample of the III^{Glc} rockets. It should be noted that detection of the *Vibrio* proteins required concentrations of anti-III^{Glc} IgG (prepared against the *S. typhimurium* III^{Glc} protein) four times greater than that used with crude extracts obtained from the enteric bacteria.

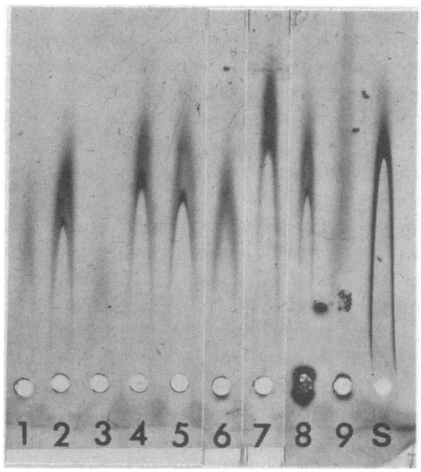

FIG. 2. Immunological detection of homologs of III^{Glc} in *Vibrio* species by rocket immunoelectrophoresis. Lanes: 1, *V. parahaemolyticus; V. cholerae* V-69; 3, *V. carchariae*; 4. *V. aestuarianus*; 5, *V. anguillarum*; 6, *V. campbellii*; 7, *V. cholerae* 599717; 8, *V. fluvialis*; 9, *V. alginolyticus*; S, *S. typhimurium* homogeneous III^{Glc}.

Similar experiments were also conducted with antibodies prepared against *S. typhimurium* enzyme I and HPr. Negative results were obtained with HPr, probably because this low-molecular-weight protein is very difficult to detect by a precipitating antibody method, even when the homologous antigen is used. Positive results were obtained with antibodies against *S. typhimurium* enzyme I, although the rocket patterns were more complex than observed with the anti-III^{Glc} antibodies. Detection of homologs of *S. typhimurium* enzyme I required eight times higher concentrations of IgG than used to detect the enzyme in extracts of enteric bacteria. The anti-enzyme I rockets obtained with crude extracts of *Vibrio* species consisted of a heavy band and one or more faint rockets (Fig. 3A). Since the faint rockets, but not the heavy one, were also obtained with preimmune IgG (Fig. 3B), we concluded that the heavy rockets showed the

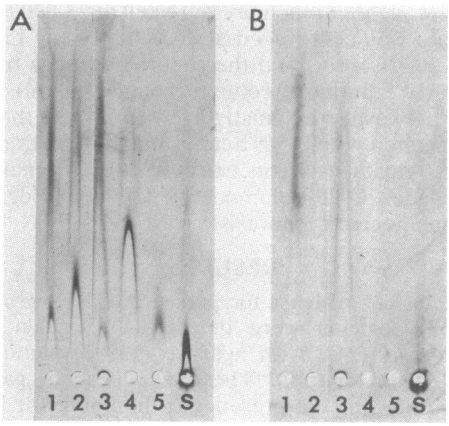

FIG. 3. Immunological detection of homologs of enzyme I in *Vibrio* species by rocket immunoelectrophoresis. The plates contain goat anti-*S. typhimurium* enzyme I (A) or preimmune IgG from the same animal (B). Lanes: 1, *V. campbellii*; 2, *V. carchariae*; 3, *V. anguillarum*; 4, *V. parahaemolyticus*; 5, *V. fluvialis*; S, *S. typhimurium* LT-2 crude extract.

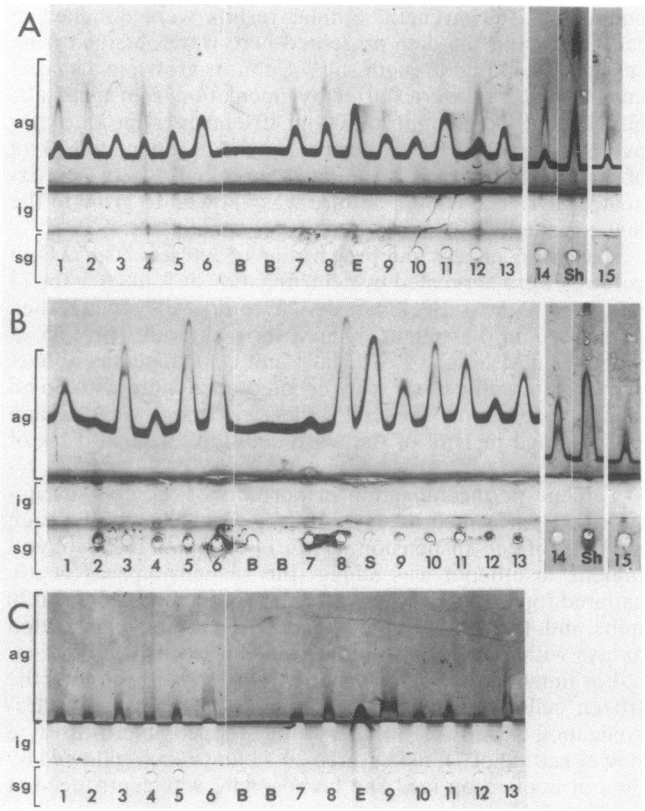

FIG. 4. Identification of putative enzyme I, III^{Glc}, and HPr immunoprecipitates by rocket-line immunoelectrophoresis. Plate A contains 0.4 mg of goat anti-*S. typhimurium* enzyme I IgG per ml in the antibody gel (ag) and 8 μg of homogeneous *E. coli* enzyme I per ml in the intermediate gel (ig); sg, sample gel. Lanes: 1, *V. metschnikovii*; 2, *V. carchariae*; 3, *V. aestuarianus*; 4, *V. parahaemolyticus*; 5, *V. cholerae* V-69; 6, *V. anguillarum*; 7, *V. alginolyticus*; 8, *V. diazotrophicus*; 9, *V. fluvialis*; 10, *V. cincinnatiensis*; 11, *V. cholerae* 599717; 12, *V. campbellii*; 13, *V. vulnificus*; 14, *V. damsela*; 15, *V. mediterranei*; E, homogeneous *E. coli* enzyme I, 0.24 μg; Sh, *S. typhimurium* homogenate; B, buffer. Plate B contains 0.2 mg of goat anti-*S. typhimurium* III^{Glc} IgG per ml in the antibody gel and 1.4 μg of homogeneous *S. typhimurium* III^{Glc} per ml in the intermediate gel. Lanes are as in plate A except S, which consisted of homogeneous *S. typhimurium* III^{Glc}, 0.12 μg. Plate C contains 0.8 mg of goat anti-*S. typhimurium* HPr IgG per ml in the antibody gel and 6 μg of homogeneous *E. coli* HPr per ml in the intermediate gel. Lanes are as in plate A except E, which consisted of homogeneous *E. coli* HPr, 0.5 μg. The line of immunoprecipitate was formed at the intersection of the antibody gel and intermediate gel in plate C because HPr rockets are carried toward the cathode by electroendo-osmosis.

presence of proteins in the *Vibrio* extracts that were homologous to enzyme I of the enteric bacteria.

The rocket-line method (18) with homogeneous antigen in the intermediate gel (1) allows the positive identification of an unknown immunoprecipitate. When the unknown antigen cross-reacts with the antibodies to the known (standard) antigen, the immunoprecipitate line formed from the standard is "lifted" to give a distinct rocket. All species tested contained antigens that interacted with the line of immunoprecipitate produced by each of the pure enteric bacterial antigens in the intermediate gel (Fig. 4). These results therefore provided significant evidence for the presence of antigenic proteins in the *Vibrio* extracts that are homologous to *S. typhimurium* enzyme I, HPr, and III^{Glc}. The variation

TABLE 2. Phosphorylation of methyl α-glucoside, 2-deoxyglucose, and fructose by toluene-permeabilized cells of various species of the genus *Vibrio*

Species	Phosphorylation (nmol of sugar-P/mg of protein/min) of[a]:					
	Methyl α-glucoside		2-Deoxyglucose		Fructose	
	PEP	ATP	PEP	ATP	PEP	ATP
V. aestuarianus	35	1.4	31	5.3	6.7	0.71
V. alginolyticus	30	1.0	21	2.2	3.4	0.65
V. anguillarum	8	0.9	NT[b]	NT	NT	NT
V. campbellii	15	2.0	NT	NT	NT	NT
V. carchariae	12	1.0	NT	NT	NT	NT
V. cholerae V-69	12	1.4	12	3.1	3.2	0.8
V. damsela	28	0.4	36	4.3	8	0.45
V. diazotrophicus	35	1.1	29	5.3	9.5	0.6
V. fluvialis	26	0.7	16	1.8	16	0.87
V. mediterranei	36	0.7	27	2.7	8.1	0.7
V. parahaemolyticus	7	0.8	NT	NT	NT	NT

[a] Cells were prepared and the rate of sugar phosphorylation was measured as described in Materials and Methods.
[b] NT, Not tested.

TABLE 3. PTS-mediated phosphorylation of methyl α-glucoside, 2-deoxyglucose, and fructose by membranes from various species of *Vibrio*

Species	Phosphorylation (nmol of sugar-P/mg of membrane protein/min) of the following in the presence or absence of III^{Glc}[a]:					
	Methyl α-glucoside		2-Deoxyglucose		Fructose	
	A	B	C	D	E	F
V. aestuarianus	13	0.02	23	0.12	0.6	0.36
V. alginolyticus	31	0.07	10	0.02	0.14	0.00
V. campbellii	13	0.10	NT[b]	NT	NT	NT
V. carchariae	11	0.09	NT	NT	NT	NT
V. cholerae V-69	42	0.08	88	0.36	1.9	0.15
V. damsela	30	0.23	28	4.3	10.3	7.5
V. diazotrophicus	25	0.34	25	0.29	1.4	0.23
V. fluvialis	30	0.23	15	0.39	7.5	1.3
V. mediterranei	14	0.15	27	0.55	1.0	0.87
V. parahaemolyticus	11	0.39	NT	NT	NT	NT

[a] Membranes were prepared as described in Materials and Methods. Each incubation mixture contained homogeneous *E. coli* enzyme I (2 U) and homogeneous *E. coli* HPr in the following concentrations: 3 μM, columns A, B, C, and E; 25 μM, columns D and F. Homogeneous *E. coli* III^{Glc} (25 μM) was used in columns A, C, and E.
[b] NT, Not tested.

in the appearance of the tips of the rockets in Fig. 4A and B may be indicative of differing degrees of antigenic homology between the various *Vibrio* and enteric bacterial antigens.

PTS activity in toluene-permeabilized cells. Preliminary experiments were conducted with several species to determine the optimal conditions for the toluene treatment; the parameters tested included the concentration of toluene used to permeabilize the cells (9), the quantity of cells incubated, the time of incubation, and the concentration of sugar substrate in the incubation mixture. Cells of *V. campbellii*, *V. carchariae*, and *V. anguillarum* lysed in the presence of toluene unless either calcium or magnesium ion was present at concentrations of about 5 to 10 mM. For this reason, 10 mM $MgCl_2$ was used with all species tested. Since optimal conditions were not determined for each cell preparation assayed, the results are considered to be qualitative and do not necessarily reflect the true PTS activity in each cell type.

All species tested were capable of phosphorylating methyl α-glucoside, 2-deoxyglucose, or fructose, and the rate of phosphorylation was at least severalfold higher when PEP rather than ATP was provided as the phosphoryl donor (Table 2). This is presumptive, not conclusive, evidence that the phosphorylation is catalyzed by a PTS (22).

Enzyme II activity in washed membranes. Membranes from all species tested were capable of phosphorylating methyl α-glucoside, 2-deoxyglucose, and fructose when supplemented with soluble PTS proteins from enteric bacteria (Table 3). In all species tested, both methyl α-glucoside and 2-deoxyglucose were phosphorylated at a higher rate in the presence of III^{Glc} than with HPr alone; a similar effect was obtained with the phosphorylation of fructose by membranes from *V. fluvialis*. These unexpected, potentially important results are discussed below.

Isolation of III^{Glc} and properties of the III^{Glc}–II-B^{Glc} system in *V. fluvialis*. The protein from crude extracts of *V. fluvialis* that gave rockets in plates with anti-*S. typhimurium* III^{Glc} was purified by affinity chromatography as described above. After chromatography on a Sephadex G-25 column, the protein was more than 90% pure as judged from electrophoresis on cellulose acetate strips (nondenaturing conditions, pH 6.0) (28). Under these conditions, the protein migrated significantly more rapidly than III^{Glc} from *S. typhimurium* (data not shown). When tested for the phosphorylation of methyl-α-glucoside in assays supplemented with enzyme I, HPr, and membranes from *E. coli*, this protein behaved similarly to III^{Glc} from enteric bacteria (Fig. 5); i.e., the rate of the reaction plotted as a function of the quantity of the *V. fluvialis* protein gave a hyperbolic curve, behavior expected from a III^{Glc} phosphocarrier protein (30, 31). The putative III^{Glc} from *V. fluvialis* behaved identically when the source of enzyme II was membranes from either *E. coli* (Fig. 5) or *V. fluvialis* (data not shown). In other words, the apparent III^{Glc} protein from *V. fluvialis* accepted a phosphoryl group from *E. coli* HPr and donated the group to methyl α-glucoside in the presence of either *E. coli* II-B^{Glc} or an apparently homologous protein in *V. fluvialis* membranes.

The protein III^{Glc} from enteric bacteria is cleaved by a

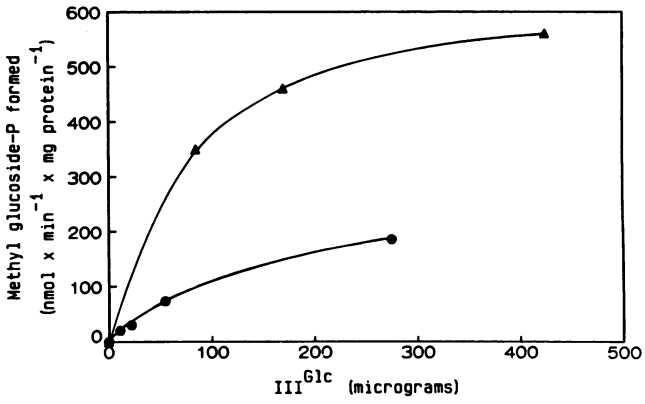

FIG. 5. III^{Glc} from *V. fluvialis* cross-reacts with *E. coli* PTS proteins in the phosphorylation of methyl α-glucoside. Incubation mixtures are described in Materials and Methods and contained homogeneous *E. coli* enzyme I, HPr, and membranes from *E. coli* 1100 as a source of II-B^{Glc}. The quantities of III^{Glc} proteins used in the assay are given on the abscissa. Methyl glucoside-P, Methyl α-D-glucoside 6-phosphate. Symbols: ▲, homogeneous III^{Glc} from *S. typhimurium*; ●, putative III^{Glc} from *V. fluvialis*.

very specific protease in the membranes from these cells (28); the cleavage occurs between amino acid residues 7 (lysine) and 8 (serine). The resulting protein is not further degraded, even after prolonged incubation with crude extracts of the bacteria. Experiments were therefore conducted with *E. coli* and *V. fluvialis* III^{Glc} and with membranes from the two species. Specific proteolysis was observed only with membranes from the enteric bacteria, but the important result was that these membranes processed *V. fluvialis* III^{Glc} as well as the III^{Glc} protein from the enteric bacteria (data not shown).

All of these results lead to the conclusion that the purified protein from *V. fluvialis* is homologous, but not identical, to III^{Glc} from the enteric bacteria.

DISCUSSION

Simple sugars are present in the oceans in submicromolar concentrations (5, 12, 15, 17, 34), and they represent a significant fraction of the total available reduced carbon (5). In addition, it is now believed that a very large proportion of the primary photosynthate produced in the sea is processed through bacteria (16, 23). Thus, marine bacteria appear to metabolize very large quantities of simple sugars, and these must be taken up by the cells by highly efficient transport systems, such as the PTS, because the extracellular sugar concentrations are so low.

The vibrios are one of the predominant groups of marine bacteria (2, 6, 41); they occupy a wide range of niches, which differ in the range of carbohydrates available (13, 41). Thus, if the mechanisms of uptake and metabolism of carbohydrates throughout the genus *Vibrio* were defined, this would provide important information on understanding a critical feature of biological energy flow in the sea.

Kubota et al. (19, 20) have definitively shown that one species of the genus, *V. parahaemolyticus*, contains the PTS. In addition, these investigators reported that enzyme I, HPr, and III^{Glc} from this species functionally complemented the corresponding proteins from *E. coli* and that mutants defective in the PTS proteins behaved phenotypically similar to *pts* mutants of the enteric bacteria.

The evidence presented here suggests that the PTS occurs widely, probably ubiquitously, throughout the genus *Vibrio*. (i) When PEP was the phosphoryl donor, toluene-permeabilized cells of all species tested showed rates of phosphorylation of methyl α-glucoside comparable to those measured in the enteric bacteria *E. coli* (9) and *S. typhimurium* (35). (ii) Each of the 15 species tested showed the presence of immunologically cross-reactive homologs of the two general PTS proteins, enzyme I and HPr, and of the sugar-specific protein III^{Glc}. (iii) The protein III^{Glc} was highly purified from *V. fluvialis* by adsorption and elution from an antibody column prepared with IgG raised against homogeneous *S. typhimurium* III^{Glc}, and the purified protein was active in phosphorylating methyl α-glucoside when supplemented with *E. coli* enzyme I, HPr, and II-B^{Glc}. (iv) Membranes from 10 different species were tested and phosphorylated fructose and analogs of glucose when supplemented with homogeneous *E. coli* enzyme I, HPr, and III^{Glc}. Our results thus show that the PTS proteins from vibrios are functionally and immunologically similar, but not identical, to those from enteric bacteria, a finding consistent with the 5S rRNA-based phylogeny of these organisms (26).

The ubiquitous distribution of glucose and fructose and the vibrios in the marine environment and the fact that the PTS appears to occur generally in the vibrios leads to speculation that the PTS plays a critical role in the marine ecosystem and carbon cycle. In this connection, we note again that the PTS (primarily III^{Glc}) in enteric bacteria regulates the catabolism of many non-PTS compounds (31, 32; Saffen et al., in press), so that the role of the PTS in the vibrios may not be confined solely to the transport and phosphorylation of PTS-sugars.

The *Vibrio* PTS may also prove to be important in connection with defining the mechanism of action of this complex system, and possibly of its evolution. Figure 1 summarizes the specificities of the two glucose PT systems in the enteric bacteria. Under proper assay conditions, methyl-α-glucoside and 2-deoxyglucose are specific substrates of the II^{Glc} and II^{Man} systems, respectively. Fructose is also a substrate, albeit a poor one (42), of the II^{Man} system (the cells must be grown on fructose to induce the specific fructose PTS).

Based on these considerations, if the sugar-specific membrane proteins of vibrios were similar to the corresponding glucose systems of the enteric bacteria, methyl α-glucoside phosphorylation should be stimulated by III^{Glc}, whereas III^{Glc} should have no effect on the rates of phosphorylation of 2-deoxyglucose or fructose. Methyl α-glucoside phosphorylation does indeed require III^{Glc} (Table 3). Table 3 also shows, however, that the vibrio membranes do not behave as expected. That is, in the seven species that were assayed, 2-deoxyglucose phosphorylation was greatly stimulated by *E. coli* III^{Glc}, often to the same extent as was the phosphorylation of methyl α-glucoside, i.e., as much as 500-fold. The results with fructose depended on the species from which the membranes were isolated; the stimulation by III^{Glc} varied from negligible to as much as 10-fold.

These data indicate that the vibrio membranes contain proteins analogous to the enteric bacterial II^{Glc}, which phosphorylates methyl α-glucoside in the presence of phospho-III^{Glc}. However, either the same protein or a similar one is also present that phosphorylates 2-deoxyglucose and, in some cases, perhaps fructose. If a single sugar-specific membrane protein catalyzes these sugar phosphorylations and interacts with phospho-III^{Glc}, this would suggest a possible evolutionary origin of the enteric bacterial genes that specify II-B^{Glc} and the three genes of the *ptsM* region (47). That is, they may have arisen from a common precursor gene that has been conserved in some of the vibrios. Recent experiments with cloned *E. coli ptsG* show that it hybridizes to the DNA from many of the vibrios (C. Bouma, unpublished data) and suggest that these hypotheses can be tested directly.

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