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Phosphoenolpyruvate:Sugar Phosphotransferase System in *Ancalomicrobium adetum*

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Ancalomicrobium adetum possesses a membrane-associated phosphoenolpyruvate:sugar phosphotransferase system, the components of which exhibited enzymatic cross-reactivity with those from *Salmonella typhimurium*.

Ancalomicrobium adetum differs from typical unicellular, gram-negative, heterotrophic bacteria. It is a gas vacuolate, budding bacterium that has several prosthecal appendages, which confer a star-shaped appearance to the organism (9). Because of its morphology and mode of reproduction, it has been classified with the budding and prosthecate bacteria (10, 11). However, it differs from known representatives of that group because it is a facultative anaerobe that ferments glucose, fructose, mannitol, and other carbohydrates.

Because of its fermentative metabolism, we decided to determine whether *A. adetum* possesses the phosphoenolpyruvate:sugar phosphotransferase system that is commonly found in other facultatively anaerobic bacteria. This complex enzyme system is involved in the transmembrane transport and phosphorylation of numerous carbohydrates in several bacterial species. It consists of two soluble energy-coupling proteins, enzyme I and HPr, as well as sugar-specific membrane constituents, the enzyme II complexes (2-4). The results summarized below show that extracts derived from *A. adetum* cells can catalyze phosphoenolpyruvate-dependent sugar phosphorylation, although corresponding activities were not detected in extracts derived from several aerobic budding or appendaged bacteria.

Mannitol- or glucose-grown bacterial cells were ruptured by passage through a French pressure cell at 10,000 lb/in^2, and cell debris was removed by low-speed centrifugation. The extracts were dialyzed overnight against 25 mM tris(hydroxymethyl)aminomethane-hydrochloride buffer (pH 7.5) containing 0.5 mM dithiothreitol and were then centrifuged at 200,000 $\times$ g for 1 h to separate particulate matter from soluble proteins. Extracts were assayed (2) before and after dialysis and before and after high-speed centrifugation. Activity was stable during dialysis, during storage at 0°C for 1 week, and during storage at −60°C for several months.

The buffer-washed membranes for *A. adetum* cells catalyzed the transfer of the phosphoryl moiety from phosphoenolpyruvate to both [^{14}C]mannitol and [^{14}C]methyl α-glucoside (Table 1). Several other potential phosphate donors, including adenosine-5′-triphosphate, guanosine-5′-triphosphate, α-glycerophosphate, 3-phosphoglycerate, creatine phosphate, and acetyl phosphate, were less effective phosphoryl donors than phosphoenolpyruvate. Ethylenediaminetetraacetate was strongly inhibitory. [^{14}C]fructose and [^{14}C]glucose were also phosphorylated in the presence of phosphoenolpyruvate, but phosphorylation of these sugars by adenosine-5′-triphosphate-dependent kinases occurred at more rapid rates. Phosphoenolpyruvate-dependent phosphorylation of [^{14}C]mannitol and [^{14}C]methyl α-glucoside was not observed with extracts from other budding and appendaged bacteria examined, including *Prosthecomicrobium pneumaticum*, *Caulobacter crescentus*, *Hyphomicrobium vulgare*, and *Rhodomicrobium vannielii*. *Pseudomonas aeruginosa*, *P. putida*, *P. fluorescens*, and several species of the *Athiorhodaceae* also appeared unable to phosphorylate these two sugars with phosphoenolpyruvate as the phosphoryl donor although they did phosphorylate fructose by a phosphoenolpyruvate-dependent mechanism (5, 8).

The phosphoenolpyruvate-dependent sugar-phosphorylating activities in *A. adetum* extracts were associated with the membrane fraction, and no activity was found in the high-speed supernatant fluid. The latter fraction did not stimulate, and inhibited slightly, the activity of the pellet. Activity was proportional to membrane protein concentration and was linear with time for more than 1 h at 30°C. Heating the membrane fraction at 90°C for 5 min destroyed the catalytic activities. Phosphoenol-

TABLE 1. *Sugar phosphorylation catalyzed by the particulate fraction of extracts derived from A. adetum cells*[a]

Carbon source for growth	Phosphoryl donor	Phosphorylation (nmol/min per g of protein) of:	
		[^{14}C]methyl α-glucoside	[^{14}C]mannitol
Glucose	None	3	5
Glucose	Phosphoenolpyruvate	2,000	87
Glucose	ATP[b]	5	11
Glucose	Glucose-6-P	51	3
Glucose	Mannitol-1-P	3	30
Mannitol	None	3	6
Mannitol	Phosphoenolpyruvate	175	410
Mannitol	ATP	5	19
Mannitol	Glucose-6-P	8	6
Mannitol	Mannitol-1-P	7	120

[a] Cells were grown aerobically in MMB medium containing glucose or mannitol (0.2%) as the sole carbohydrate (9, 11), harvested, washed three times with modified Hutner salts solution, frozen, and resuspended for rupture in a French pressure cell. The extracts were prepared for assay of ^{14}C-labeled sugar phosphorylation as described in the text. The assay tubes contained (in a final volume of 250 μl): 50 μM ^{14}C-labeled sugar (10 μCi/μmol), 10 mM phosphate donor, 50 mM potassium phosphate buffer (pH 7.4), 10 mM $MgCl_2$, 20 mM KF, 1 mM dithiothreitol, and buffer-washed membranes from cells grown in the presence of the sugar indicated above. Incubations were for 1 h at 30°C. Sugar phosphate formation was measured by employing the anion-exchange resin procedure described previously (2).

[b] ATP, Adenosine-5'-triphosphate.

pyruvate-dependent phosphorylation of [^{14}C]-methyl α-glucoside occurred with much higher specific activity in glucose-grown cells than in mannitol-grown cells. Conversely, mannitol phosphorylation activity was induced by growth in the presence of mannitol (Table 1). Similar activities were observed when the cells were grown under aerobic and anaerobic conditions. Additionally, inducible activities responsible for the transfer of the phosphoryl moiety of glucose-6-P to [^{14}C]methyl α-glucoside and of mannitol-1-P to [^{14}C]mannitol were demonstrated (Table 1). The radioactive products of these reactions were characterized as sugar phosphates on the basis of their chromatographic and electrophoretic properties and by their sensitivities to hydrolysis by crystalline *Escherichia coli* alkaline phosphatase (Sigma Chemical Co., St. Louis, Mo.) (6). Since the sugar-P:sugar transphosphorylation reactions are specifically catalyzed by the enzyme II complexes of the eubacterial phosphotransferase system in the absence of enzyme I and HPr (6), the results suggest that the inducible phosphotransferase proteins in *A. adetum* are the sugar-specific enzyme II complexes.

Although the soluble protein fraction from *A. adetum* cells did not complement extracts derived from enzyme I- or HPr-deficient mutants of *Salmonella typhimurium* (7), purified enzyme I and HPr from *S. typhimurium* were found to stimulate phosphoenolpyruvate-dependent mannitol phosphorylation about twofold in the presence of the particulate fraction from *A. adetum* (data not shown). Moreover, extraction of the *A. adetum* membrane fraction with butanol and urea as described by Kundig and Roseman (3) greatly reduced phosphoenolpyruvate:[^{14}C]mannitol phosphoryl transfer activity without reducing the mannitol-1-P:[^{14}C]mannitol transphosphorylation activity (Table 2). Addition of the soluble protein fraction of *A. adetum* cell extracts to the butanol-urea-extracted membranes partially restored phosphoenolpyruvate-dependent activity, but purified enzyme I and HPr from *S. typhimurium* were more effective. These enzymes did not stimulate mannitol-1-P:[^{14}C]mannitol transphosphorylation (data not shown).

The results summarized above suggest that *A. adetum* possesses a phosphoenolpyru-

TABLE 2. *Phosphorylation of [^{14}C]mannitol catalyzed by butanol- and urea-extracted membranes isolated from mannitol-grown A. adetum cells*[a]

Phosphoryl donor	Soluble proteins added	Phosphorylation of [^{14}C]mannitol (nmol/min per mg of protein)
None	None	5
Phosphoenolpyruvate	None	6
Phosphoenolpyruvate	*A. adetum* soluble proteins	29
Phosphoenolpyruvate	*S. typhimurium* exzyme I + *S. typhimurium* HPr	170
ATP[b]	None	6
Glucose-6-P	None	4
Mannitol-1-P	None	130

[a] Washed membranes were prepared and extracted with butanol and urea as described by Kundig and Roseman (3). Assays were performed as described in the footnote to Table 1. Soluble proteins, when present, were included at the following concentrations: *A. adetum* soluble proteins, 1 mg/ml; *S. typhimurium* HPr (purified through the Sephadex G-75 column [1]), 17 μg/ml; *S. typhimurium* enzyme I (purified about 50-fold from the crude extract by an unpublished procedure), 70 μg/ml.

[b] ATP, Adenosine 5'-triphosphate.

vate:sugar phosphotransferase system similar to that found in other gram-negative bacteria. The system appears to differ from those found in previously studied bacterial species in that the entire enzyme complex is membrane associated. Cross-complementation of the components of the phosphotransferase systems of *A. adetum* and *S. typhimurium* was an unexpected result in view of the substantial differences between these two organisms.

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