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Presence of Protein Constituents of the Gram-Positive Bacterial Phosphotransferase Regulatory System in *Acholeplasma laidlawii*

CHRISTIAN HOISCHEN, ARNOUD DIJKSTRA, SHLOMO ROTTEM,† JONATHAN REIZER, AND MILTON H. SAIER, JR.*

Department of Biology, University of California at San Diego, La Jolla, California 92093-0116

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***Acholeplasma* species have been reported to lack a functional phosphoenolpyruvate:sugar phosphotransferase system (PTS). We show here that *Acholeplasma laidlawii* possesses activities of enzyme I, HPr, HPr(ser) kinase, and HPr(ser-P) phosphatase but lacks detectable activities of enzymes II of the PTS. HPr from this organism was purified, and the regulatory properties of the kinase and phosphatase were characterized and shown to differ from those of previously studied bacteria. The results suggest the presence of an incomplete PTS in *A. laidlawii* which has the potential to function in a unique regulatory capacity.**

Sugars are detected, transported, and phosphorylated in numerous bacterial species by a phosphoenolpyruvate (PEP): sugar phosphotransferase system (PTS) (12, 19, 23). The PTS consists of a protein phosphoryl transfer chain including two energy coupling proteins or protein domains, enzyme I and HPr, as well as the sugar-specific proteins, enzymes II (29). The PTS also functions in several regulatory capacities (1, 25, 26, 30). In gram-positive bacteria, HPr is phosphorylated by an ATP-dependent, fructose-1,6-bisphosphate (FBP)-activated kinase on a seryl residue, serine 46, as well as by the PEP-dependent, enzyme I-catalyzed reaction which derivatizes a histidyl residue, histidine 15 (14, 18). This regulatory system has been found in all gram-positive bacteria known to possess a PTS (14, 19), including heterofermentative lactobacilli which apparently lack enzymes I and II (16, 17, 22). Evidence suggesting that seryl phosphorylation of HPr functions in catabolite repression in *Bacillus subtilis* (30, 31), has recently been presented, but the functional significance of this reaction in other gram-positive bacteria has not been investigated.

Pioneering studies by Cirillo and Razin as well as Tarshis and his collaborators have shown that the small, wall-less mycoplasmas possess a functional PTS, whereas *Acholeplasma* species apparently lack such a system (3, 4, 17, 19). We have reinvestigated these claims and have confirmed that *Acholeplasma laidlawii* lacks a functional PTS. However, our analyses show that *A. laidlawii*, while lacking an intact PTS, nevertheless does possess some of the constituents of this system. While we have been unable to detect enzyme II activities in this organism, we do find functional enzyme I and HPr. Additionally, we detected levels of an HPr kinase and HPr(ser-P) phosphatase which are comparable to those found in other gram-positive bacteria. In some respects, the PTS regulatory system of *A. laidlawii* resembles that in *Lactobacillus brevis* (17, 22). However, the *Acholeplasma* system exhibits unique features: enzyme I is present, and the allosteric regulatory responses of the kinase to metabolites are different from those reported for other gram-positive bacteria.

* Corresponding author.

† Permanent address: Department of Membrane and Ultrastructure Research, Haddasa Medical School, The Hebrew University, Jerusalem, Israel.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The following bacterial species and strains were used in this study: *A. laidlawii* (strain OR), *Mycoplasma gallisepticum* (strain A5969), *Mycoplasma capricolum* (strain California kid), and *Staphylococcus aureus* (strain S797A [*ptsH*] and strain S710A [*ptsI*] (10). *A. laidlawii*, *M. gallisepticum*, and *M. capricolum* were grown aerobically without shaking at 37°C in complex medium (pH 8.0) consisting of brain heart infusion broth (13 g/liter), yeast extract (7 g/liter), Bacto Peptone (5 g/liter) (Difco Laboratories, Detroit, Mich.), NaCl (2.5 g/liter), HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (25 mmol/liter), potassium phosphate (10 mmol/liter), heat-inactivated (1 h at 56°C) horse serum (50 ml/liter) and ampicillin (200 mg/liter). Sugar, when present, was included at a concentration of 40 mmol/liter. Cells were harvested in the late logarithmic growth phase (10,000 × *g* for 15 min at 4°C) and washed three times in 10 mM HEPES buffer (pH 7.5) containing 250 mM NaCl. The cells were then lysed by resuspension in a minimal volume of 20 mM Tris-HCl buffer (pH 7.2) containing 5 mM $MgCl_2$, 3 mM dithiothreitol (DTT), and 0.1 mM phenylmethylsulfonyl fluoride (PMSF) (buffer A) followed by two passages through an Aminco French pressure cell at 100,000 lb/in^2. Cell debris and unbroken cells were removed by centrifugation (10 min at 10,000 × *g*), and the supernatants were used after dialysis against buffer A. Membranes were prepared by centrifugation for 120 min at 10,000 × *g*, rinsed with buffer A, resuspended in a minimal volume of the same buffer, and used for HPr(ser) kinase assays as described below. Transphosphorylation assays were performed with membrane preparations that were washed twice and resuspended in buffer A. *S. aureus* S797A (*ptsH*) and S710A (*ptsI*) were grown as described previously (19).

Enzyme assays. Sugar phosphorylation assays were performed essentially as described previously (11, 27, 28). Assay mixtures (final volume, 100 μl) contained 50 mM potassium phosphate buffer (pH 7.4), 12.5 mM $MgCl_2$, 25 mM KF, 2.5 mM DTT, 10 μM ^{14}C-labeled sugar (5 μCi/μmol), and either 5 mM PEP or 5 mM ATP. Enzyme I and HPr activities in *A. laidlawii* and *M. capricolum* were determined by complementation assays using 10 μM [^{14}C]thiomethyl β-galactoside ([^{14}C]TMG) (5 μCi/μmol) as the phosphoryl acceptor and 5 mM PEP as the phosphoryl donor with extracts of *S. aureus* S710A (*ptsI*) and S797A (*ptsH*), respectively, as described previously (17). Sugar transphosphorylation assays were con-

ducted with 10 μM ^{14}C-labeled sugar (5 μCi/μmol) and 10 mM sugar phosphate as described previously (27, 28).

The standard assay mixture (17) for ATP-dependent phosphorylation of HPr contained (final volume, 27 μl) 20 mM Tris-HCl buffer (pH 7.2), 2 mM $MgCl_2$, 5 mM KF, 3 mM DTT, 0.7 mM [γ-^{32}P]ATP (specific activity, 500 to 2,000 cpm/pmol), and purified HPr (20 μM) as indicated. For quantitative determination of HPr kinase activity, the radiolabeled HPr protein bands were cut out of the dried gels and counted in a liquid scintillation counter with 4 ml of Aqua mix (ICN, Irvine, Calif.). Alternatively, proteins were separated by nondenaturing polyacrylamide gel electrophoresis (PAGE) (15% polyacrylamide).

Purified HPr of *B. subtilis* (155 μg) was phosphorylated (90 min at 37°C) in a standard phosphorylation reaction mixture (final volume, 400 μl) containing washed membranes of *A. laidlawii* (180 μg of protein) as a source of ATP-dependent HPr kinase, 5 mM gluconate-6-P as an allosteric activator, and 0.7 mM [γ-^{32}P]ATP (specific activity, 1,000 cpm/pmol). The phosphorylated HPr was washed 10 times with 400 μl of standard phosphorylation buffer by ultrafiltration (Ultrafree-MC, type PLCC; 5,000-Da molecular size cutoff; Millipore, Bedford, Mass.) to eliminate [γ-^{32}P]ATP and resuspended in 300 μl of the same buffer. HPr(ser-P) phosphatase assay mixtures (final volume, 40 μl) contained phosphorylation buffer, [^{32}P]HPr(ser-P), and when indicated, P_i or PP_i as an activator or inhibitor of the phosphatase reaction. Phosphorylated HPr was identified by autoradiography following separation by sodium dodecyl sulfate (SDS)-PAGE, and residual [^{32}P]HPr was quantitated as described above.

For assays of β-glucosidase or phospho-β-glucosidase activities, the standard assay mixture contained the following (in 0.1 ml): Tris-HCl (pH 7.2), 20 mM; $MgCl_2$, 5 mM; DTT, 3 mM; and *p*-nitrophenyl-β-glucoside-6-P or *p*-nitrophenyl-β-glucoside, 1 mM. The reactions were started by adding an *A. laidlawii* crude extract and incubated at 37°C. After 20 min, the reactions were stopped by the addition of 25 μl of SDS (20%) and 1 ml of Na_2CO_3 (0.5 M), and A_{420} was determined.

Protein purification. Wild-type HPr proteins of *B. subtilis* and *A. laidlawii* and the various mutant HPr proteins and enzyme I of *B. subtilis* were purified as previously described (20, 21). HPr(ser) kinase of *A. laidlawii* was purified as follows: supernatant (200 ml) after high-speed centrifugation (120 min at 100,000 × *g*) of ruptured cells of *A. laidlawii* grown with glucose was diluted with 30 mM morpholine ethanesulfonic acid (MES) buffer (pH 6.2) containing 5 mM $MgCl_2$, 3 mM DTT, and 0.1 mM PMSF (buffer B) to a final volume of 2 liters and loaded onto a phosphocellulose column (24-ml bed volume) preequilibrated with the same buffer. The column was washed with 250 ml of buffer B, and proteins were then eluted with a linear concentration gradient (120 ml; 0 to 0.5 M) of potassium phosphate buffer (pH 6.5) in buffer B. Forty fractions (3 ml each) were collected and analyzed for HPr(ser) kinase activity, which eluted at approximately 210 mM potassium phosphate. Active fractions (30 ml) were pooled, dialyzed against buffer B, diluted to 250 ml with the same buffer, and then applied to an Affi-Gel Blue column (9-ml bed volume) that had been preequilibrated with the same buffer. The column was washed with buffer A, and HPr(ser) kinase was then eluted with a linear gradient (90 ml; 0 to 0.05 M) of ATP in the same buffer. The main HPr(ser) kinase activity eluted in fractions (3 ml) containing 35 to 40 mM ATP. Residual HPr(ser) kinase was subsequently eluted with 0.5 M potassium phosphate buffer (pH 7.2; 15 ml) containing 5 mM $MgCl_2$, 3 mM DTT, and 0.1 mM PMSF. Protein levels were determined by the method of Bradford (2).

RESULTS

Presence of PTS proteins in *A. laidlawii*. Table 1 summarizes experiments in which crude extracts from *M. capricolum* or *A. laidlawii* were examined for sugar phosphorylation by employing PEP as a potential phosphoryl donor. [^{14}C]TMG was used as the substrate in complementation assays (see above). Enzyme I and HPr activities were readily demonstrable in both the *M. capricolum* and *A. laidlawii* extracts, and the activities were comparable.

Table 2 summarizes data demonstrating the abilities of crude extracts from *A. laidlawii* and *M. capricolum* to phosphorylate either [^{14}C]2-deoxyglucose, [^{14}C]2-deoxyglucose, or [^{14}C]fructose with either ATP or PEP as the potential phosphoryl donor. It can be seen that in contrast to *M. capricolum*, which phosphorylated [^{14}C]glucose and [^{14}C]fructose with PEP but only slightly with ATP, *A. laidlawii* preferentially phosphorylated the three sugars with ATP. In fact, virtually no phosphorylation of either 2-deoxyglucose or glucose was observed when PEP served as the phosphoryl donor.

In order to further substantiate the conclusion that mycoplasma species but not *A. laidlawii* possess enzymes II of the PTS, the transphosphorylation reaction, which is catalyzed exclusively by the membranal enzymes II, independently of the soluble energy coupling proteins of the PTS (27, 28) was studied. *M. capricolum* exhibited both glucose-6-P:[^{14}C]glucose and fructose-1-P:[^{14}C]fructose transphosphorylation activities. A glucose-6-P:[^{14}C]glucose transphosphorylation activity was similarly observed with isolated membranes of *M. gallisepticum*. By contrast, these activities were barely detectable in isolated membranes from *A. laidlawii* (<2% of the activity observed for *M. capricolum* or *M. gallisepticum* membranes; data not shown). As expected, other sugar phosphates could not serve as phosphoryl donors in any of the membrane preparations tested.

A. laidlawii is capable of utilizing β-glucosides such as cellobiose as carbon sources for growth. [^{14}C]cellobiose is not available commercially, but one can evaluate whether a PTS or non-PTS utilization mechanism is operative by determining the nature of the substrate for which the β-glucosidic bond is hydrolyzed. In β-glucoside- and β-galactoside-metabolizing bacteria investigated to date, those which possess a PTS always

TABLE 1. Enzyme I and HPr activities in *A. laidlawii* and *M. capricolum* detected by complementation assays with *ptsI* and *ptsH* mutants of *S. aureus*[a]

Assay system	[^{14}C]TMG phosphorylation[b] (pmol/30 min)
S. aureus ptsI	12
S. aureus ptsI + enzyme I (*B. subtilis*)	278
S. aureus ptsH	4
S. aureus ptsH + HPr (*B. subtilis*)	174
A. laidlawii	4
A. laidlawii + *S. aureus ptsI*	59
A. laidlawii + *S. aureus ptsH*	104
M. capricolum	4
M. capricolum + *S. aureus ptsI*	53
M. capricolum + *S. aureus ptsH*	68

[a] Cell extracts of *A. laidlawii* and *M. capricolum* were prepared from cells grown on glucose. Assay mixtures with the indicated extracts or purified PTS proteins contained the following protein concentrations (in micrograms per 0.1 milliliter): *S. aureus ptsI* mutant, 110; *S. aureus ptsH* mutant, 80; purified enzyme I of *B. subtilis*, 4; purified HPr of *B. subtilis*, 6.7; *A. laidlawii*, 24; and *M. capricolum*, 5 to 12. The protein content in the control experiments which contained *A. laidlawii* or *M. capricolum* (with no additions) was 110 μg.

[b] PEP-dependent phosphorylation of [^{14}C]TMG.

TABLE 2. PEP- and ATP-dependent sugar phosphorylation activity of crude extracts of *A. laidlawii* and *M. capricolum*[a]

Extract	Phosphorylation activity of sugar (pmol/30 min)								
	[^{14}C]2-deoxyglucose			[^{14}C]glucose			[^{14}C]fructose		
	PEP	ATP	No donor	PEP	ATP	No donor	PEP	ATP	No donor
A. laidlawii	3	145	3	29	7,200	20	24	43	18
M. capricolum	195	29	14	ND[b]	ND	ND	247	32	24

[a] Assays were conducted as described in Materials and Methods with PEP or ATP as the phosphoryl donor. [^{14}C]2-deoxyglucose and [^{14}C]glucose phosphorylation assays were performed with extracts derived from glucose-grown cells, whereas [^{14}C]fructose phosphorylation assays were performed with extracts derived from fructose-grown cultures. The following protein concentrations (in micrograms per 0.1 milliliter) were used: *A. laidlawii* (glucose grown), 48; *A. laidlawii* (fructose grown), 55; *M. capricolum* (glucose grown), 118; and *M. capricolum* (fructose grown), 62.

[b] ND, not determined.

possess phosphoglycosidases, while those which utilize the saccharides by a non-PTS mechanism generally possess typical glycosidases.

β-Glucosidase assays were performed with either *p*-nitrophenyl-β-glucoside or *p*-nitrophenyl-β-glucoside-6-phosphate as the substrate. Both substrates were readily hydrolyzed by the *A. laidlawii* extract after growth in the presence of cellobiose. However, when either vanadate (10 mM) or potassium fluoride (20 mM) was included in the assay mixture, the activity with phospho-*p*-nitrophenyl-β-glucoside was abolished. By contrast, the activity of the *A. laidlawii* extract observed with *p*-nitrophenyl-β-glucoside was not inhibited by these agents (data not shown). Known phosphatases are strongly inhibited by these two agents (32). The purified *Escherichia coli* phospho-β-glucosidase (19a) was not inhibited by either vanadate or potassium fluoride, as expected. These agents presumably inhibited a *p*-nitrophenyl-β-glucoside-6-P phosphatase. The results therefore provide convincing evidence that *A. laidlawii* lacks a functional phospho-β-glucosidase and therefore by inference, a PTS for cellobiose.

To confirm the presence of HPr in *A. laidlawii* extracts, this protein was purified to near homogeneity and characterized. The HPr protein exhibited typical HPr-like properties. It had an apparent molecular mass of approximately 10,000 Da, as determined by gel filtration and SDS-PAGE, and it exhibited considerable heat stability (50% loss of activity after a 5-min incubation at 100°C). It catalyzed transfer of the phosphoryl group of phospho enzyme I to IIA^{Glc} of *B. subtilis* (21, 29; unpublished results), and it was phosphorylated by ATP and HPr kinase (see below).

A. laidlawii enzyme I was found to exhibit characteristics of other enzymes I. Thus, it phosphorylated purified HPr from *B. subtilis* or *A. laidlawii* and exhibited heat lability: it exhibited a 43% loss of activity after a 5-min incubation at 55°C and 84% loss after a corresponding incubation at 65°C.

Properties of *A. laidlawii* HPr(ser) kinase and HPr(ser-P) phosphatase. The purified HPr of *A. laidlawii* could be phosphorylated with [^{32}P]ATP as the substrate and an extract from this organism providing HPr kinase activity (Fig. 1, lanes 1). The resultant ^{32}P-labeled derivatized HPr was acid stable and base labile, similar to the well-characterized HPr(ser-P) of *B. subtilis* (Fig. 1).

The HPr(ser) kinase of *A. laidlawii* was found to be loosely associated with the membrane (~30%), as are the HPr(ser) kinases of other gram-positive organisms (6, 15). The cytoplasmic HPr kinase was partially purified from extracts of *A. laidlawii*. Two major bands of 45 and 90 kDa, as well as three minor bands in the 60-kDa range, were observed (data not shown). This partially purified kinase preparation was active in the phosphorylation of HPr from *B. subtilis* (Fig. 2, gel B, lane 3). Surprisingly, although the HPr of *A. laidlawii* was readily phosphorylated with a crude extract of *A. laidlawii* (Fig. 2, gel A, lane 2), it could be only weakly phosphorylated with the purified kinase from *A. laidlawii* (Fig. 2, gel B, lane 2) or *L. brevis* (Fig. 2, gel C, lane 1). Poor phosphorylation would be expected if the purified *A. laidlawii* HPr were already phosphorylated on serine 46. The presence of an HPr(ser-P) phosphatase in crude extracts of *A. laidlawii* (see below) would be expected to generate the native substrate, i.e., free HPr, for phosphorylation by the kinase.

The kinase of *A. laidlawii* was shown to be capable of phosphorylating the *B. subtilis* HPr at seryl residue 46. Although wild-type HPr and the H15A site-directed mutant protein of *B. subtilis* were readily phosphorylated by the ATP-dependent HPr kinase, no phosphoprotein product was detected when the S46A mutant protein of this organism was employed (data not shown). Not surprisingly, the purified HPr of *Bacillus stearothermophilus* was also phosphorylated by the *Acholeplasma* kinase. Altogether, these results provide compelling evidence for the conclusion that the *Acholeplasma* kinase phosphorylates heterologous HPr proteins as well as the endogenous substrate protein at seryl residue 46. They also

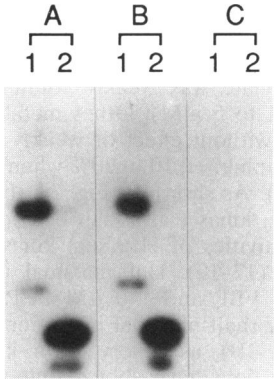

FIG. 1. Identification of HPr(ser)-P in *A. laidlawii* by nondenaturing PAGE (15% polyacrylamide) and selective hydrolytic procedures. The autoradiograms show the purified HPr protein of *A. laidlawii* (3 μg per lane) (lanes 1), and for comparison, the purified HPr from *B. subtilis* (5 μg per lane) (lanes 2), all phosphorylated by the *Acholeplasma* ATP-dependent HPr kinase. Gel A was washed with methanolic NaOH (0.1 N NaOH in 50% methanol) at 25°C. Duplicate gels were treated for 45 min in boiling 15% trichloroacetic acid (gel B) or washed for 45 min (60°C) in 1 N NaOH (gel C). Phosphorylation of HPr protein was performed for 60 min under standard assay conditions with *A. laidlawii* crude extract protein (16 μg of protein) as the source of the kinase activity.

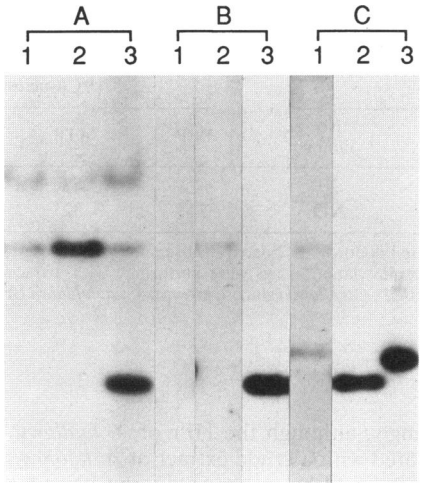

FIG. 2. ATP-dependent phosphorylation of different HPr proteins by crude and purified HPr kinases of *A. laidlawii* and *L. brevis*. Shown are autoradiograms of nondenaturing polyacrylamide gels (15% polyacrylamide). Gel A shows ATP-dependent phosphorylation by a crude extract of *A. laidlawii* (12 μg of protein per assay) without exogenous HPr (lane 1) and after the addition of purified HPr (2 μg per assay) from *A. laidlawii* (lane 2) or *B. subtilis* (lane 3). Gel B shows the ATP-dependent phosphorylation by purified HPr kinase of *A. laidlawii* without HPr (lane 1) and with purified HPr (2 μg per assay) of *A. laidlawii* (lane 2) or *B. subtilis* (lane 3). Gel C shows the ATP-dependent phosphorylation by purified *L. brevis* HPr kinase of purified HPr (2 μg per assay) from *A. laidlawii* (lane 1), *B. subtilis* (lane 2), or *L. brevis* (lane 3). Assays were performed for 60 min at 37°C under standard conditions. Autoradiograms were developed following 2- to 36-h incubations at −70°C.

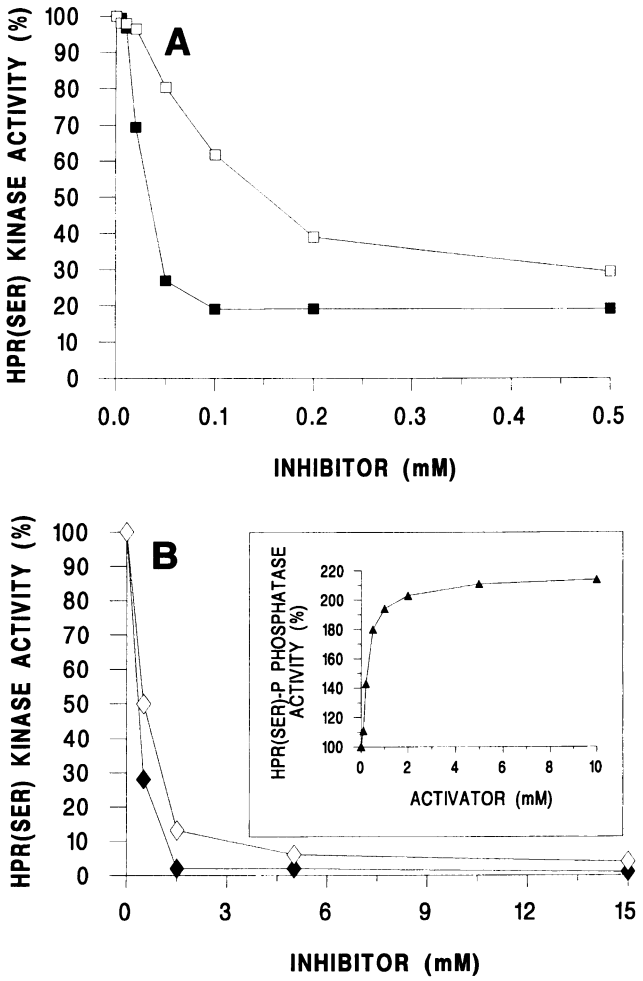

FIG. 3. Regulation of *A. laidlawii* HPr kinase and HPr(ser-P) phosphatase. ATP-dependent HPr kinase assays were performed under standard assay conditions for 30 min with 16 μg of *A. laidlawii* membrane protein per assay. Metabolites were added as indicated. 100% activity represents the activity of HPr kinase with no additions. (A) Inhibitory effect of FBP (■) and PEP (□). (B) inhibition of HPr(ser) kinase by phosphate (◆) and PP_i (◇). (Insert) Activation of HPr(ser-P) phosphatase by P_i. The phosphatase assay was performed as described in Materials and Methods. 100% phosphatase activity corresponds to the amount of HPr(ser-P) dephosphorylated after a 10-min incubation period under standard conditions (no addition).

demonstrate the broad specificity of this kinase for various gram-positive HPrs, similar to the previously reported specificities of HPr(ser) kinases from other gram-positive organisms (16–19).

Studies of the regulatory effects of various compounds on the activity of the partially purified HPr(ser) kinase from *A. laidlawii* surprisingly revealed that FBP was not stimulatory but was instead inhibitory. PEP also inhibited activity, although gluconate-6-phosphate was weakly stimulatory (10 to 40% stimulation with 1 to 5 mM). Other metabolites tested were either essentially without effect or weakly inhibitory (several sugar phosphates inhibited 10 to 20% when present at concentrations of 5 mM). As shown in Fig. 3A, both FBP and PEP inhibited HPr(ser) kinase, although both compounds readily stimulated the activities of HPr(ser) kinases of other gram-positive bacteria (17–19). Half-maximal inhibition was observed at 30 μM FBP and 140 μM PEP. P_i and PP_i were strongly inhibitory (half-maximal inhibition at 0.4 mM P_i and 0.5 mM PP_i Fig. 3B), as observed for kinases from other organisms. Other data have shown that the activity of the *Acholeplasma* HPr(ser) kinase was dependent upon the presence of divalent cations and was strongly inhibited by arsenate (data not shown).

Extracts of *A. laidlawii* exhibited HPr(ser-P) phosphatase activity comparable to that found in other gram-positive bacteria. The soluble activity was activated by P_i but inhibited by PP_i. The stimulatory effect of P_i was at most twofold, with a K_a of about 0.3 mM (see insert in Fig. 3B). 10 mM PP_i gave maximal inhibition (data not shown). These results are in qualitative agreement with those reported for the HPr(ser-P) phosphatase of *Enterococcus faecalis* (7).

DISCUSSION

Most HPr(ser) kinases of gram-positive bacteria are allosterically activated by FBP and to a lesser extent by gluconate-6-P and other phosphorylated metabolites. Phosphorylation of HPr on serine 46 by ATP strongly inhibits phosphorylation of this protein on histidine 15, as catalyzed by the PEP-dependent enzyme I of the PTS (7). Conversely, ATP-dependent phosphorylation of serine 46 is strongly inhibited by prior phosphorylation of HPr on the catalytic histidine (15). The potential physiological significance of these opposing phosphorylation events on PTS function have been discussed (7) and experimentally tested (14, 20, 21). Surprisingly, an inhibitory effect of HPr(ser) phosphorylation on the activity of the PTS could not be demonstrated in vivo (9, 20, 21). These observations and the fact that heterofermentative lactobacilli possess HPr, its ATP-dependent kinase, and HPr(ser-P) phosphatase but lack an

apparent PTS activity (17, 22) led to the postulate that HPr(ser) phosphorylation functions in the regulation of non-PTS functions (17, 18). Indeed, evidence suggesting that in *B. subtilis*, this phosphorylation event controls one form of catabolite repression (18, 24, 30).

Acholeplasma species use glycolysis as the principal pathway of carbohydrate metabolism with a unique PP_i-dependent phosphofructokinase (5). A functional PTS was not found in *A. laidlawii* because of the absence of enzyme II complexes, but we discovered that this organism does possess HPr and the enzymes which catalyze the cyclic interconversion of its two derivatized forms, the ATP-dependent kinase, HPr(ser-P) phosphatase, and PEP-dependent enzyme I. Because the PEP-dependent enzyme I and ATP-dependent kinase catalyze opposing reactions, it can be postulated that their activities exert opposite regulatory effects. That is, while phosphorylation of HPr on histidine 15 may regulate a particular process as a result of transfer of the phosphoryl group to as yet unknown target protein(s), phosphorylation of HPr at serine 46 would be expected to inhibit that same process. While the precise targets of these postulated opposing regulatory events have yet to be identified, it can be expected that they will differ from those in other characterized gram-positive bacteria. Thus, only in *A. laidlawii* does FBP exert an inhibitory effect on the ATP-dependent phosphorylation of HPr. The shape of the inhibition curve shown in Fig. 3A is not characteristic of a competitive process and consequently must reflect allosteric binding of the inhibitor. Allosteric activation of other HPr kinases has been reported. Similarly, PEP inhibits the *Acholeplasma* kinase by a noncompetitive mechanism (Fig. 3A). This result is particularly surprising in view of the fact that the responses of the *Acholeplasma* kinase and phosphatase to P_i (inhibitory and stimulatory, respectively) are the same as for other gram-positive bacteria (6, 8, 15). Perhaps the *Acholeplasma* kinase is subject to activation by an as yet unidentified metabolite. In any case, the results clearly show that different bacteria have different regulatory responses to metabolites, and these observations suggest, in turn, that different physiological systems are the targets of regulation.

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