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Mycoplasma Phosphoenolpyruvate-Dependent Sugar Phosphotransferase System: Glucose-Negative Mutant and Regulation of Intracellular Cyclic AMP

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Glucose-negative mutants of *Mycoplasma capricolum* were selected for growth on fructose in the presence of the toxic glucose analog α-methyl-D-glucopyranoside. The mutants are defective in the phosphoenolpyruvate:sugar phosphotransferase system for glucose. One mutant, *pts-4*, was studied in detail. It lacks the glucose-specific, membrane-bound enzyme II, II^{Glc}, as well as the general, low-molecular-weight, phosphocarrier protein, HPr. In place of the latter, however, it has a fructose-specific protein, HPr^{Fru}. Consistent with these changes, the mutant lost the ability to grow on glucosamine and maltose but retained its ability to grow on sucrose. In the glucose-negative mutant, glucose did not regulate the intracellular concentration of cyclic AMP. The intracellular concentration of cyclic AMP in *M. capricolum* is regulated by the presence of metabolizable sugars. In the wild-type, both glucose and fructose reduced the intracellular concentration of cyclic AMP; however, in the glucose-negative mutant, glucose no longer regulated the intracellular level of cyclic AMP.

Most of the phosphoenolpyruvate-dependent sugar phosphotransferase systems (PTS) studied so far consist of four types of proteins, two general, nonspecific proteins that are always cytoplasmic (enzyme I and HPr) and two sugar-specific proteins that may be membrane bound (IIA and IIB) or may consist of one soluble (III) and one membrane-bound (IIB') component (14). In *Mycoplasma capri* (2) and *M. capricolum* (6, 7), enzyme I and HPr are the only soluble components. The membrane-bound, sugar-specific enzyme II activities for the various sugars are, therefore, presumed to be of the IIA/IIB type. In *M. capri*, the enzymes II for glucose, fructose, and mannose are constitutive (2). In *M. capricolum*, the glucose-specific activity is constitutive, whereas fructose-specific activity is inducible.

For certain membrane reconstitution experiments, it was desirable to have cells containing the fructose-specific enzyme II complex but lacking the glucose-specific complex. For this purpose, mutagenized cells were selected by growth on a fructose medium containing the toxic glucose analog α-methyl-D-glucopyranoside (α-MG). Since α-MG toxicity depends upon its phosphorylation by the glucose-specific PTS, cells that grow on α-MG plus fructose were expected to be enzyme II, glucose negative. Several glucose-negative, fructose-positive mutants were isolated by this procedure, and one was analyzed in detail. This mutant presented a double alteration: a loss of the glucose-specific enzyme II glucose complex, as expected, and the presence of a fructose-specific HPr in place of the nonspecific HPr. The occurrence of fructose-specific HPr has been observed in *Aerobacter aerogenes* (4, 23) and *Salmonella typhimurium* (20).

The loss of the glucose-specific components of the PTS in the mutants also results in the loss of the ability of glucose to regulate the intracellular level of cyclic AMP (cAMP). Thus, in fructose-grown wild-type cells that contain both glucose- and fructose-specific PTS activity, the presence of both sugars causes a decrease in the intracellular concentration of cAMP. In the fructose-grown mutant cells, only fructose regulates the cAMP concentration.

MATERIALS AND METHODS

Organisms and growth conditions. *M. capricolum* (kid strain) was kindly provided by Joseph Tully of the National Institutes of Health. The microorganisms were grown in modified Edwards medium at pH 8.0. The medium contained 0.042 M NaCl, 0.014 M K_2HPO_4, 0.5% (wt/vol) D-glucose, fructose, sucrose, or maltose, 0.5% (wt/vol) peptone, 0.7% (wt/vol) yeast extract, and 1.3% (wt/vol) heart infusion broth. The medium was supplemented with 20 ml of bovine serum fraction A (Grand Island Biological Co., Grand Island,

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N.Y.) per liter, 28.0 mg of DNA per liter, and 10^6 U of penicillin G (Sigma Chemical Co., St. Louis, Mo.) per liter. The organisms were grown in stationary, 2-liter Erlenmeyer flasks containing 500 ml of liquid medium at 37°C. The cells were grown for a period of 20 h. The cells were harvested when the pH of the medium had fallen to between 5.5 and 6.0. The culture was rapidly chilled to 0°C, and the cells were harvested with a Sorvall RC2-B centrifuge and an HS-4 rotor at 9,000 × *g* for 15 min. The harvested cells were washed once with a wash medium containing 0.25 M NaCl and 0.01 M $MgCl_2$. The washed cells were used directly for fractionation of PTS components. All subsequent steps were carried out at 0 to 4°C.

Preparation of the crude extracts. Washed cells were suspended in β-buffer [150 mM NaCl, 50 mM tris(hydroxymethyl)aminomethane-hydrochloride (pH 7.4), 1 mM ethylenediaminetetraacetic acid, 10 mM β-mercaptoethanol, and 0.1 mM dithiothreitol]. A typical preparation contained 1.0 to 1.25 g of wet cells in 10 ml of β-buffer. A crude enzyme extract was then prepared from this cell suspension by ultrasonic oscillation and the removal of unbroken cells and cellular debris by subsequent centrifugation at 15,000 × *g* for 20 min in a Sorvall SS-24 rotor.

Separation of membranes and soluble fraction. The crude extracts were centrifuged at 102,000 × *g* for 60 min at 4°C in a Beckman model L-5-65 ultracentrifuge using a 65 rotor. The supernatant solution was carefully separated and recentrifuged for an additional 60 min as described above to concentrate membrane fragments. The pelleted membranes were resuspended with the aid of a glass homogenizer (Thomas, Philadelphia, Pa.) and repelleted at 102,000 × *g* for 60 min at 4°C. At the end of this centrifugation, the translucent membrane fraction was thoroughly resuspended in β-buffer and stored at −20°C. The supernatant from the second centrifugation was added to the previously collected supernatant fraction. The washed membrane material was the source of enzyme II in all PTS assays described elsewhere in this communication.

Fractionation of enzyme I and HPr. The supernatant fraction obtained by centrifugation at 102,000 × *g* was used to separate enzyme I and HPr; all steps of the fractionation were carried out at 0 to 4°C. The crude soluble fraction was adjusted to 100% saturation of $(NH_4)_2SO_4$ by addition of crystalline enzyme-grade ammonium sulfate (Schwarz/Mann, Orangeburg, N.Y.) over a period of 30 to 45 min with constant stirring in a cold room. The apparent pH of the mixture, measured with a glass electrode, was maintained at 7.4, and the saturated solution was stirred overnight at 4°C. The precipitated proteins were pelleted by centrifugation at 30,000 × *g* for 30 min and resuspended in a minimal volume of β-buffer. This ammonium sulfate-precipitated fraction was applied to a Bio-Gel P-10 column (1.5 by 30 cm) preequilibrated with β-buffer at a flow rate of about 0.6 ml/min to separate enzyme I and HPr according to the Jaffor Ullah and Cirillo procedure (6).

Isolation of α-MG-resistant mutants of *M. capricolum*. The wild-type kid strain of *M. capricolum* was grown in 10 ml of liquid medium (pH 8.0) for 24 h at 37°C. The cells were harvested when the optical density of the medium reached 0.54 unit at 540 nm, by a Bausch and Lomb Spectronic 20 spectrophotometer. The cells were pelleted in a Sorvall RC-2B centrifuge and SS-34 rotor at 15,000 × *g* for 10 min. The pelleted cells were suspended in 1.0 ml of liquid medium supplemented with 0.5% (wt/vol) fructose and 100 μg of nitrosoguanidine (Mann Research Laboratories) and incubated in stationary test tubes for 2 h at 37°C. The treated cells were harvested, washed with 5 ml of sugar-free medium, and suspended in 1.0 ml of sugar-free growth medium. This cell suspension was used to inoculate 5 ml of liquid culture supplemented with 5% (wt/vol) α-MG and 0.5% (wt/vol) fructose or glucose. After 24 h of incubation at 37°C, growth was observed only in the fructose-supplemented medium. The cells were maintained by daily transfers in fructose medium. After several transfers in fructose medium, the cells from a culture with an optical density of 0.48 unit at 540 nm were diluted 10^6 times, spread on solid growth medium supplemented with 5% (wt/vol) α-MG and 0.5% (wt/vol) fructose, and incubated at 37°C. Spontaneous or mutagen-induced α-MG-resistant colonies appeared after about 48 h. Separate colonies were picked after 4 days, using sterilized toothpicks, to inoculate 5 ml of liquid medium supplemented with glucose or fructose. Several mutants (*pts-1* through *pts-7*) were isolated that grew on fructose but not on glucose. Mutant *pts-4* was characterized as shown below, and the rest were saved for future investigation.

Carbohydrate fermentation by *M. capricolum* and α-MG-resistant mutant. The wild-type kid strain and the *pts-4* mutant were tested for their ability to utilize different carbohydrates. Culture tubes with 5 ml of growth medium were supplemented with carbohydrates at a concentration from 0.1 to 0.5% (wt/vol). Wild-type and the mutant cells were grown in carbohydrate-free medium before inoculation into sugar-supplemented medium. Growth was determined after 24, 48, and 72 h.

Enzyme assay. The PTS assay, a modification of that of Kundig and Roseman (8), has been described previously (6). The HPr and enzyme I fractions are not significantly contaminated with each other or with enzyme II; however, enzyme II preparations (i.e., washed membranes) are constantly contaminated with low levels of enzyme I and HPr. Specific activity is expressed as nanomoles of sugar phosphorylated per milligram of protein per minute at 37°C. The specific activity of individual components was determined under conditions in which activity depended linearly on that component.

Extraction of cAMP. Liquid cultures (5 ml) of the wild-type and the mutant, having an optical density of 0.54 to 0.60 unit at 540 nm, were used to inoculate 500 ml of liquid medium in 2-liter Erlenmeyer flasks. The cells were grown for 8, 12, 16, 20, and 24 h. The medium for the wild-type cells was supplemented with glucose or fructose, and the medium for the mutant was supplemented with fructose. The microorganisms were harvested separately as described before. The pelleted cells were suspended in 5.0 ml of 5 mM sodium phosphate buffer (pH 8.0) and heated in a boiling water bath for 10 min. The tubes were chilled in ice and centrifuged. The super-

natants were saved and adjusted to 5.0 ml with 5 mM sodium phosphate. The extracts were assayed for cAMP.

cAMP assay. cAMP determination was carried out by a modification of the procedure of Hesse et al. (5). Washed erythrocyte ghost membranes were prepared as described by Fairbanks et al. (3) from outdated human blood from a blood bank. All steps were performed at 0 to 4°C. The membranes were suspended in 5 mM sodium phosphate buffer (pH 8.0) at approximately 4 mg of protein per ml and stored in small batches at −20°C. The membranes were treated at 0°C for 2 h with 1 M NH_4Cl in a solution containing 10 mg of bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.) per ml, as described by Rubin et al. (16). The treated membranes were sedimented by centrifugation at 42,000 × *g* for 30 min and used the same day after suspension in 5 mM phosphate buffer (pH 8.0) at approximately 4 mg of protein per ml. The cyclic AMP binding was assayed in a total volume of 0.2 ml containing 50 mM potassium phosphate buffer (pH 7.5); 10 mM $MgCl_2$; 0.83 pmol of [8-^{3}H]-cAMP (30 Ci/mmol); and 20 μl of assay sample. The assay was initiated by the addition of the treated membrane suspension containing approximately 40 μg of protein. After 60 min of incubation at 0°C, the mixture was diluted with 1 ml of cold 20 mM potassium phosphate buffer (pH 6), and the cells were collected with suction on a membrane filter (25-mm diameter; Millipore Corp., Bedford, Mass.).

The assay tube was rinsed with 20 ml of cold buffer, which was then passed over the filter. The filter was dried and counted in a scintillation spectrometer (Intertechnique model SL-30), after the addition of 10 ml of ACS scintillation fluid (Amersham/Searle, Arlington Heights, Ill.).

Analytical techniques. The protein concentration was determined by the method of Lowry et al. (9), using bovine serum albumin as the standard. Glucose and fructose concentrations were determined colorimetrically by the method of Nelson and Somogyi (11), using glucose as a standard.

Source of materials. Phosphoenolpyruvate tricyclohexamine salt, ATP disodium salt, dithiothreitol, and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, Mo. The anion-exchange resins, AG 1-X2 (50 to 100 mesh), and Bio-Gel P-10 polyacrylamide beads were purchased from Bio-Rad Laboratories, Richmond, Calif. [*U*-^{14}C]-labeled α-methyl-D-glucopyranoside, fructose, and glucose and [8-^{3}H]cAMP were purchased from Amersham/Searle, England. All other chemicals were obtained from available commercial resources and were of analytical or reagent grade.

RESULTS

Selection of α-MG-resistant mutants. α-MG-resistant mutants were selected by growing nitrosoguanidine-treated cells in the presence of α-MG plus fructose. Wild-type cells are inhibited by α-MG; by selecting cells that grew in the presence of a mixture of α-MG and fructose, mutants were isolated that were glucose negative and fructose positive. The growth of one isolate, *pts-4*, was tested on several carbon sources. The wild type is able to grow on glucose, fructose, glucosamine, maltose, and sucrose. The mutant was unable to grow in glucose, glucosamine, and maltose but continued to grow as well as the wild type on fructose and sucrose. The pattern of growth on these sugars was consistent with the glucose-negative character of the mutant.

PTS activity of an α-MG-resistant strain (*pts-4*). As expected, the PTS activity of the α-MG-resistant strains selected on fructose medium was negative for α-MG and positive for fructose. The α-MG PTS activity of crude extracts of one of these strains, *pts-4*, was less than 1.0% of the wild-type level, while the PTS activity for fructose in this strain was three- to fourfold higher than that of fructose-grown, wild-type cells (Table 1). The PTS activity for fructose was inducible (Table 1).

To determine which component(s) of the PTS was defective in the mutant, the isolated components (enzyme II, enzyme I, and HPr) of the mutant were tested individually with the complementary components from wild-type cells. The results of the complementation tests (Table 2) show that both enzyme II and HPr of the mutant were defective for α-MG phosphorylation. Thus, when the enzyme II from the mutant was substituted for the enzyme II from the wild type, the specific activity ratio for α-MG versus fructose phosphorylation decreased from 1.94 for the wild-type enzyme II to 0.12 for the mutant enzyme II. This represented a 16-fold decrease in the relative specific activity for α-MG. Similarly, when the HPr from the mutant was substituted for the HPr from the wild type, the

TABLE 1. *PTS activity of crude extracts of wild-type and mutant cells*

Growth sugar[b]	Sp act[a] (nmol/mg of protein per min at 37°C)			
	Wild type		Mutant (*pts-4*)	
	α-MG	Fructose	α-MG	Fructose
Glucose	0.75	0.07		
Fructose	0.72	0.35	<0.008	1.23

[a] The assays were carried out using a low-speed supernatant (30,000 × *g* for 20 min) of sonically treated material added to an assay mixture containing 2 μmol of $MgCl_2$, 20 μmol of K_2HPO_4, 4 μmol of KF, 4 μmol of phosphoenolpyruvate (tricyclohexylamine salt), 0.5 μmol of dithiothreitol, 0.5 μmol of spermine tetrachloride, and 0.25 μmol of α-[^{14}C]MG or [^{14}C]fructose (0.5 μCi/μmol) in a final volume of 250 μl at pH 7.5. The assay was carried out at 37°C for 1 h.

[b] Wild-type cells were grown in glucose or fructose medium; mutant cells could be grown only in fructose medium.

TABLE 2. *Complementation using PTS components of fructose-grown mutant and wild-type cells*

Source of PTS components[a]			Sp act (nmol/mg of protein per min at 38°C)		
EII	EI	HPr	α-MG	Fructose	α-MG/fructose
W	W	W	0.68	0.35	1.94
m	m	m	<0.008	1.14	<0.01
m	W	W	0.09	0.74	0.12
W	m	W	0.63	0.35	1.80
W	W	m	0.10	0.44	0.23
W	m	m	0.08	0.60	0.13
m	W	m	<0.008	1.07	<0.01
m	m	W	0.09	0.93	0.10

[a] W, Wild type; m, mutant. Enzyme I and HPr were separated from the soluble fraction by filtration through a Bio-Gel P-10 column. Washed membranes were used as a source of enzyme II. The assay mixtures contained 1.0 to 1.4 mg of washed membrane protein as a source of enzyme II, 600 to 700 μg of protein of the enzyme I fraction, and 3 to 10 μg of protein of the HPr fraction per ml. Assay conditions as described in Table 1, footnote *a*.

α-MG/fructose specific activity ratio decreased from 1.94 to 0.23, a 9-fold decrease in the relative specific activity for α-MG. Only in the case of enzyme I were the enzymes from the mutant and the wild type able to be substituted for each other with no change of the relative specific activity ratio, namely 1.94 and 1.8, respectively.

The PTS components from the fructose-grown wild-type cells were added singly to the complementary components from the mutant. The results confirm that more than one of the components of the mutant system are defective in α-MG phosphorylation, since none of the components from wild-type cells, when added singly, restored α-MG phosphorylation to the wild-type level.

Heat stability of fructose-specific HPr. The observation that the fructose-grown, glucose-negative mycoplasma mutant has a fructose-specific HPr is supported by a similar observation with fructose-grown *ptsH* mutants of *S. typhimurium* by Saier et al. (20). These authors reported that the fructose-induced HPr of the mutant is heat labile relative to that of wild-type cells. This is not so for mycoplasma HPr; the HPr activity of the mutant was as fully resistant to boiling as wild-type HPr. Furthermore, the chromatographic characteristics of the fructose-specific HPr were also similar to those of the nonspecific HPr of the wild type. It eluted in the 10,000-dalton range from Bio-Gel P-10 columns and was not retained by diethylaminoethyl columns at pH 7.5 (6; data not shown).

Presence of HPr^{Fru} in wild-type cells? The demonstration in the mutants of a fructose-specific HPr (HPr^{Fru}) raised the question of whether this component is constitutive or induced with the fructose-specific enzyme II in fructose-grown wild-type cells. In an attempt to answer these questions, the HPr fractions from glucose- or fructose-grown cells were tested with the membrane fraction (enzyme II) from fructose-grown cells. The ratio of the specific activity for α-MG and fructose was the same (ca. 2.0), whether the HPr fraction came from glucose- or fructose-grown cells. This excludes the possibility that the HPr^{Fru} is an inducible component, for if it were, the relative specific activity for fructose phosphorylation would be higher with the HPr fraction of fructose-grown cells. A constitutive HPr^{Fru}, however, is not excluded.

The absence of ATP-dependent sugar kinases. The results of the growth experiments showed that the wild-type cells grow on glucose, maltose, and sucrose, while the mutant grows only on fructose and sucrose. The inability of the mutant to grow on maltose shows that glucose can only be used if there is a functional PTS. In organisms like *E. coli* or *M. mycoides* var. *capri*, which have a cytoplasmic, glucose-specific, ATP-dependent hexokinase or glucokinase in addition to a glucose-specific PTS, inability of the PTS mutant to grow on maltose would be evidence for extracellular hydrolysis of maltose. Therefore, assays for a cytoplasmic, glucose-specific, ATP-dependent kinase were carried out in both wild-type and mutant cells of *M. capricolum*. The results of these experiments (Table 3) show that neither the wild type nor the mutant have ATP-dependent kinase activity. We can, therefore, reach no conclusions

TABLE 3. *PTS versus ATP-dependent kinase activity of crude cell extracts*[a]

Growth sugar	Phosphoryl donor	Sp act (nmol/mg of protein per min at 37°C)	
		Glucose	Fructose
Wild type			
Glucose	ATP	<0.008	<0.004
Glucose	PEP	0.75	0.075
Maltose	ATP	0.035	0.06
Maltose	PEP	1.3	0.03
Sucrose	ATP	<0.008	<0.004
Sucrose	PEP	1.5	0.8
Mutant[b]			
Sucrose	ATP	<0.008	<0.004
Sucrose	PEP	<0.008	0.6

[a] Enzyme preparations and assay conditions as described in Table 1, except for the use of either phosphoenolpyruvate (PEP) or ATP as phosphoryl donors.

[b] The mutant did not grow on either glucose or maltose.

from the effect of the PTS mutation on maltose utilization on the site of maltose hydrolysis. The answer to this question awaits direct experiments on maltose transport and metabolism.

Regulation of the concentration of intracellular cAMP by PTS sugars. In other bacteria, the presence of PTS substrates inhibits the induction of enzymes required for the utilization of alternate energy sources (12). Induction inhibition results from a combination of the reduction in the intracellular cAMP level and inhibition of the uptake of the inducer (i.e., inducer exclusion [18]). The following experiments show that, as in other bacteria, the PTS substrates fructose and glucose reduce the level of intracellular cAMP. The data of Fig. 1 and 2 show how the intracellular concentration of cAMP varied during growth in glucose and fructose. The intracellular level of cAMP bore an inverse relationship to the concentration of glucose in the medium during growth in a medium with an initial extracellular glucose concentration of 0.2% (Fig. 1).

Figure 2 shows the time course of the intracellular concentration of cAMP in glucose and fructose cultures in which the starting sugar concentration was reduced to 0.1%. In this latter case, there was no detectable sugar left in the culture medium after 8 h. Note that in both cases the intracellular concentration of cAMP rose after the exhaustion of sugar from the medium; however, the maximum level of cAMP reached in the fructose-grown cells was about half that obtained in glucose-grown cells. The level of cAMP was constantly lower in fructose-grown cells. The significance of this phenomenon is unknown.

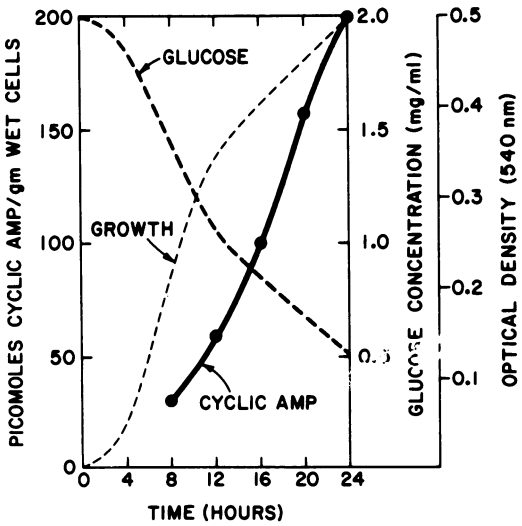

FIG. 1. *Variation of intracellular cAMP content and the glucose concentration of the medium during growth.*

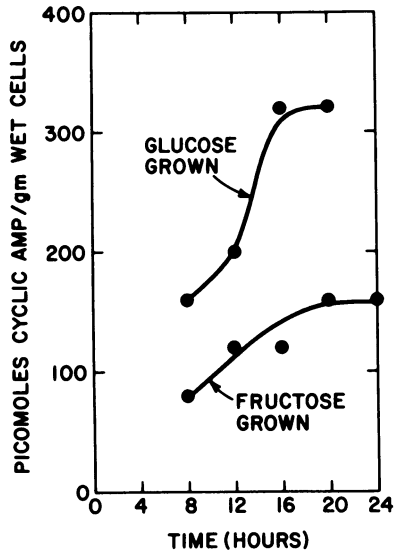

FIG. 2. *Variation of intracellular cAMP content during growth in glucose and fructose. Because of a high inoculum and a low initial sugar concentration (1 mg/ml), both sugars were exhausted by 8 h of growth. Each point represents the analysis of cells from a 500-ml culture.*

The experiments of Fig. 1 and 2 have shown that the presence of metabolizable sugars in the culture medium during growth appears to depress the intracellular concentration of cAMP. However, if a fermentable sugar was added to cells containing high concentrations of cAMP because of the exhaustion of sugar from the medium, the cAMP content of the cells dropped precipitously. Both glucose and fructose exhibited this ability to decrease the intracellular level of cAMP when added to cells taken from 24-h cultures grown in the presence of an initial fructose concentration of 0.1%. The cells had a maximal level of cAMP, since the sugar of the medium was completely used up. The dramatic reduction in the level of cAMP was followed by a rise as the glucose or fructose was consumed by metabolism. Since the ability of glucose and fructose to reduce the level of cAMP in *E. coli* and *S. typhimurium* depends upon a functional PTS (18), these sugars were added to the glucose-negative mutant cells that lacked a functional PTS for glucose but were normal for fructose. Only fructose was able to reduce the intracellular level of cAMP in these cells (Table 4). Thus, a functional PTS is required for a PTS sugar to be able to reduce the intracellular concentration of cAMP; glucose is not metabolized by the cells and has no effect on the level of cAMP.

TABLE 4. *Effect of fructose and glucose on the level of intracellular cAMP*[a]

Time after sugar addition (h)	Extracellular sugar (mg/ml)		Intracellular cAMP (pmol/g [wet wt] of cells)	
	Fructose	Glucose	Fructose-supplemented cells	Glucose-supplemented cells
1	0.85	1.10	50	200
2	0.6	1.08	100	200
3	0.3	1.08	180	200

[a] Glucose-negative mutant cultures were grown to the maximum stationary phase in a medium initially containing 0.1% fructose. At 24 h, no detectable fructose remained in the medium. Additional fructose or glucose was added to a final level of 1.0 mg/ml, and the incubation was continued at 37°C. The cultures were harvested at 1-h intervals, and the levels of sugar in the medium and cAMP in the cells were measured.

DISCUSSION

The phosphoenolpyruvate (PEP)-dependent sugar phosphotransferase system of *M. capricolum* can be represented to consist of two soluble, general proteins, enzyme I and HPr, and a sugar-specific enzyme II complex, which carry out sugar phosphorylation according to the following scheme:

$$\text{HPr} + \text{PEP} \xrightarrow{\text{EI}} \text{P}\sim\text{HPr} + \text{pyruvate}$$

$$\text{P}\sim\text{HPr} + \text{sugar} \xrightarrow{\text{EII complex}} \text{HPr} + \text{sugar-P}$$

The selection condition used to identify α-MG-resistant mutants, namely, ability to grow on fructose in the presence of α-MG, was expected to isolate enzyme II mutants, since enzyme I and HPr, according to the scheme presented above, are required for growth on fructose. However, the mutant analyzed in this report owes its resistance to α-MG to two modifications: (i) the expected loss of the α-MG (i.e., glucose-specific) enzyme II activity, and (ii) the replacement of the nonspecific HPr by a fructose-specific phosphocarrier protein, HPr^{Fru}.

The existence of sugar-specific phosphocarrier proteins that can replace HPr in the phosphoryl transfer reactions has been reported in other organisms. The first of these was reported by Anderson et al. (4, 23), who described a fructose-specific phosphocarrier protein, the K_m factor, in *Enterobacter* (*Aerobacter*) *aerogenes* that replaces HPr and shows a lower apparent K_m for fructose. The physical characteristics of the K_m protein are sufficiently different from those of HPr that it was readily separated from HPr by standard chromatographic techniques (4).

A fructose-induced phosphocarrier protein that can substitute for HPr was recently reported by Saier et al. (20). These authors showed that HPr-negative mutants of *S. typhimurium* (*ptsH*) unexpectedly could ferment and grow on fructose. They reported that growth of the *ptsH* mutants on fructose induces the synthesis of a heat-labile protein that can substitute for HPr in the in vitro phosphorylation of a number of other sugars, including α-MG.

The phosphocarrier protein of the fructose-grown, α-MG-resistant *M. capricolum* differs from the fructose-induced phosphocarrier of *S. typhimurium* in its substrate specificity. The mycoplasma protein has greatly reduced activity for α-MG; however, its molecular weight (judged by its elution from a Bio-Gel P-10 column) and heat stability are unchanged from that of the general HPr of glucose-grown cells. There is no evidence that this phosphocarrier, HPr^{Fru}, is induced when wild-type cells are grown on fructose, since the substrate specificity of the HPr fraction does not change. This can mean either that the HPr^{Fru} is not normally made in wild-type cells, whether they are grown in glucose or in fructose, or that HPr^{Fru} is a constitutive component together with the general HPr. The appearance of HPr activity in the mutant would then arise by either its synthesis in place of the missing HPr or its continued synthesis in spite of the loss of the HPr, respectively. Future purification and characterization of HPr^{Fru} will provide the basis for identifying the presence or absence of HPr^{Fru} in wild-type cells.

One of the reasons for choosing to study the PTS of mycoplasmas was the possibility that in these small-genome organisms the PTS, which is so complex in higher bacteria, might be simpler (i.e., reduced to its simplest limit). However, the PTS of mycoplasmas seems to be as complex as it is in higher bacteria (6, 7). The observations reported in this paper, that fructose utilization involves an inducible enzyme II complex as well as a specific phosphocarrier protein that can replace the general HPr, show that even in this complexity the mycoplasma PTS is similar to that of the higher bacteria.

The phosphotransferase system has always seemed to be too complex to be serving simply as a sugar transport system (17). The occurrence of a PTS of equal complexity in mycoplasmas appears to be even more extravagant. The mycoplasmas approach the theoretical minimum size for cellular organization and have the smallest genome size of all cellular organisms, about one half that of *E. coli* (21, 22). For an organism with this small a genome size to commit so much of its genome to so complex a system suggests a more fundamental role for the PTS than merely sugar transport. In *E. coli* and *S. typhi-*

murium, there is now increasing evidence that the PTS functions to regulate cell growth and the induction of enzyme synthesis (12). In these organisms, the phosphorylated proteins of the phosphoryl transport chain have been proposed to regulate induced enzyme synthesis by controlling the level of intracellular cAMP and by regulating inducer permease activity (12–14, 17, 18). For PTS sugars (sugars phosphorylated by the PTS), these regulatory effects require a functional enzyme II. No reports on the occurrence of cAMP in mycoplasma have previously appeared in the literature (12, 15). It was, therefore, decided to look for cAMP in *M. capricolum* and to study the effects of PTS sugars on its intracellular concentration. cAMP was found in *M. capricolum*, and the effect of glucose on the intracellular concentration of cAMP was similar to that originally reported by Makman and Sutherland for *E. coli* in 1965 (10) and since reported by many others (12). As in other bacteria, the intracellular level of cAMP remains low during growth while free glucose and fructose are present in the medium. However, the intracellular level of cAMP rises when the glucose (or fructose) is exhausted from the medium. If glucose or fructose is added back to cells with a high level of cAMP, the level of cAMP falls rapidly. Finally, the experiments with the glucose-negative strain show that glucose metabolism is required for glucose to be able to regulate the intracellular concentrations of cAMP. The mechanisms of these controls in *M. capricolum* are still to be explored. In *S. typhimurium* and *E. coli*, PTS sugars control the intracellular concentration of cAMP by a combination of the inhibition of cAMP synthesis and stimulation of nucleotide secretion (19). Which of these processes (or both) is involved in *M. capricolum* remains to be determined.

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