Identification and characterization of phosphoenolpyruvate:fructose phosphotransferase systems in three *Streptomyces* species

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***Streptomyces lividans*, *S. coelicolor* and *S. griseofuscus* were examined for the presence of the enzymes of the phosphoenolpyruvate:sugar phosphotransferase system (PTS). All three species were shown to possess Enzyme I, HPr and fructose-specific Enzyme II (II^{fr}) activities. In *S. lividans* and *S. coelicolor*, all three PTS enzymes were fructose-inducible, but in *S. griseofuscus* the system was expressed constitutively. These organisms apparently lack the HPr(Ser) kinase and HPr(Ser-P) phosphatase that characterize low-GC Gram-positive bacteria.**

Keywords: sugar transport, phosphorylation, phosphotransferase system, *Streptomyces*, regulation

INTRODUCTION

Streptomyces species are high-GC, Gram-positive, strictly aerobic soil actinomycetes. They differentiate with the development of fungal-like mycelia and spores (Schauer *et al.*, 1988; Hodgson, 1992). They represent one of the most important groups of organisms for medical and industrial applications (Chater & Hopwood, 1989, 1993) as they produce a diversity of antibiotics (Baltz & Seno, 1988; Chater, 1990) and extracellular enzymes (Nagaso *et al.*, 1988; McCarthy & Williams, 1992; Strickler *et al.*, 1992; Vats-Mehta *et al.*, 1990). Differentiation and the production of secondary metabolites, including antibiotics, are regulated by interrelated, complex mechanisms (Hopwood, 1988; Hong *et al.*, 1991; Distler *et al.*, 1992; Geistlich *et al.*, 1992; Ishizuka *et al.*, 1992; Ueda *et al.*, 1993). Moreover, these bacteria synthesize proteins that render them resistant to the antibiotics they produce. Horizontal transmission of the genetic material encoding these proteins is apparently in part responsible for the spread of drug resistance in bacterial populations (Mosher & Vining, 1992; Parr & Saier, 1992; Reizer *et al.*, 1992b; Schoner *et al.*, 1992). In order to understand the interrelationships between the syntheses of secondary metabolites and the metabolism of carbohydrates in these

bacteria, it is important to understand the pathways responsible for sugar catabolism (Martin & Demain, 1980; Hodgson, 1982; Vilches *et al.*, 1990; Delic *et al.*, 1992).

The phosphoenolpyruvate:sugar phosphotransferase system (PTS) is known to function in carbohydrate transport, chemotaxis, and a variety of regulatory capacities in diverse bacteria (Reizer *et al.*, 1988b; Titgemeyer, 1993). More than 20 different carbohydrates are transported via the PTS which exclusively uses phosphoenolpyruvate (PEP) as the phosphoryl donor in a phosphoryl transfer chain that involves the two general energy-coupling proteins, Enzyme I and HPr, as well as the sugar-specific, membrane-bound Enzyme II complexes. The Enzyme IIC permeases translocate the sugar substrates into the cytoplasmic compartment of the bacterial cell concomitant with phosphorylation (Saier & Reizer, 1992; Postma *et al.*, 1993).

Different types of regulatory phenomena such as catabolite repression, inducer exclusion and inducer expulsion have been described and characterized in enteric bacteria (Saier, 1989, 1993) and low-GC Gram-positive bacteria (Reizer *et al.*, 1993; Ye *et al.*, 1994a, b, c, d). In Gram-negative enteric bacteria, catabolite repression and the cytoplasmic levels of the inducing carbohydrates are regulated by the phosphorylation state of the IIA^{Glc} protein of the PTS (Saier, 1989; Saier & Reizer, 1992). By contrast, in low-GC Gram-positive bacteria, an ATP-

Abbreviations: DTP, diphosphoryl transfer protein; -P, phosphate; PEP, phosphoenolpyruvate; PTS, phosphotransferase system; TMG, thiomethyl β -galactoside (methyl β -D-thiogalactopyranoside).

dependent protein kinase that phosphorylates HPr of the PTS on seryl residue 46 is involved in the regulation of similar physiological processes (Ye *et al.*, 1994a, b, c, d). Moreover, in Gram-negative bacteria, PTS proteins apparently play regulatory roles in nitrogen utilization (Reizer *et al.*, 1992a) and β -hydroxybutyrate production (Pries *et al.*, 1991).

Although species of *Streptomyces* are able to utilize a large number of sugars for growth (Hodgson, 1980, 1982), knowledge concerning the pathways of carbohydrate metabolism and their regulation is still limited (Romano, 1986). Inducible or constitutive uptake has been reported for several sugars (Sabater *et al.*, 1972; Sabater & Asensio, 1973; Hodgson, 1982). Bacteria utilizing glucose, mannose and fructose exhibit activities for ATP-dependent glucokinase, fructokinase and mannokinase (Sabater *et al.*, 1972; Ikeda *et al.*, 1984; Angell *et al.*, 1992) suggesting that transport systems for these sugars are not of the PTS type. At least five different groups have reported evidence leading to the conclusion that the PTS is lacking from the genus *Streptomyces* (Sabater *et al.*, 1972; Romano & Margiotta, 1980; Novotna & Hostálék, 1985; Garcia-Dominguez *et al.*, 1989; L. Dijkhuizen, personal communication in Angell *et al.*, 1992). However, the PTS has been reported to be present in numerous low-GC Gram-positive bacteria (Reizer *et al.*, 1988a; Hoischen *et al.*, 1993; Mitchell *et al.*, 1993). It has also been found in a few high-GC Gram-positive bacteria including *Arthrobacter pyridinolis*, *A. ureafaciens*, *Corynebacterium glutamicum* and *Brevibacterium helvolum* (Reizer *et al.*, 1988a; V. P. Juban, L.-F. Wu, J. Reizer, C. Blanco & M. H. Saier, Jr, unpublished results). We have therefore re-examined the possibility that *Streptomyces* species possess a PTS by assaying for the presence of individual components of the system in three species of this genus. We chose *S. coelicolor* A3(2) and *S. lividans* TK23, which are genetically well characterized, as well as *S. griseofuscus* C581, a distantly-related fast-growing streptomycete. Our studies have led to the identification of two general energy-coupling proteins of the PTS, Enzyme I and HPr (Titgemeyer *et al.*, 1994), as well as fructose-specific Enzyme II complexes in all three strains examined. The properties of these PTSs are presented, and the system in *S. griseofuscus* is shown to differ significantly from those in *S. coelicolor* and *S. lividans*.

METHODS

Materials. These were commercially obtained and were of the highest purity available. [^{14}C]Fructose was purified free of anionic radioactive impurities by ion-exchange chromatography employing Bio-Rad AG1X2 (50–100 mesh) as described previously (Kundig & Roseman, 1971; Saier *et al.*, 1976).

Bacterial strains and growth conditions. *S. lividans* TK23, *S. coelicolor* A3(2)T2 and *S. griseofuscus* C581 were obtained from P. Solenberg and J. Tang (Eli Lilly Research Labs, Lilly Corporate Center, Indianapolis, IN 46285, USA). For preparation of crude cell extracts as well as cytoplasmic and membrane fractions, all three *Streptomyces* strains were grown for 24–72 h with vigorous shaking at 37 °C (500 ml volumes in 2 l Erlenmeyer flasks) in tryptic soy broth without dextrose (Difco). Sugars, when present, were added to a final concentration of 0.4%. Cells were

harvested by centrifugation in the early stationary phase of growth.

Growth responses to various carbon sources were recorded using salts medium 63 (Miller, 1972; Sabater *et al.*, 1972) containing 1% (w/v) carbohydrate and 15 mM asparagine (Vilches *et al.*, 1990). Of the carbon sources tested, all three *Streptomyces* strains were found to utilize fructose and glucose most avidly. Mannitol, galactose, xylose, raffinose, glycerol and succinate were also used by all three strains. Maltose and melibiose were utilized by *S. coelicolor* and *S. lividans* but not by *S. griseofuscus*. Glucitol was not utilized to an appreciable extent by any of these *Streptomyces* species after 3 d vigorous shaking at 37 °C.

PEP- and ATP-dependent *in vitro* phosphorylation experiments. Cell-free extracts were prepared by passage of washed cells through a French press at 10000 p.s.i. (69 kPa) and stored in aliquots at –70 °C in 20 mM Tris/HCl, pH 7.5, 3 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride (PMSF). Enzyme I and HPr were assayed by measuring [methyl- ^{14}C]methyl β -D-thiogalactopyranoside ([^{14}C]TMG) phosphorylation in the presence of complementing mutant extracts of *Staphylococcus aureus* that express the lactose operon constitutively but lack either Enzyme I or HPr, respectively [strains S710A (*ptsI*) or S797A (*ptsH*), respectively]. The assay procedure of Kundig & Roseman (1971), as modified by Saier *et al.* (1977), was used with [^{14}C]TMG (10 or 20 μM) at a specific activity of 5 $\mu\text{Ci } \mu\text{mol}^{-1}$ (185 kBq μmol^{-1}). The fructose-specific Enzyme II (II^{Fru}) preparations were assayed for PEP-dependent and ATP-dependent phosphorylation reactions using dialysed crude extracts of the three *Streptomyces* strains, while salt-washed membranes were used for the assay of fructose-1-P-[^{14}C]fructose transphosphorylation activities essentially as described previously (Saier *et al.*, 1977).

The salt-washed membranes used for assay of fructose-1-P:[^{14}C]fructose transphosphorylation activity were prepared by a modification of the procedure of Borkovich & Simon (1991). Membranes were washed twice by suspension and centrifugation with a solution containing 20 mM Tris/HCl, pH 7.5, 3 mM dithiothreitol, 0.1 mM PMSF and 2 M KCl. The washed membranes were centrifuged at 200000 g for 30 min at 4 °C, resuspended to a final protein concentration of between 20 and 40 mg ml $^{-1}$ in the same solution lacking KCl and stored at –70 °C. [^{14}C]Fructose (20 μM ; specific activity 5 $\mu\text{Ci } \mu\text{mol}^{-1}$, 185 kBq μmol^{-1}) was used for these assays. Phosphorylation of [^{14}C]mannitol, methyl α -[^{14}C]glucoside, 2-deoxy[^{14}C]glucose and [^{14}C]glucose (all at 5 $\mu\text{Ci } \mu\text{mol}^{-1}$) was assayed employing the same conditions. [^{32}P]PEP- and [^{32}P]ATP-dependent phosphorylation of *Streptomyces* PTS proteins or of purified PTS proteins from *Bacillus subtilis* was measured as described by Reizer *et al.* (1984, 1989). The specific activities of these two radioactive substrates were approximately 500 $\mu\text{Ci } \mu\text{mol}^{-1}$ (18.5 MBq μmol^{-1}), and they were used at a final concentration of 0.2 mM. Protein was determined by the method of Bradford (1976). Additional experimental details are provided in the legends to the figures and tables.

Partial purification of Enzyme I and HPr. Gel filtration was performed on French-pressed extracts derived from fructose-grown cells of the three *Streptomyces* species. A membrane-free extract of 0.1 ml (3–5 mg protein ml $^{-1}$) was loaded onto a Superose 6 HR10/30 column attached to a Pharmacia FPLC system. Proteins were eluted with a buffer containing 20 mM Tris/HCl, pH 7.5, 3 mM dithiothreitol, 0.1 mM PMSF and 15 mM KCl. The flow rate was 0.5 ml min $^{-1}$, and fractions of 0.2 ml were collected. For the determination of the molecular masses of Enzyme I and HPr of the three *Streptomyces* species,

Table 1. Identification of Enzyme I and HPr in extracts of three *Streptomyces* species by complementation assays

ptsH and *ptsI* mutant extracts of *Staph. aureus* were used at 130 µg protein for the *ptsH* extract and 90 µg protein for the *ptsI* extract per assay solution of 100 µl, with the *Streptomyces* extracts present in limiting amounts (10–600 µg protein). The *Streptomyces* extracts were prepared from cells grown either with (+) or without (–) fructose. Values were determined in triplicate and varied from the mean by less than 10%.

Enzyme assayed	Complementing <i>Staph. aureus</i> extract	<i>Streptomyces</i> extract	Phosphorylation activity [pmol [¹⁴ C]TMG-P min ⁻¹ (mg protein) ⁻¹]	
			– Fructose	+ Fructose
Enzyme I		<i>S. coelicolor</i>	≤ 2	≤ 2
		<i>S. lividans</i>	≤ 2	≤ 2
		<i>S. griseofuscus</i>	≤ 2	≤ 2
		<i>ptsH</i>	≤ 2	
		<i>ptsI</i>	≤ 2	
		<i>ptsI</i> + <i>ptsH</i>	1200	
	<i>ptsI</i>	<i>S. coelicolor</i>	90	730
		<i>S. lividans</i>	100	410
		<i>S. griseofuscus</i>	50	110
	HPr	<i>ptsH</i>	≤ 2	27
		<i>ptsH</i>	≤ 2	20
		<i>ptsH</i>	≤ 2	17

standard marker proteins were run separately under the same conditions.

RESULTS

Complementation assays for Enzyme I and HPr

Table 1 presents the results of complementation assays for the two energy-coupling proteins of the PTS, Enzyme I and HPr. The three *Streptomyces* extracts exhibited no detectable TMG phosphorylation activity in the presence of PEP [< 2 pmol TMG-P formed min⁻¹ (mg protein)⁻¹] (Table 1). The *S. aureus ptsH* and *ptsI* mutant extracts alone exhibited negligible activity; when these two mutant extracts were mixed, a high level of activity was observed (Table 1). Addition of extracts from broth-grown cultures of any one of the *Streptomyces* strains gave low activity for Enzyme I and non-detectable activity for HPr (Table 1). Growth in the presence of fructose enhanced both activities in all three *Streptomyces* species. The inductive effect of fructose on Enzyme I activity was maximal for *S. coelicolor* and minimal for *S. griseofuscus* (Table 1).

Fractionation of Enzyme I and HPr

Membranes were removed from the extracts by centrifugation, and the Enzyme I and HPr activities were shown to reside in the soluble fractions. These were passed through a calibrated gel filtration column. Extracts of all three *Streptomyces* strains exhibited HPr activities that eluted in the same position (estimated molecular mass 11 kDa). The *S. lividans* and *S. coelicolor* Enzymes I both eluted with an apparent molecular mass of 130 kDa ($\pm 10\%$) as expected for dimeric Enzyme I, but the *S.*

griseofuscus Enzyme I eluted as a protein of 70 kDa ($\pm 15\%$) as expected for monomeric Enzyme I (Grenier *et al.*, 1985). Since all three extracts were prepared in the same way, the results suggest a difference between the *S. griseofuscus* Enzyme I and those from *S. lividans* and *S. coelicolor*. It is worth noting that the latter two species are believed to be closely related while *S. griseofuscus* is more distant.

[³²P]PEP-dependent protein phosphorylation assays

The identity of Enzyme I and HPr in *Streptomyces* was confirmed by [³²P]PEP-dependent protein phosphorylation assays employing the protein fractions that were partially purified by gel filtration. As shown in Fig. 1 for *S. lividans*, a protein corresponding in position to Enzyme I from *Bacillus subtilis* could be phosphorylated with [³²P]PEP as the phosphoryl donor in the absence of any other protein fraction (lane 1). The *Streptomyces* Enzyme I could phosphorylate *B. subtilis* HPr (lane 2).

In lanes 5, 10 and 11 of Fig. 1, additional bands migrate just below the band corresponding to Enzyme I of *S. lividans*. These are most probably degradation products of Enzyme I. Such degradation products have been observed previously in other bacteria (LiCalsi *et al.*, 1991). *S. lividans* HPr was not phosphorylated by [³²P]PEP unless *B. subtilis* or *S. lividans* Enzyme I was added (Fig. 1, lanes 3–5, 10, 11). Two phosphorylated protein bands appeared in the size range between 7 and 15 kDa, as expected for HPr proteins (Reizer *et al.*, 1988a). One co-migrated with *B. subtilis* HPr while the other migrated slightly faster in the SDS gels. The presence of two bands could be explained either by the presence of two distinct species of HPr

Fig. 1. Phosphorylation of partially purified protein fractions from *S. lividans* and of homogeneous *B. subtilis* proteins using [³²P]PEP as the phosphoryl donor. The *S. lividans* Enzyme I and HPr were partially purified by gel filtration as described in Methods. *B. subtilis* proteins were purified to homogeneity as described previously (Reizer *et al.*, 1984, 1989). Protein additions were as indicated below the figure (F20, fraction from the gel filtration column of approximate molecular mass 20 kDa; F30, fraction from the gel filtration column of approximate molecular mass 30 kDa). The positions of standard *B. subtilis* Enzyme I, IIA^{Glc} and HPr are indicated to the left of the figure. Positions of *S. lividans* Enzyme I, IIA? and HPr are indicated to the far right of the figure.

proteins or by partial proteolytic degradation of a single HPr protein.

S. lividans Enzyme I and HPr phosphorylated the glucose-specific Enzyme IIA of *B. subtilis* (IIA^{Glc}; Fig. 1, lane 8), and the same phosphorylated protein was observed when *S. lividans* Enzyme I was added to the IIA^{Glc} protein with *B. subtilis* HPr (lane 9). These results show that the *B. subtilis* and *S. lividans* PTS proteins exhibit cross-reactivities. In lanes 10 and 11, *S. lividans* fractions containing proteins in the size range 20–30 kDa (F20, lane 10) and 30–40 kDa (F30, lane 11) were detected in the presence of *S. lividans* Enzyme I and HPr. At least one protein band (IIA?, lane 10) was phosphorylated, migrating just above the band corresponding to the IIA^{Glc} protein of *B. subtilis*. This may correspond to a *Streptomyces* IIA protein.

The partially purified Enzyme I of *S. coelicolor* was examined with respect to its capacity to phosphorylate various purified HPr and HPr-like proteins (data not shown). It could phosphorylate HPr proteins of *B. subtilis* and *S. coelicolor* as well as the diphosphoryl transfer protein [DTP; FruB(MH)] of *Salmonella typhimurium* (Sutrina *et al.*, 1988; Wu *et al.*, 1990). The phosphorylated

HPr of *S. coelicolor* co-migrated with one of the two putative *S. lividans* phospho-HPr proteins as well as phospho-HPr of *B. subtilis* (see Fig. 1). The HPr of *Escherichia coli* was not phosphorylated under the same conditions. These results suggest that the *S. coelicolor* Enzyme I exhibits specificity with respect to its phosphoryl transfer activity. It prefers Gram-positive HPr or the fructose-specific HPr domain (FPr) of the enteric bacterial DTP to the general HPr protein of enteric organisms. This last observation is consistent with the probable specificity of the *Streptomyces* PTS for fructose.

Apparent absence of HPr(Ser) kinase in the *Streptomyces* spp.

All low-GC Gram-positive bacteria so far examined possess the metabolite-activated protein kinase that transfers the γ -phosphoryl moiety of ATP to serine-46 of the PTS phosphocarrier protein, HPr (Deutscher & Saier, 1983; Reizer *et al.*, 1988b). This kinase is believed to function in catabolite repression, regulation of the activities of sugar:H⁺ symport permeases, regulation of a cytoplasmic sugar-phosphate phosphatase and regulation of the activities of the PTS itself (Saier *et al.*, 1994; Ye *et al.*, 1994a, b, c, d). As a result of the latter three processes, it regulates cytoplasmic inducer accumulation. We examined the three species of *Streptomyces* for the presence of this enzyme by incubating [³²P]ATP with cell-free extracts of these bacteria under a variety of conditions. The possibility of phosphorylation of endogenous *Streptomyces* HPr and the heterologous, exogenously added *B. subtilis* HPr was tested in the presence and absence of the various metabolites (fructose 1,6-diphosphate, gluconate 6-phosphate and 2-phosphoglycerate) that activate the kinase in low-GC Gram-positive bacteria. Surprisingly, phosphorylation of HPr could not be detected, even though other proteins in the extracts became phosphorylated. Positive controls established that under the same conditions, the kinase of *B. subtilis* exhibited the expected activities.

PEP- and ATP-dependent sugar phosphorylation

Attempts were made to demonstrate convincing PEP-dependent phosphorylation of various radioactive sugars. These sugars were selected based on the ability of the three *Streptomyces* species to utilize sugars (see Methods). PEP-dependent phosphorylation experiments were conducted in order to identify PTS activities towards fructose, glucose and mannitol. The fructose PTS is most widely distributed and may be the archetypal system (Saier *et al.*, 1985) while the glucose- and mannitol-specific systems are common to many Gram-negative and Gram-positive bacteria. The radioactive sugars used were glucose, methyl α -glucoside, 2-deoxyglucose, mannitol and fructose, and cells were grown in nutrient medium without a sugar or in the presence of glucose, fructose or mannitol. No phosphorylation was observed when methyl α -glucoside was the phosphoryl acceptor. When glucose or 2-deoxyglucose was the phosphoryl acceptor, no stimulation over background was observed when PEP

Table 2. PEP- and ATP-dependent phosphorylation of [14 C]fructose in dialysed extracts of *Streptomyces* species grown with and without fructose

In vitro phosphorylation of [14 C]fructose was carried out for 20 min at 37 °C using 80 µg protein of membrane-free cytoplasmic extract combined with 8 µg of membrane fraction per assay solution of 100 µl. Activity was detected only when PEP or ATP was present (50 mM final concentration). Values were determined in triplicate and varied from the mean by less than 10%. PEP-dependent phosphorylation activities decreased rapidly when extracts were stored on ice for longer than 20 min, while ATP-dependent phosphorylation activity remained stable. Therefore, PEP-dependent phosphorylation was assayed immediately after thawing of the extracts.

<i>Streptomyces</i> species	Sugar present during growth	Phosphorylation activity [pmol fructose-P min ⁻¹ (mg protein) ⁻¹]	
		Phosphoryl donor:	
		PEP	ATP
<i>S. lividans</i>	None	0	61
	Fructose	36	71
<i>S. coelicolor</i>	None	1.5	12
	Fructose	41	40
<i>S. griseofuscus</i>	None	2.5	15
	Fructose	4	10

was added, although ATP strongly stimulated phosphorylation. With mannitol as the phosphoryl acceptor, activity over background was again observed only when ATP was added. By contrast, when fructose was used as the substrate, phosphorylation was observed when either PEP or ATP was added, and in *S. lividans* and *S. coelicolor*, the PEP-dependent activity was inducible by growth in the presence of fructose (Table 2). In *S. lividans* and *S. griseofuscus*, ATP-dependent fructose phosphorylation activity was expressed constitutively, while in *S. coelicolor* it was inducible. In several experiments, the PEP-dependent

activity was as much as twofold higher than the ATP-dependent activity, and increasing the ratio of membrane protein to soluble protein tended to increase the PEP-dependent activity relative to the ATP-dependent activity (data not shown).

The ATP-dependent fructose phosphorylation activity proved to reside in the soluble fraction of the extract, while the PEP-dependent activity required both the soluble and the membrane fractions (data not shown). This observation suggested that a fructose-inducible, fructose-specific Enzyme II might be present in the membranes of these *Streptomyces* species. In order to confirm the presence of fructose Enzyme II activity in the *Streptomyces* extracts, the highly specific transphosphorylation assay was applied to salt-washed membrane preparations derived from cells grown with and without fructose. Fructose-1-P:[14 C]fructose transphosphorylation activity was assayed as a function of pH (Fig. 2). Both *S. coelicolor* and *S. lividans* exhibited fructose-inducible transphosphorylation activities (up to fivefold induction; Figs 2a and b) while *S. griseofuscus* exhibited low activity that was weakly fructose-repressible (Fig. 2c). These results are in general agreement with the PEP-dependent phosphorylation results presented in Table 2. No detectable transphosphorylation activity was observed when fructose-1-P was replaced by fructose-6-P, glucose-6-P or glucose-1-P in all three species examined (data not shown). This fact is reflective of the phosphoryl acceptor/donor specificity of the fructose-specific fructose-1-P-forming Enzymes II in other bacteria (see, for example, Mitchell *et al.*, 1993).

DISCUSSION

The observations reported above demonstrate the presence of a fructose-specific PTS consisting of Enzyme I, HPr and a fructose-1-phosphate-forming Enzyme II complex in three species of *Streptomyces*. Other sugar-specific proteins of the PTS such as those comprising Enzyme II complexes specific for glucose and mannitol are probably not present.

In previous reports several groups failed to detect the PTS in species of *Streptomyces* (see Introduction). In contrast to these reports we have used approaches that

Fig. 2. Activity/pH profiles for the fructose-1-P:[14 C]fructose transphosphorylation reaction measured with salt-washed membranes from (a) *S. coelicolor*, (b) *S. lividans* and (c) *S. griseofuscus* grown in the presence (■) or absence (□) of fructose. Membrane preparation and assay procedures were as described in Methods.

allowed us to assay for the individual components of the PTS phosphoryl transfer chain. Evidence for the presence of Enzyme I and HPr could be easily obtained using the complementation assay with defined *Staphylococcus aureus* mutants described in previous publications (Reizer *et al.*, 1988a; Mitchell *et al.*, 1993). The presence of a fructose-specific Enzyme II was clearly established by the detection of fructose-inducible PEP-dependent phosphorylation activity as well as fructose:fructose-1-P transphosphorylation activity.

Sabater *et al.* (1972) reported ATP-dependent phosphorylation of fructose, but they were unable to detect PEP-dependent phosphorylation of fructose in cell extracts of *S. violaceoruber*, which is genetically very closely related to *S. lividans* and *S. coelicolor*. Since we found that the PEP-dependent phosphorylation activity was unstable, losing activity when extracts were kept longer than 2 h at 0 °C, it is possible that protease activities in the *Streptomyces* extracts accounted for the negative results reported by Sabater *et al.* (1972). In *S. aureofaciens*, both PEP- and ATP-dependent fructose phosphorylation activities have been reported by Novotna & Hostálék (1985). The nonlinear kinetics of PEP-dependent phosphorylation activity, and the inability to detect fructose-1-phosphate kinase activity, led these investigators to conclude that a PTS is not operative. When we assayed for the activity of fructose-1-P kinase in *Streptomyces* crude extracts, we detected weak activity employing conditions that were optimal for the *E. coli* enzyme (data not shown). No attempt was made to optimize this assay for the *Streptomyces* enzyme.

Other investigators who attempted to identify PTS activities in *Streptomyces* species focused on the glucose-PTS in *S. clavuligerus* (Garcia-Dominguez *et al.*, 1989) and *S. griseus* (Romano & Margiotta, 1980). Their negative results were confirmed by our experiments. It should be noted that confirmatory experiments designed to detect immunological cross-reactivity using antibodies raised against purified Enzyme I or HPr yielded positive results when extracts derived from *S. griseus*, *S. albus* and *S. lividans* were examined (U. Wehmeier, personal communication).

Our inability to detect HPr(Ser) kinase activity in extracts derived from the three *Streptomyces* species examined under conditions that resulted in the facile detection of this activity in low-GC Gram-positive bacteria led us to believe that this protein, which plays an important regulatory role in low-GC Gram-positive bacteria (Reizer *et al.*, 1993; Ye *et al.*, 1994a, b), may not be present in *Streptomyces* species. However, other published results suggest that the PTS in *Streptomyces* may be involved in regulatory phenomena, such as catabolite repression (Hodgson, 1982, 1992; Delic *et al.*, 1992; Angell *et al.*, 1992) and the control of antibiotic biosynthesis (Distler *et al.*, 1992). In one case, that of oleandomycin production by *S. antibioticus*, the highest level of antibiotic production was observed when fructose served as the carbon and energy source (Vilches *et al.*, 1990). Furthermore, nutritional factors are known to influence antibiotic pro-

duction in several cases (Cortes *et al.*, 1986; Vilches *et al.*, 1990). The presence of a complete PEP-dependent PTS suggests that one or several of the PTS proteins may interact directly with other enzymes, and thereby control their activities, or they may function catalytically to phosphorylate target proteins, thereby acting as a protein kinase system. Both possibilities have been demonstrated for other bacteria (Saier, 1993).

The discovery of a fructose-specific PTS in *Streptomyces* species as well as our inability to detect an HPr(Ser) kinase leads to the possibility that components of the PTS function in *Streptomyces* to regulate carbon metabolism, secondary metabolite biosynthesis, antibiotic excretion and/or differentiation by novel mechanisms, dissimilar from those currently recognized in enteric and low-GC Gram-positive bacteria. Further studies will be required to elucidate the role(s) of the PTS in regulatory phenomena in the industrially and medically important bacterial genus *Streptomyces*.

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