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Regulation of Glycogen Concentration by the Histidine-Containing Phosphocarrier Protein HPr in *Escherichia coli*

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In addition to effecting the catalysis of sugar uptake, the bacterial phosphoenolpyruvate:sugar phosphotransferase system regulates a variety of physiological processes. In a previous paper [Seok *et al.*, (1997) J. Biol. Chem. 272, 26511-26521], we reported the interaction with and allosteric regulation of *Escherichia coli* glycogen phosphorylase activity by the histidine-containing phosphocarrier protein HPr *in vitro*. Here, we show that the specific interaction between HPr and glycogen phosphorylase occurs *in vivo*. To address the physiological role of the HPr-glycogen phosphorylase complex, intracellular glycogen levels were measured in *E. coli* strains transformed with various plasmids. While glycogen accumulated during the transition between exponential and stationary growth phases in wild-type cells, it did not accumulate in cells overproducing HPr or its inactive mutant regardless of the growth stage. From these results, we conclude that HPr mediates crosstalk between sugar uptake through the phosphoenolpyruvate:sugar phosphotransferase system and glycogen breakdown. The evolutionary significance of the HPr-glycogen phosphorylase complex is suggested.

Key words: allosteric regulation, *Escherichia coli* PTS, HPr, glycogen phosphorylase, protein-protein interaction

Bacteria have the capacity to efficiently adapt to environmental changes by switching on and off the utilization of a large number of carbon sources. One system that plays an important role in this adaptation response is the phosphoenolpyruvate : sugar phosphotransferase system (PTS). The PTS is composed of two general cytoplasmic proteins, enzyme I (EI) and histidine phosphocarrier protein HPr, that are used for all sugars and, in addition, some sugar-specific components collectively known as enzymes II (17). The primary function of this multifunctional system is transport of hexoses or hexitols across the cytoplasmic membrane by a mechanism that couples translocation to phosphorylation of the substrate. Glucose-specific enzyme II of *Escherichia coli* consists of two subunits, soluble enzyme IIA^{Glc} ($EIIA^{Glc}$) and membrane-bound enzyme $IICB^{Glc}$ ($EIICB^{Glc}$). Thus, glucose transport in *E. coli* involves three soluble PTS components (EI, HPr, and $EIIA^{Glc}$, encoded by the *ptsHIcrr* operon) and one membrane-bound protein, $EIICB^{Glc}$ (encoded by the *ptsG* gene). Glucose uptake entails sequential phosphoryl transfer via the PTS, as follows: Phosphoenolpyruvate (PEP) $\Rightarrow$ EI $\Rightarrow$ HPr $\Rightarrow$ $EIIA^{Glc}$ $\Rightarrow$ $EIICB^{Glc}$ $\Rightarrow$ glucose.

Since the PTS was discovered in *E. coli* nearly 35 years ago (6), additional studies have documented other metabolic roles of this system. The regulatory functions of PTS depend on the phosphorylation state of the involved component that increases in the absence and decreases in the presence of a PTS sugar substrate. The ratio of phosphorylated to dephosphorylated proteins in turn serves as signal input for the control of these physiological processes. Unphosphorylated EI interacts with and regulates the autophosphorylation activity of CheA to trigger chemotaxis towards PTS carbohydrates (9). Unphosphorylated HPr interacts with and stimulates the activity of glycogen phosphorylase (GP) (25). Unphosphorylated $EIIA^{Glc}$ inhibits glycerol kinase and several sugar permeases by a mechanism termed inducer exclusion (17, 26). Phosphorylated $EIIA^{Glc}$ stimulates cAMP synthesis (13). As expected for a system as central to bacterial metabolism as the PTS, synthesis of the PTS proteins is regulated in a highly sophisticated way. The main transcriptional regulator of the operons encoding the PTS was recently shown to be Mlc (5, 15, 16). Furthermore, it has also been reported that unphosphorylated $EIICB^{Glc}$ recruits global repressor Mlc to induce the Mlc regulon in the presence of glucose (8, 11, 30).

Although we fished out GP using HPr as the bait and confirmed interaction with and allosteric regulation of GP activity by HPr in *E. coli* (25), the physiological role of the HPr-glycogen phosphorylase complex had not been determined. In this report, we address the physiological

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importance of the HPr-glycogen phosphorylase complex by determining intracellular glycogen levels in *E. coli* strains transformed with various plasmids designed to overproduce GP, HPr and HPr (H15A). The evolutionary significance of the physical interaction between HPr and GP and the metabolic crosstalk between glucose uptake and glycogen breakdown is discussed.

Materials and Methods

Strains and culture conditions

E. coli strain GI698 (7) encodes the gene for the λ *cI* repressor under control of the *trp* promoter. This strain was grown in synthetic medium as described previously (24). For determination of the intracellular concentration of glycogen, transformants of *E. coli* strain GI698 with appropriate plasmids [pRE1 for the control (21), pGP for overproduction of GP (25), pSP100 for overproduction of HPr (3), or pSP100 (H15A) for overproduction of HPr (H15A) (25)] were cultured at 30°C in synthetic minimal medium containing per liter: 1 g $(NH_4)_2SO_4$, 10.5 g K_2HPO_4, 4.5 g KH_2PO_4, 1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.1 g ampicillin, 30 mg thiamine, 0.1 g tryptophan, 30 mg threonine, 30 mg methionine, 30 mg histidine, and 5 g glucose.

Proteins

E. coli GI698 transformed with pRE1-*ptsHIcrr* (11) was used for the overproduction of the soluble PTS proteins (EI, HPr, and $EIIA^{Glc}$) and these proteins were purified as described previously (24). Glycogen phosphorylase was expressed and purified using GI698 transformed with expression vector pGP as described previously (25).

Measurement of protein-protein interaction

The real-time interactions of glycogen phosphorylase and PTS proteins were monitored by SPR detection using a BIAcore 3000 (Pharmacia Biosensor AB, Uppsala, Sweden). PTS proteins were immobilized onto the carboxymethylated dextran surface of a CM5 sensor chip (11, 25). Enzyme I (70 μl, 20 μg/ml), HPr (70 μl, 100 μg/ml), or $EIIA^{Glc}$ (70 μl, 100 μg/ml) in coupling buffer (10 mM sodium-formate, pH 4.0) was allowed to flow over a sensor chip at 10 μl/min to couple the protein to the matrix by an NHS/EDC reaction (70 μl mix). Unreacted *N*-hydroxysuccinimide was inactivated by injecting 70 μl of 1 M ethanolamine-HCl, pH 8.0. A blank surface was prepared by activation and inactivation of the sensor chip without any protein immobilization. Assuming that 1000 response units (RU) corresponds to a surface concentration of 1 ng/mm^2, EI, HPr, and $EIIA^{Glc}$ were immobilized to a surface concentration of 2.9, 1.4, and 2.0 ng/mm^2, respectively. The standard running buffer was 10 mM HEPES (pH 7.4), 150 mM NaCl, and 0.005% Tween 20, and all reagents were introduced at a flow rate of 10 μl/min. The sensor surface was regenerated between injections by allowing 10 μl of water to flow over it to remove the bound analyte.

Determination of glycogen and glucose

Intracellular glycogen levels were measured according to Parrou and François (12) and glucose concentrations were measured using glucose oxidase mixture (Sigma) following the supplier's instruction with some modifications. Cells (corresponding to the number of cells in 2 ml of culture at A_{600} = 1.0) were withdrawn every two hours and centrifuged at 10,000 × *g* for 5 min. Cell pellets were used to assay intracellular glycogen, while supernatants were used to determine glucose remaining in the culture media. Each harvested cell pellet was quickly washed with 10 mM Tris-HCl, pH 7.5, containing 100 mM NaCl, resuspended in 0.25 ml of 0.25 M Na_2CO_3 using a screw-cap Eppendorf tube, and incubated at 95°C for 4 h to make cells permeable to amyloglucosidase. The mixture was brought to pH 5.2 by addition of 0.15 ml 1 M acetic acid and 0.6 ml of 0.2 M sodium acetate, pH 5.2. This suspension was incubated at 57°C overnight with 1.2 units of *Aspergillus niger* amyloglucosidase (Boehringer Manheim) and centrifuged at 10,000 × *g* for 5 min. Glucose, originating from glycogen, was determined on 20 μl aliquots of supernatant solution (without dilution) by addition of 200 μl combined enzyme-color reagent solution, incubating at 37°C for 40 min, and reading at 450 nm in a ELISA reader apparatus. To prepare combined enzyme-color reagent solution, 200 mg of enzyme mixture containing 100 units of *Aspergilus niger* glucose oxidase, 20 Purpurogalin units of horse radish peroxidase and buffer salts was dissolved in 20 ml of distilled water, and mixed with 320 μl *O*-dianisidine dihyrdochloride solution (2.5 mg/ml in distilled water). To determine glucose in the medium, each aliquot of culture broth was diluted 100-fold in water, and 20 μl of diluted solution was mixed with 200 μl combined enzyme-color reagent solution and assayed as described above.

Results

Highly specific interaction between HPr and glycogen phosphorylase

Previously we found that glycogen phosphorylase (GP) in *E. coli* is regulated by HPr. To confirm whether GP specifically interacts with HPr but no other PTS proteins, homogeneous GP was tested for binding to different immobilized protein surfaces (Fig. 1) prepared as described under Materials and Methods. GP (8 μg/ml) was allowed to flow (rate of 10 μl/min) over each surface for 10 min. GP showed the expected binding to immobilized HPr, but not to immobilized EI or $EIIA^{Glc}$. A blank surface (see Materials and Methods) showed no interaction with puri-

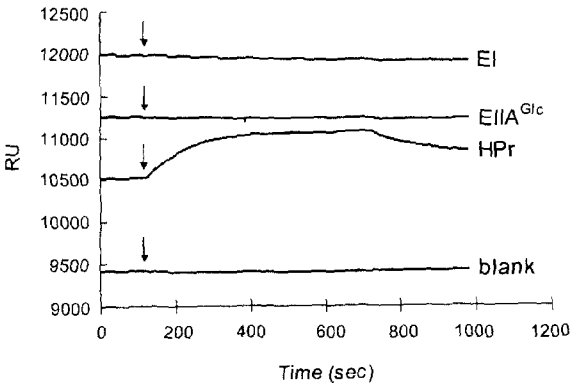

Fig. 1. Interaction of the purified *E. coli* glycogen phosphorylase with different surfaces. A CM-5 sensor chip surface was immobilized with EI, $EIIA^{Glc}$, HPr, and no protein (blank) as described in Materials and Methods. The purified glycogen phosphorylase (8 μg/ml in the standard running buffer) was tested for binding to these different surfaces. Arrows indicate the starting points of injection. RU, response units.

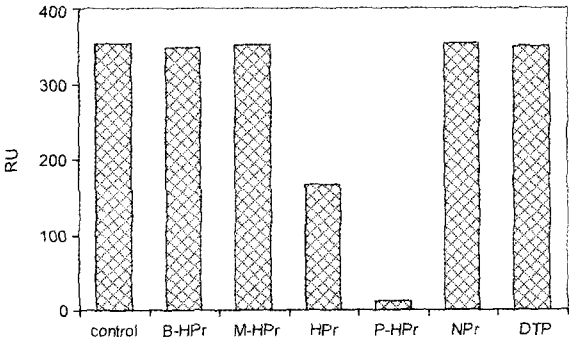

Fig. 2. Competition for binding of glycogen phosphorylase to immobilized HPr. Proteins (2 μg/ml each) were preincubated with GP (2 μg/ml) in standard running buffer supplemented with 1 mM $MgCl_2$ and 1 mM dithiothreitol, then the mixtures were allowed to flow over immobilized HPr. To phosphorylate HPr, 1 mM PEP and 1 μg/ml of EI were included in the incubation mixture. The increase in number of response units (RU) obtained during the 10 min monitoring period for each mixture was plotted as a bar graph: control, no competitor; B-HPr, *B. subtilis* HPr; M-HPr, *M. capricolum* HPr; HPr, *E. coli* HPr; P-HPr, *E. coli* phospho-HPr; NPr, *E. coli* NPr; DTP, *E. coli* DTP as a competitor.

fied GP.

Since *E. coli* HPr shares a high degree of identity in amino acid sequence with *E. coli* NPr (encoded by a *rpoN*-neighboring gene) and *E. coli* DTP (the diphosphoryl transfer protein encoded by the *fruB* gene) as well as HPrs from other bacteria, it was of interest to test the binding of GP to those proteins. A competition approach was taken to further evaluate the specificity of the protein interaction with GP. Fig. 2 shows that, if free *E. coli* HPr was included in the test solution with GP, the binding of GP to immobilized HPr was reduced. *E. coli* GP was preincubated with the following proteins (2 μg/ml) in the standard running buffer: *B. subtilis* HPr (B-HPr) or *M. capricolum* HPr (M-HPr), *E. coli* NPr (NPr), *E. coli* DTP (DTP), *E. coli* HPr (HPr), or *E. coli* HPr under conditions where it became phosphorylated (P-HPr; see Fig. 2, legend), and each mixture was allowed to flow (10 μg/ml) over the immobilized HPr surface. The response unit (10 minute monitoring period) was compared to the signal obtained when GP was tested alone (control). *E. coli* HPr competed with immobilized HPr for the binding with GP and gave about 60% competition compared to the control. However, HPrs from other bacterial strains did not compete for GP binding. It should be noted that neither NPr nor DTP from *E. coli* interacts with *E. coli* GP, although NPr (18) and DTP (22) also accept a phosphoryl group from EI in *E. coli*. Neither EI nor $EIIA^{Glc}$ influenced the binding of GP to immobilized HPr (data not shown), as expected from the results in Fig. 1. Frequently, strength of the protein-protein interaction is modulated by the phosphorylation state of subunits. Because *E. coli* GP lacks the phosphorylation site characteristic of yeast and mammalian GPs, the effect of phosphorylating HPr on its affinity for GP was checked by a competition experiment (Fig. 2, column P-HPr). When HPr (2 μg/ml) was preincubated with GP under conditions where it became phosphorylated, it almost completely eliminated the binding. This result agrees with our previous finding that P-HPr has about 4 times higher affinity towards GP than dephospho-HPr (25). These data support the conclusion that the binding of *E. coli* GP is highly specific for *E. coli* HPr regardless of its phosphorylation state.

HPr interacts with and regulates activity of glycogen phosphorylase in vivo

Because the cellular concentration of HPr in *E. coli* is significantly higher than that of GP (2, 10), and both dephospho- and phospho-HPr have high affinity for GP, it was assumed that, *in vivo*, *E. coli* GP might be always bound to HPr and that physiological perturbations that shift the ratio of HPr to P-HPr lead to changes in the activity of GP and intracellular level of glycogen (25).

If GP interacts with HPr *in vivo* as tightly as reported previously for *in vitro* (25), overexpression of GP might sequester HPr and inhibit the transport of PTS sugars. To test this question, the phenotype of *E. coli* GI698 strains overexpressing GP and HPr was examined on MacConkey agar plates containing various PTS and non-PTS sugar substrates (Table 1). In pRE1-based plasmids used in this work, genes are under the control of the strong λP_L promoter-cII ribosome binding site combination (21). Colonies of GI698 harboring pRE1 or pSP100 (pRE1-based recombinant plasmid constructed for the overexpression of *E. coli* HPr) on plates containing readily fermentable sugars showed a reddish-purple color 8 hr after spotting at 30 °C. When non-PTS sugars such as lactose, maltose, and sucrose were used as the substrate, there were no remarkable differences between GI698 harboring pGP (pRE1-based recombinant plasmid constructed for

Table 1. Fermentation patterns of *E. coli* strains transformed with indicated plasmids on various sugar substrates

Sugars added	GI698/pRE1	GI698/pSP100	GI698/pGP
PTS sugars			
Glucose	++	++	-
Mannose	++	++	-
Mannitol	++	++	-
Non-PTS sugars			
Lactose	+	+	+
Maltose	++	++	++
Sucrose	-	-	-

Fermentation rate was checked on the basis of color development on a MacConkey agar plate containing indicated sugars (1%), and each experiment was repeated three times. −, no fermentation; +, slow fermentation; ++, fast fermentation.

the overexpression of *E. coli* GP) and the strain containing pRE1 or pSP100. When PTS sugars were included in MacConkey plates, however, GP overexpression seriously retarded the fermentation rate of those PTS sugars regardless of the sugar substrates (Table 1). These results indicate that HPr tightly interacts with GP *in vivo* to regulate activity of glycogen phosphorylase.

Since it is established that mutants, in which the phosphorylatable residue His15 has been replaced with Ala or Glu, as well as the wild-type HPr, activate GP activity (25), we employed another approach to verify that HPr interacts with and regulates the activity of GP *in vivo*. If the inhibition of the PTS sugar transport by overexpression of GP, as shown in Table 1, is solely due to sequestration of HPr, overexpression of either wild-type HPr or its mutant at His15, in turn, should change the intracellular level of glycogen. We examined this question using *E. coli* strain GI698 (7, 24) transformed with pRE1, pGP, pSP100, and pSP100(H15A) (Fig. 3). Intracellular glycogen concentration was measured using amyloglucosidase- and glucose oxidase-coupled assay as described in Materials and Methods. In cells overproducing GP as well as in those transformed with the control vector pRE1, glycogen was accumulated during the transition from exponential growth into the stationary phase, as reported for the wild-type cells of *E. coli* and other glycogen-accumulating microorganisms (19). However, in cells overproducing HPr and HPr(H15A), glycogen was never accumulated regardless of the growth phase (Fig. 3C). The negative effect of overproduction of HPr(H15A) on glycogen accumulation was stronger than that of HPr, indicating that some of overproduced HPr exists in the phospho-form. These results suggest that the amount of unphosphorylated HPr but not that of GP is critical for the regulation of the intracellular concentration of glycogen. It should be noted that there was little difference in the amount of glucose remaining in media between cultures of tested cells (Fig. 3B) except GI698 transformed with pSP100(H15A). In the case of cells over-

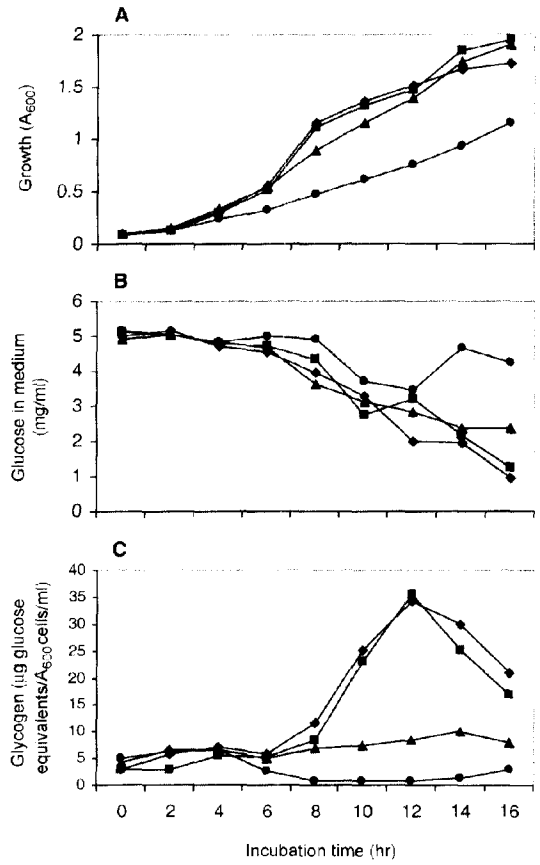

Fig. 3. HPr regulates the intracellular level of glycogen during the growth cycle in *E. coli*. Intracellular glycogen level and glucose concentration in the media were measured as described in Materials and Methods using amyloglucosidase- and glucose oxidase-coupled procedure. (A), (B), and (C) show growth rates, the amounts of glucose remaining in the medium, and intracellular glycogen concentrations of cells harboring different plasmids, respectively: Rectangle, GI698/pRE1 as a control; diamond, GI698/pGP; triangle, GI698/pSP100; and circle, GI698/pSP100(H15A).

producing HPr(H15A), growth was retarded and the glucose consumption rate was slower than other cells (Fig. 3A & 3B).

However, it may be argued that overproduction of HPr or its mutant might exert an effect on the phosphorylation state of $EIIA^{Glc}$, and the changed level of P-$EIIA^{Glc}$ might alter the cellular concentration of cAMP. Since the cAMP receptor protein-cAMP complex regulates *glgCAP* expression, we clarified that the decreased level of glycogen in cells overproducing HPr and its mutant was due to the direct interaction of HPr with GP but not dependent on cAMP. The cellular level of glycogen in the wild-type cells and those harboring pSP100 was not affected by the addition of 0.5 mM cAMP in the culture medium (data not shown). These results support that overproduction of HPr or its mutant HPr(H15A) markedly diminishes the accumulation of glycogen by activating GP through the physical interaction *in vivo*.

Discussion

Physiological role of HPr-glycogen phosphorylase interaction

Glycogen and other similar α1,4-glucans have been reported in over 40 different bacterial species; the concentration of glycogen accumulated can be over 50% of the cell dry weight (19). In *E. coli*, a single operon, *glgCAP*, encodes genes for ADP-glucose pyrophosphorylase, glycogen synthase, and GP (32). Synthesis of glycogen involves ADP-glucose pyrophosphorylase and glycogen synthase, while breakdown involves GP. Regulation of *glgCAP* expression occurs coordinately. Positive and negative regulations are exerted by cAMP and the CsrA gene product, respectively (23, 32). GP catalyzes the breakdown of glycogen into glucose-1-phosphate while the PTS effects the uptake of sugar substrates, like glucose, and hence both play a central role in carbohydrate metabolism.

In this report, employing an amyloglucosidase- and glucose oxidase-coupled procedure, we showed that HPr regulates the intracellular concentration of glycogen through interaction with and allosteric regulation of GP activity *in vivo*, and thus, crosstalk between these two important carbon metabolic pathways is mediated by the phosphorylation state of HPr of the *E. coli* PTS (see results in Table 1 and Fig. 3). Furthermore, it was shown that the amount of unphosphorylated HPr but not that of GP is critical for the regulation of the intracellular glycogen level. Because the phosphocarrier function of HPr in the PTS allows the protein to exist in both phospho- and dephospho-forms, data in this study suggest that glycogen degrades in the presence of glucose.

Many studies suggest that glycogen provides carbon and energy to prolong viability during the stationary phase by sparing essential macromolecular components such as proteins and rRNA from turnover (19, 28, 32). Many bacteria and yeast accumulate glycogen in either the stationary phase or stress conditions such as nitrogen or sulfur starvation, heat shock or osmotic stress. In *E. coli*, the rate of growth and quantity of glycogen accumulated are inversely related when cells are grown in nitrogen-limited, glucose-containing media. The ratio of phosphorylated to dephosphorylated forms of soluble PTS proteins changes depending on growth phase, and phosphorylated forms might prevail under starvation conditions. The phosphorylated form of $EIIA^{Glc}$ will increase cellular cAMP levels. Consistent with the concept that cAMP levels rise in the stationary phase (13), *glg* operon expression is increased in this phase (19, 32). Because regulation of *glgCAP* expression occurs coordinately, there must be other mechanism(s) for the regulation of glycogen breakdown distinct from its synthesis to explain the selective accumulation of glycogen in the stationary phase (2). This differential regulation is accomplished by HPr in *E. coli*.

During the transition from stationary phase to a new round of growth, the demand for biosynthetic metabolism increases and a number of metabolic changes occur. *E. coli* as well as many other microorganisms rapidly degrade available glycogen to accommodate the increased growth rate. The decrease in cellular cAMP level characteristic of the entry into logarithmic growth phase (13) turns off the expression of the *glgCAP* operon, and glycogen synthase activity preferentially decays. The uptake of a PTS sugar should promote a shift in the state of HPr in the direction of dephospho-HPr, resulting in an activation of GP. Consequently, effective recovery from the stationary phase of growth may be enhanced by the energy derived from the utilization of stored glycogen. There are several reports supporting this assumption. One study examined synchronized *E. coli* B1 cells (14). At the beginning of cell division, the level of cellular glucose is low while that of glycogen is high. As cell division progresses, the level of glucose increases while that of glycogen drops possibly via glucose uptake and glycogen breakdown. Other studies suggest that exponential-phase glycogen recycling is essential for growth in *Mycobacterium smegmatis* (1) and that glycogen is required for an increase in the cell division cycle and cell viability in *Saccharomyces cerevisiae* (27). Obviously, further clarification of the role of bacterial glycogen is important.

Evolutionary importance of the HPr-glycogen phosphorylase interaction

In addition to the physiological role of the physical interaction between HPr and GP, that is, regulation of glycogen hydrolysis by sensing the carbon sources available in its environment, its significance can be considered from the evolutionary aspect. Alpha-glucan phosphorylase has been examined from a variety of organisms including bacteria, yeast, slime mold, plants, insects, and vertebrates including humans. These proteins share similar enzymatic properties. All of them catalyze the phosphorylation of the α,1-4 glucosyl link between glucose residues at the non-reducing end of the glucosyl chain and utilize the 5-phosphate group of the cofactor pyridoxal phosphate (PLP) in catalysis (4). They exhibit similar pH dependence and reaction kinetics, and require the dimeric state for activity (31). Furthermore, the amino acid sequences of phosphorylases are highly conserved with more than 40% sequence identity regardless of the source (19), and the overall three-dimensional structures show similar folds for rabbit muscle and yeast GPs and *E. coli* MP (20, 31). The initiating events for activation or expression of phosphorylases also have a common factor, cAMP, which in eukaryotes acts to stimulate cAMP-dependent protein kinase for phosphorylase kinase activation and in the bacterial system to relieve catabolite repression through interaction with CRP. The cAMP regulatory binding subunit of cAMP-dependent protein kinase is homologous in sequence

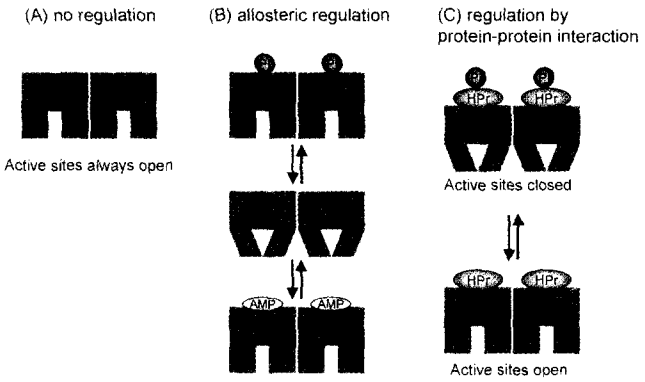

Fig. 4. Evolutionary diversity of the regulation modes of glycogen phosphorylases. *E. coli* maltodextrin phosphorylase and other bacterial and plant glycogen phosphorylases are known to be active in the absence of phosphorylation and AMP and have, thus far, shown no regulatory properties (A). The yeast enzyme requires phosphorylation for maximal activity, but is insensitive to activation by AMP, while mammalian enzymes are activated by phosphorylation or AMP (B). In the case of *E. coli* GP lacking the phosphorylation site characteristic of yeast and mammalian glycogen phosphorylases, the highly specific and high affinity interaction with HPr is the major basis for activity regulation (C).

and structure to CRP, indicating a common evolutionary origin in response to cAMP (29). In both cases, cAMP is synthesized in response to external stimuli, hormonal stimulation for the mammalian system and nutrient deprivation for the bacteria. Nevertheless, phosphorylases differ in their modes of regulation from species to species as summarized in Fig. 4. *E. coli* GP lacks the phosphorylation site characteristic of yeast and mammalian GPs. In the case of *E. coli* GP, the highly specific and high affinity interaction with HPr is the major basis for activity regulation (Fig. 4C). *E. coli* MP and other bacterial and plant phosphorylases are active in the absence of phosphorylation and AMP (Fig. 4A) and have, thus far, shown no regulatory properties (4). The yeast enzyme requires phosphorylation for maximal activity, but is insensitive to activation by AMP (4), while mammalian enzymes are activated by phosphorylation or AMP (Fig. 4B). Crystallographic studies of yeast and rabbit muscle GPs and *E. coli* MP have elucidated the structural characteristics and changes undergone by a protein in response to phosphorylation and to various allosteric effectors. (4, 20, 31). This argues that *E. coli* HPr evolved in a unique way to regulate glycogen hydrolysis by sensing the availability of carbon sources in its environment.

We have shown that HPr mediates crosstalk between two important pathways for carbon metabolism, i.e., uptake of sugar substrates and breakdown of the storage compound, glycogen. The changes that occurred during the separate evolutionary development of these enzymes have led to different properties in terms of regulatory patterns and three-dimensional structures. The regulation of GP activity by the phosphorylation state of HPr is proposed to be the bacterial analogy to the covalent phosphorylation-dephosphorylation cascade characteristic of eukaryotic GPs. Clearly, X-ray crystallographic studies of the HPr-GP complex will enhance our understanding of the details of the interaction of GP with HPr. Further clarification of the role of bacterial glycogen is also needed to understand the physiological basis for activation of glycogen breakdown by unphosphorylated HPr in the presence of PTS sugars. Furthermore, it should be clarified whether regulation of GP activity by interaction with HPr is the general mechanism for regulation of glycogen concentration in other glycogen-storing bacteria.

Acknowledgments

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