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Properties of Two Sugar Phosphate Phosphatases from *Streptococcus bovis* and Their Potential Involvement in Inducer Expulsion

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***Streptococcus bovis* possesses two sugar phosphate phosphatases (Pases). Pase I is a soluble enzyme that is inhibited by the membrane fractions from lactose-grown cells and is insensitive to activation by S46D HPr, an analog of HPr(ser-P) of the sugar phosphotransferase system. Pase II is a membrane-associated enzyme that can be activated 10-fold by S46D HPr, and it appears to play a role in inducer expulsion.**

Streptococcus bovis is a rapidly growing, opportunistic bacterium that outgrows other bacteria when carbohydrates are plentiful (3). Gilbert and Hall (2) reported that *S. bovis* had a lactose operon similar to that of *Escherichia coli*. However, recent work has indicated that more than 20 strains of *S. bovis* have phosphotransferase systems for lactose transport (1, 3a). *S. bovis* JB1 uses glucose preferentially, has a diauxic pattern of glucose and lactose utilization, and will expel the nonmetabolizable lactose analog, methyl-b-D-thiogalactopyranoside (TMG), when glucose is added (1).

Inducer expulsion is a common method of regulation in low G+C bacteria (7). Because sugars accumulate intracellularly as sugar phosphates, inducer expulsion must involve a cytoplasmic dephosphorylation event. In *Streptococcus pyogenes* and *Lactococcus lactis*, glucose-dependent increases in intracellular fructose-1,6-bis-phosphate cause an allosteric activation of an ATP-dependent protein kinase (8, 12). The activated kinase then phosphorylates a seryl residue in phosphocarrier protein HPr. Because a mutant HPr with an aspartate rather than a serine at position 46 (S46D HPr; see reference 9) was able to promote TMG expulsion from membrane vesicles of *L. lactis* and a mutant having an alanine at position 46 (S46A HPr) could not, Ye et al. (12) hypothesized that serine-phosphorylated HPr activates the sugar phosphate phosphatase (Pase) and triggers TMG expulsion.

Pases have been purified from *Lactobacillus casei* (4) and *L. lactis* (11), and *L. lactis* appears to have two phosphatases (13). Pase I is a largely soluble enzyme similar to the one previously described by Thompson and Chassy (11). Pase II is a peripherally membrane-associated, strongly acidic, small, and heat-stable protein that is stimulated 10-fold by HPr(ser-P) or the S46D HPr mutant protein (13). The soluble Pase from *L. casei* was inactivated by a membrane component, but the significance of this phosphatase was not readily apparent (4).

S. bovis cells were grown anaerobically (1) with lactose (4 g/liter) as an energy source, harvested in the mid-logarithmic phase of growth by centrifugation (10,000 × *g* for 10 min at 4°C), washed twice in buffer (100 mM Tris HCl [pH 7.0], 10 mM $MgCl_2$, and 5 mM dithiothreitol), and broken with a French pressure cell (three passes at 10,000 lb/in_2 each). The protein concentration in the cell-free supernatant was determined by the method of Lowry et al. (6). Because TMG-6-phosphate is not commercially available, it was prepared by the method of Robrish et al. (10). Pase activity was determined by the method of Lowry and Lopez (5).

Extracts of *S. bovis* cells grown on lactose had three-fold more Pase I activity than those of glucose-grown cells (data not shown). The crude extracts from lactose-grown cells were ultracentrifuged (265,000 × *g* for 45 min at 4°C), and most of the Pase activity was found in the supernatant. This activity was designated Pase I. The particulate Pase could not be readily solubilized in buffer, and it was designated Pase II. Both Pases showed activity against a variety of sugar phosphates (2-deoxyglucose-6-P, fructose-1-P, glucose-6-P, galactose-6-P, and mannitol-1-P). No activity towards phosphoenolpyruvate was de-

TABLE 1. Fractionation and stimulation by S46D HPr of phosphatase activities in a crude extract of *S. bovis* JB1 and in various partially purified fractions[a]

Condition	Phosphatase activity (nmol min^{-1} mg of $protein^{-1}$)	Activation (fold)
Crude extract	11.0 ± 0.3	0
HPr (100 mM)	13.0 ± 0.1	1.2
S46A HPr (100 mM)	12.0 ± 0.2	1.1
S46D HPr (50 mM)	20.0 ± 0.3	1.8
Water-soluble fraction	34.0 ± 0.1	0
S46A HPr (100 mM)	36.0 ± 0.5	1.1
S46D HPr (50 mM)	35.0 ± 0.3	1.0
Membrane fraction	40.0 ± 0.8	1.0
S46A HPr (100 mM)	40.0 ± 1.1	1.0
S46D HPr (50 mM)	280.0 ± 33.0	6.9
Urea-butanol supernatant	80.0 ± 5.0	1.0
S46A HPr (100 mM)	90.0 ± 11.0	1.1
S46D HPr (50 mM)	710.0 ± 32.0	8.9
Pase I (DEAE-Sephacel I, peak 1)	180.0 ± 40.0	1.0
S46A HPr (100 mM)	210.0 ± 20.0	1.2
S46D HPr (50 mM)	240.0 ± 60.0	1.3
Pase II (DEAE-Sephacel I, peak 2)	230.0 ± 40.0	1.0
S46A HPr (100 mM)	240.0 ± 30.0	1.1
S46D HPr (50 mM)	2,600.0 ± 580.0	11.3

[a] Assay conditions were as reported previously (13), with mannitol-1-P (20 mM) being used as the substrate. The values for three experiments were averaged, and activities are reported as means ± standard deviations.

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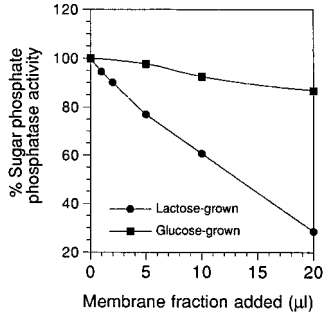

FIG. 1. The effect of added membranes from lactose-grown (0.11 mg of protein per μl) or glucose-grown (0.09 mg of protein per μl) cells on Pase (0.11 mg of protein per assay) activity. The membranes were incubated with the soluble fraction, and activity was measured after 30 min at 39°C with 20 mM fructose-1-phosphate.

tected. The affinity constants (K_m) for fructose-1-phosphate, 2-deoxyglucose-6-phosphate, and glucose-6-phosphate were all greater than 10 mM. Pase I activities for fructose-1-phosphate, mannitol-1-phosphate, and TMG-6-phosphate were similar, and fructose-1-phosphate and mannitol-1-phosphate were routinely used as substrates.

Ultracentrifuged extracts had 3.5-fold more activity than the crude extracts (Table 1), and this result suggested that the membrane fraction might be inhibiting Pase I. This hypothesis was supported by the observation that Pase I was strongly inhibited by the addition of the membrane fraction. Because membranes from lactose-grown cells but not those from glucose-grown cells inhibited Pase I (Fig. 1), it appeared that this membrane-mediated inhibition was a specific effect. Inhibition of Pase I activity could be relieved by high-speed centrifugation to remove membranes, and therefore the possibility that a membrane-bound protease could inhibit activity could not be ruled out. S46D HPr and S46A HPr had no effect on Pase I (Table 1).

Pase II had substrate specificities similar to those of Pase I (data not shown). Pase II was, however, stimulated 10-fold by S46D HPr. S46A HPr had no effect. Pase from the membrane pellets could be solubilized by urea-butanol treatment (13). The solubilized extract, when eluted from DEAE-Sephacel, yielded two activity peaks (Fig. 2). The Pase activity that eluted at a low salt concentration (Pase I) was not stimulated by S46D HPr, but the peak that eluted at a high salt concentration (Pase II) was stimulated 11-fold by 50 μM S46D HPr (Table 1). Membrane-derived Pase I and the soluble-fraction Pase had

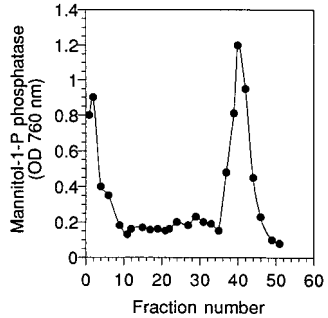

FIG. 2. Elution profile of Pase I and Pase II from a DEAE-Sephacel ion exchange column. The conditions were as described by Ye and Saier (13). Elution was effected with a linear salt gradient from 0.1 to 0.4 M NaCl. Pase II eluted at an estimated salt concentration of 0.3 M. OD, optical density.

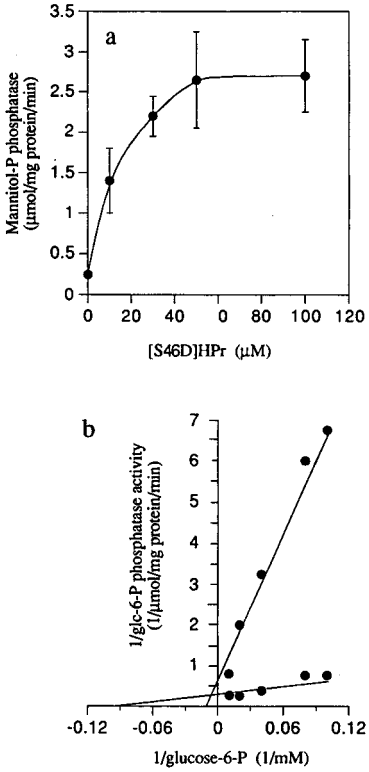

FIG. 3. Kinetic properties of Pase II. (a) Activation of Pase II as a function of the S46D HPr concentration. The substrate was 20 mM mannitol-1-P. The assay conditions were 50 mM MES (morpholineethanesulfonic acid) buffer, pH 7.0, and 20 mM $MgCl_2$ at 40°C as described by Ye and Saier (13). The K_m for S46D HPr was calculated to be 9 μM. Error bars show standard deviations. (b) Lineweaver-Burk double reciprocal plots of the glucose-6-P hydrolysis rates with and without S46D HPr (30 μM). The calculated K_ms and maximum rates of metabolism at 39°C were as follows: without S46D HPr, 70 mM and 570 nmol min^{-1} mg of $protein^{-1}$, respectively; with S46D HPr, 11 mM and 1,750 nmol min^{-1} mg of $protein^{-1}$, respectively.

similar substrate specificities and were both inhibited by the membrane fraction of lactose-grown cells (data not shown). Pase II could be saturated with S46D HPr, and 10 μM S46D HPr gave a half-maximal stimulation (Fig. 3). S46D HPr increased the maximum rate of metabolism and decreased the K_m of the enzyme for sugar phosphate. Similar results were previously reported for the Pase II of *L. lactis* (13).

On the basis of these and previous results (1), it appears that *S. bovis* has a method of inducer expulsion that is similar to that of *L. lactis*. The role of Pase I and its inhibition by membranes from lactose-grown cells are not yet clear, but they might involve a mechanism that provides for the sugar specificity of inducer expulsion. It would not be advantageous for cells growing on lactose to dephosphorylate lactose-6-phosphate and expel lactose.

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