JOURNAL OF BACTERIOLOGY, Aug. 2002, p. 4205–4210 Vol. 184, No. 15
0021-9193/02/$04.00+0 DOI: 10.1128/JB.184.15.4205–4210.2002

Cooperativity in Signal Transfer through the Uhp System of *Escherichia coli*

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Received 19 February 2002/Accepted 7 May 2002

The UhpABC regulatory system in enterobacteria controls the expression of the hexose phosphate transporter UhpT. Signaling is initiated through sensing of extracellular glucose 6-phosphate by membrane-bound UhpC, which in turn modulates the histidine-protein kinase UhpB. Together with the cytoplasmic response regulator UhpA, they constitute a typical two-component regulatory system based on His-to-Asp phosphoryl transfer. Activated (i.e., phosphorylated) UhpA binds to the promoter region of *uhpT*, resulting in initiation of transcription. We have investigated the contribution of transmembrane signaling (through UhpBC) and intracellular activation (through UhpA) to the overall Uhp response (UhpT expression) in vivo. UhpA activation could be made independent of transmembrane signaling when Δ*uhpBC* cells were grown on pyruvate. Inorganic phosphate interfered with glucose 6-phosphate-dependent, UhpBC-mediated, as well as pyruvate-mediated activation of UhpA. The relationship between the concentration of inducer (glucose 6-phosphate) and the Uhp induction rate was nonhyperbolic, indicating positive cooperativity. The degree of cooperativity was affected by the carbon or energy source available to the cells for growth. As pyruvate-mediated activation of UhpA in Δ*uhpBC* cells could result in considerably stronger UhpT expression than glucose 6-phosphate-dependent activation through UhpBC, the observed positive cooperativity for the overall pathway in wild-type cells may reflect the previously described cooperative binding of UhpA to the *uhpT* promoter (J. L. Dahl et al., J. Biol. Chem. 272:1910–1919, 1997).

Bacteria must be able to accurately respond to a large number of extracellular signals in their continuously changing surroundings. Therefore, they have evolved sophisticated sensory mechanisms coupled to intracellular signal transduction pathways. The majority of these prokaryotic intracellular signaling routes are based on the reversible phosphorylation of sensor kinases and response regulators in so-called two-component regulatory systems (23, 28, 30). According to this concept, sensory input affects autophosphorylation on a conserved histidine residue in the transmitter module of the kinase. The phosphoryl group is subsequently transferred to a conserved aspartate residue in the (amino-terminal) receiver domain of the cognate response regulator. This covalent modification elicits a molecular switch, and above a certain threshold amount of phosphorylated regulator, a specific response is turned on, which is in most cases gene transcription via (enhanced) affinity of the carboxyl-terminal output domain of the regulator for the target promoter sequence.

To provoke a cellular response, the environmental physical or chemical stimulus has to cross the membrane. Therefore, in most cases the first step in a signal transduction cascade is the activation of a membrane-bound protein. In order to be able to quantitatively study specific transmembrane (receptor) signaling leading to cellular activation, the first (ligand-induced) step and the subsequent response should be well defined and straightforward to quantify. However, despite the huge number of characterized signaling pathways, such examples are not abundant.

An exception is the Uhp regulatory system in *Escherichia coli* (for a review, see reference 13). This signaling pathway is triggered by external glucose 6-phosphate, which is recognized by the membrane-bound receptor protein UhpC. UhpC interacts with a second membrane-bound protein, UhpB, the kinase/phosphatase of the Uhp two-component system. Sensing of glucose 6-phosphate is supposed to cause a conformational change in the supposed UhpBC complex, leading to histidine autophosphorylation in the (cytoplasmic) transmitter domain of UhpB. Upon phosphoryl transfer, the response regulator UhpA (presumably phosphorylated on the conserved Asp-54) shows enhanced affinity for the *uhpT* promoter and, together with the catabolite gene activator protein, initiates *uhpT* transcription. Glucose 6-phosphate is the only ligand that can activate signaling through UhpABC, which results in UhpT expression and thus in the uptake of a broad range of phosphorylated sugars by this transport protein, in exchange for intracellular phosphate. This enables enterobacteria to use these compounds as a carbon and energy source.

In this study we used *E. coli* strains with a chromosomal *uhpT-lacZ* fusion. Such strains facilitated quantitative measurements of the output of signaling through the Uhp system (i.e., *uhpT* transcription rates measured through β-galactosidase expression), as a function of the added glucose 6-phosphate concentration [G6P], together with the effect of inorganic phosphate (Pi) and the carbon or energy source used for growth. We obtained stimulus-response relationships indicative of positive cooperativity in the Uhp signaling pathway. Furthermore, Uhp induction rates reached significantly higher levels in Δ*uhpBC* cells, via pyruvate-mediated activation of

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UhpA, than in wild-type cells (maximally) induced with glucose 6-phosphate.

It has been shown previously in vitro that binding of UhpA to the *uhpT* promoter exhibits positive cooperativity and that binding occurs with much higher affinity when UhpA is phosphorylated (5, 6, 22). Therefore, the overall positive cooperativity derived from our whole-cell assay may reflect the contribution at the level of transcription regulation affected by signaling through UhpBC and/or phosphoryl transfer from UhpB to UhpA and/or the energy status. We discuss our findings in light of the cooperative phenomena observed in chemotaxis regulation (29).

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *E. coli* strains (K-12 derivatives) used in this study were RK5115 [Φ(*uhpT-lacZ*) Δ*uhpT*], RK5163 [Φ(*uhpT-lacZ*)], and RK1307 [Δ*uhp*(B60-C437) Φ(*uhpT-lacZ*[Km])], kindly provided by R. J. Kadner (27, 34). Cells were grown aerobically at 37°C in Luria-Bertani (LB) medium (20) or in MOPS (morpholinepropanesulfonic acid) minimal medium (21) containing either 30 mM succinate, 10 mM glucose, or 40 mM pyruvate as the carbon source, 14 mM NH_4Cl as the nitrogen source, and (unless indicated otherwise) 2 mM K_2HPO_4 as the phosphate source and supplemented with thiamine (20 μg ml^{-1}).

Uhp induction. Overnight cultures (RK5115) in LB or MOPS minimal medium were diluted in the same fresh medium to an optical density at 600 nm (OD_{600}) of ~0.075 and grown until mid-exponential phase. Prior to inducing the Uhp system with glucose 6-phosphate (Boehringer Mannheim), the main culture was distributed into appropriate volumes in small culture flasks, which were incubated in a 37°C shaking water bath. At an OD_{600} of 0.8 to 0.9, glucose 6-phosphate from a 50-fold-concentrated stock solution was added. To study Uhp inhibition, potassium phosphate (pH 7.5; 25-fold concentrated) was added 12.5 min after addition of glucose 6-phosphate.

To measure Uhp induction in RK1307, cells were grown in MOPS minimal medium with 30 mM succinate and the indicated phosphate concentration. Overnight-grown cells were harvested by centrifugation (10 min at 3,000 × *g*) and resuspended to a final OD_{600} of 0.1 in MOPS minimal medium with 40 mM pyruvate and the same phosphate concentration. Culture aliquots were collected on ice at the indicated time points for determination of OD_{600} and for measurement of β-galactosidase activity. Samples were stored at −80°C.

Uhp reporter assay. β-Galactosidase (*uhpT-lacZ*) activity was assayed as described before (20) with only minor modifications. Hydrolysis of *o*-nitrophenyl-β-D-galactopyranoside, the substrate for β-galactosidase, was assayed in cells permeabilized with sodium dodecyl sulfate-chloroform in Z buffer (20). Reactions were stopped by addition of 1 M Na_2CO_3. The resulting absorbance (*O*-nitrophenol formed) was measured spectrophotometrically (OD_{420}).

Data analysis. β-Galactosidase activities (Miller units [MU]), normalized for cell density (OD_{600}), were calculated by using the equation (OD_{420} × 1,000)/(reaction time in minutes × culture volume in milliliters × OD_{600}). The induction rate (V_{Uhp}, in MU per minute) per glucose 6-phosphate concentration was obtained by calculating the slope of the linear trendline through the MU-versus-time data series between 5 and ~40 min after glucose 6-phosphate addition. Activity determinations were performed on culture samples obtained from at least two independent induction experiments. Where indicated, a statistical evaluation of three experiments (mean ± standard deviation [SD]) is shown. Plotted data (glucose 6-phosphate concentration versus V_{Uhp}) were fitted (using Origin 6.0, Microcal Software) to the Michaelis-Menten equation, $V_{Uhp} = V_{max} \times [G6P]/(K + [G6P])$ as well as to the Hill equation, $V_{Uhp} = V_{max} \times ([G6P])^n/[(K)^n + ([G6P])^n]$, where K is $K_{0.5}^{G6P}$ and n is the Hill coefficient (n_H). For calculations assuming competitive inhibition, the $K^{G6P,\,Pi} = K^{G6P} \times (1 + [Pi]/K_i^{Pi})$ relationship was used, derived from Michaelis-Menten (Lineweaver-Burk) kinetics, in which the Pi concentration is the inhibiting phosphate concentration and K_i^{Pi} is the inhibition constant.

RESULTS

Energy- and carbon source-dependent Uhp induction. Addition of increasing amounts of glucose 6-phosphate to exponentially growing *E. coli* RK5115 (*uhpT-lacZ* Δ*uhpT*) cultures gave rise to a linear increase in β-galactosidase activity. As expected, it was observed that it took about 2 min after initiation of Uhp signaling before β-galactosidase activity could be detected (not shown). This reporter of UhpT expression reached higher levels in a strain in which functional UhpT was missing, due to the absence of concomitant uptake of inducer by the synthesized transporter, which occurs in RK5163 after 10 to 15 min (data not shown) (27). When the induction rate of *uhpT*-driven β-galactosidase expression (V_{Uhp}) was plotted as a function of the glucose 6-phosphate concentration, this resulted in the dose-response curves shown in Fig. 1A (succinate as the carbon source) and Fig. 1B (glucose as the carbon source), both in MOPS minimal medium, and in Fig. 1C in LB medium.

Half-maximal induction rates were reached at 1 to 2.5 μM glucose 6-phosphate (called $K_{0.5}^{G6P}$). For comparison, the three curves are depicted in one graph in Fig. 1D. In MOPS minimal medium, growth on succinate resulted in higher induction rates (V_{max} = 20 MU min^{-1}), albeit with a higher threshold sensitivity (i.e., lower initial rates; $K_{0.5}^{G6P}$ = 2.2 μM), than obtained during growth on glucose ($K_{0.5}^{G6P}$ = 1.3 μM; V_{max} = 10 MU min^{-1}). The 50% reduction in maximal induction during growth on glucose has been measured previously and can be ascribed to catabolite repression (18). Cells grown in rich medium (LB) showed the same sensitivity ($K_{0.5}^{G6P}$ = 1.2 μM) as in MOPS minimal medium with glucose, but reached similar maximal Uhp induction rates (19 MU min^{-1}) as in MOPS minimal medium with succinate.

The dose-response curves obtained appeared to be sigmoid rather than hyperbolic, i.e., deviated from ideal Michaelis-Menten kinetics (dotted lines in Fig. 1A to C, with $K_{0.5}^{G6P}$ and V_{max} as mentioned above). Because this implied an allosteric effect, the data were fitted to a sigmoidal function, i.e., the Hill equation (lines in Fig. 1), as well as linearized into a Hill plot (not shown). The magnitude of n_H in the Hill equation, being the slope of the line in the Hill plot ($\log [V/(V_{max} - V])$ versus $n \times \log[G6P] - n \times \log K$), is related to the type of cooperative behavior and can be greater than (positive cooperativity), less than (negative cooperativity), or equal to (no cooperativity) 1. We obtained Hill coefficients (± SD; $R^2 \geq 0.99$) of 2.11 (± 0.03) in LB and of 2.78 (± 0.08) and 1.95 (± 0.17) for cells grown on succinate and glucose as the carbon source in MOPS minimal medium, respectively. These values indicate positive cooperativity in overall Uhp induction kinetics, with a cooperative strength depending on the carbon and energy source.

Inhibition of Uhp induction. Induction studies were performed on cells grown in a culture medium with a relatively low phosphate concentration (2 mM) compared to other (minimal) media (21), since a high concentration of phosphate in the medium inhibits Uhp induction either partly or completely (depending on the concentration of glucose 6-phosphate and phosphate); maximal induction is reached at higher concentrations of glucose 6-phosphate (data not shown) (27). It has been proposed that this (competitive) inhibition may occur at the regulatory sensor (27), later established to be UhpC (35).

We addressed this (supposedly extracellular) regulatory inhibition in more detail to investigate whether it could be used to control the intracellular signal transfer through UhpABC. Therefore, we added phosphate to cultures in which *uhpT* was already induced and followed the subsequent response over

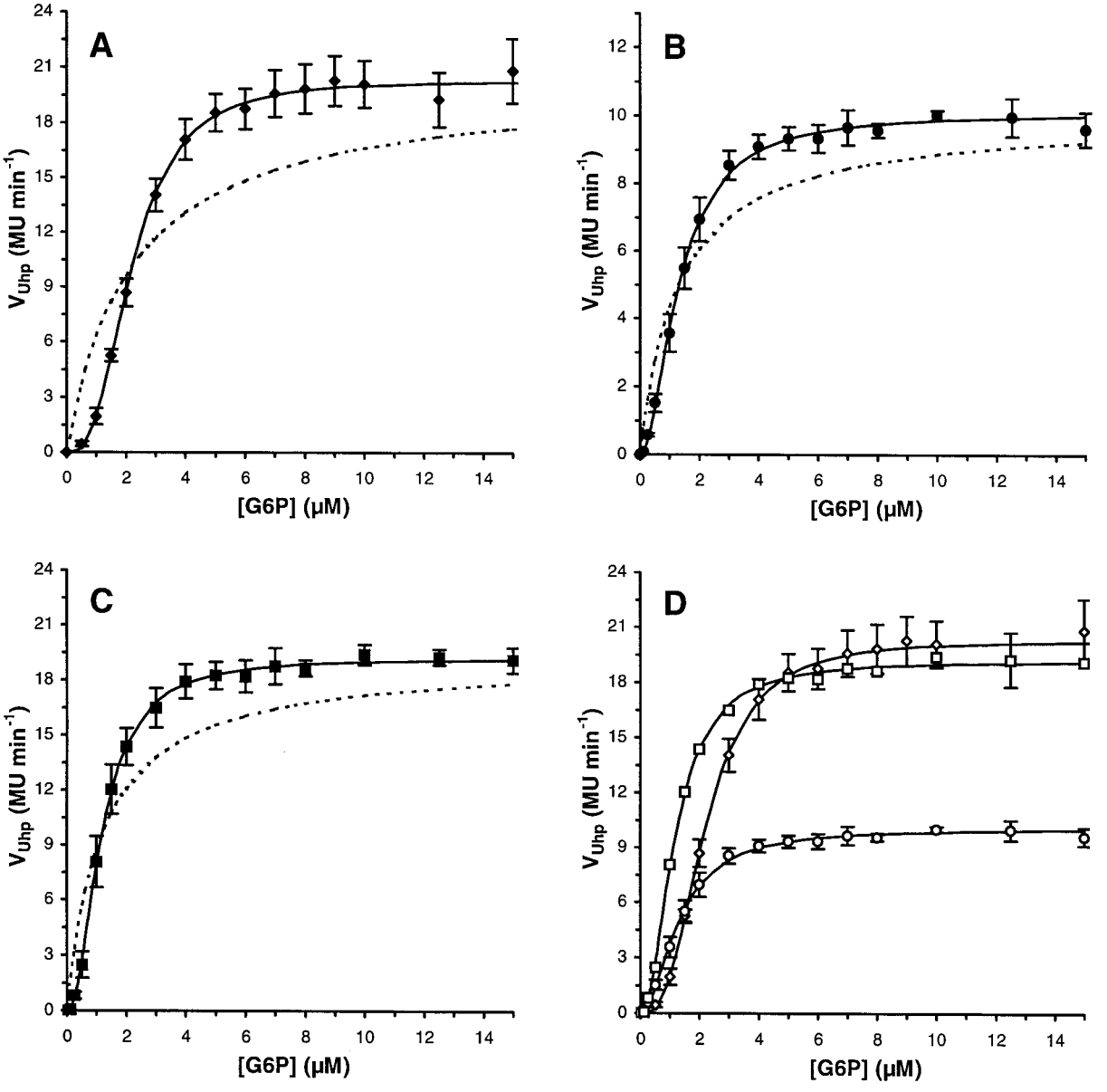

FIG. 1. Uhp induction rates in *E. coli* RK5115. Rates (V_{Uhp}) per glucose 6-phosphate concentration were obtained by determining the slope of the linear trendline of the β-galactosidase activity-versus-time data between 5 and 30 min after glucose 6-phosphate addition. Error bars represent the SD of three independent experiments (mean ± SD). Uhp induction rates for *E. coli* RK5115 in MOPS minimal medium with 30 mM succinate (♦) (A), MOPS minimal medium with 10 mM glucose (●) (B), Luria-Bertani medium (■) (C). The data in panels A to C are compiled in panel D (now with open symbols). Each correlation was fitted (solid lines) to the Hill equation, as described in Materials and Methods. The dotted curve in graphs A to C represents a Michaelis-Menten curve with the same $K_{0.5}^{G6P}$ and V_{max} as the Hill fit.

time (Fig. 2). Inhibition occurred within 3 min following addition of phosphate, as shown in Fig. 2A for an inducing glucose 6-phosphate concentration of 5 μM and three concentrations of phosphate. We established a correlation between the added minimal phosphate concentration for total inhibition ($[Pi]_0$ when $V_{Uhp} = 0$) and the glucose 6-phosphate concentration, i.e., $[Pi]_0 = 2{,}000 \times [G6P]$. Thus, we were able to determine the affected induction rates at different glucose 6-phosphate concentrations for added phosphate concentrations below each $[Pi]_0$ (Fig. 2B). This resulted in an apparent K_i^{Pi} for competitive inhibition of 2.9 ± 0.7 mM (via $K^{G6P, Pi} = K_{0.5}^{G6P} \times [1 + [Pi]/K_i^{Pi}]$) for cells grown in MOPS minimal medium with succinate as the carbon source and an initial phosphate concentration in the medium of 2 mM.

Uhp induction stimulated by growth on pyruvate. Extracellular glucose 6-phosphate is the only known inducing signal for the Uhp system in wild-type *E. coli*. However, recently we showed that UhpT was expressed in cells without the UhpBC sensor and in the absence of glucose 6-phosphate when cells were grown on pyruvate (33). This phenomenon strongly indicates that UhpA can use acetyl phosphate as phosphoryl donor in vivo, like many other response regulators, and thus can be

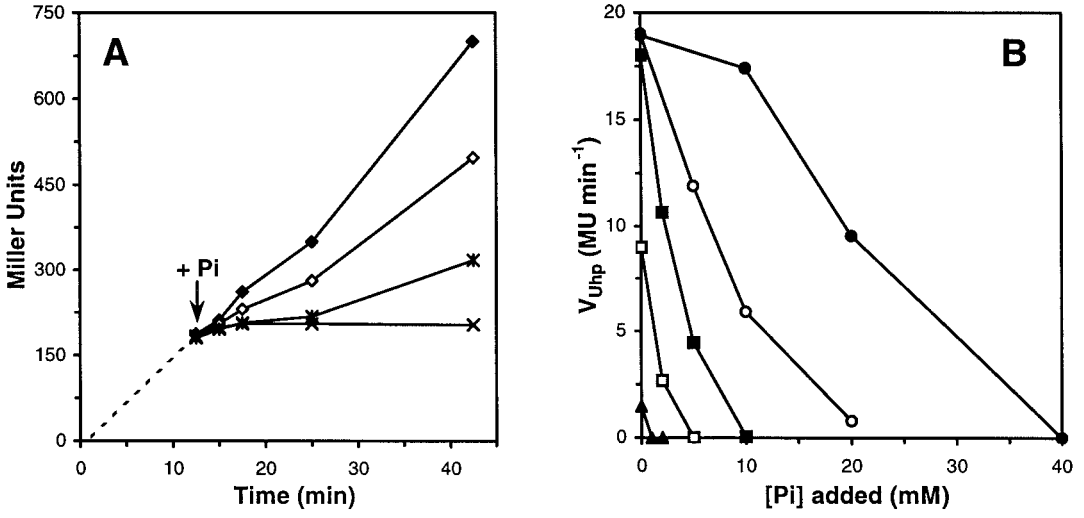

FIG. 2. Inhibition of Uhp induction by addition of inorganic phosphate. (A) RK5115 cells in MOPS minimal medium with 30 mM succinate were induced with 5 μM glucose 6-phosphate at time zero. Phosphate was added at 12.5 min (arrow), and samples were analyzed as described in Materials and Methods. Symbols: ♦, 0 mM Pi; ◇, 2 mM Pi; ∗, 5 mM Pi; ×, 10 mM Pi. (B) Rates (V_{Uhp}) at various glucose 6-phosphate concentrations were obtained by determining the slope of the linear trendlines of β-galactosidase activity versus time, as in A, between 15 and 42.5 min for each phosphate concentration tested. Glucose 6-phosphate concentrations: ▲, 1 μM; □, 2.5 μM; ■, 5 μM; ○, 10 μM; ●, 20 μM. $[Pi]_0$, intersection of the line representing V_{Uhp} with the horizontal axis ($V_{Uhp} = 0$).

activated in the absence of kinase and phosphatase activity by UhpB. Acetyl phosphate is present in large amounts during growth on pyruvate (17) due to an increased flux into the Pta-AckA (phosphotransacetylase-acetate kinase) pathway (10).

Here, we have further characterized this pyruvate-stimulated Uhp induction in cells with a Δ*uhpBC* background (*uhpT-lacZ*). As shown in Fig. 3, Uhp was induced after 30 min upon switching the carbon source to pyruvate and reached a maximum in the early exponential growth phase, in agreement with the observed growth phase-dependent synthesis of acetyl phosphate (24). The Uhp induction rate (up to 40 MU min^{-1}) as well as the maximum expression level (MU_{max}) in pyruvate-grown Δ*uhpBC* cells was considerably higher than in wild-type cells. We also addressed the effect of the concentration of inorganic phosphate in the growth medium. Uhp induction appeared to be more sensitive to suboptimal levels of phosphate for growth, in a phase of growth (i.e., early exponential) at which these phosphate concentrations were not limiting growth yet (Fig. 3). High phosphate concentrations decreased maximal Uhp induction rates in Δ*uhpBC* cells but did not block Uhp induction as in wild-type cells.

Next, we addressed Uhp induction in early-exponential-phase (OD_{600} ~0.3) wild-type cells (RK5115) grown on pyruvate (compared with growth on succinate) and a low optimal phosphate concentration (400 μM) upon addition of glucose 6-phosphate to find out whether Uhp induction rates were affected when UhpA was activated by UhpB (cells plus glucose 6-phosphate, i.e., kinase on) and (supposedly) acetyl phosphate simultaneously. In contrast to pyruvate-grown Δ*uhpBC* cells (Fig. 3), pyruvate-grown wild-type cells showed very low (basal) UhpT expression (20 to 40 MU in the absence of glucose 6-phosphate, though not zero like during growth on succinate). But their sensitivity to glucose 6-phosphate (K^{suc}/K^{pyr} = 1.31 ± 0.17 for $K_{0.5}{}^{G6P}$) and Uhp induction rates were higher (V^{pyr}/V^{suc} = 1.15 ± 0.08 at V_{max}) than in succinate-grown wild-type cells. When they were grown in the presence of 2 mM phosphate and induced at an OD_{600} of 0.8 to 0.9, the pyruvate-mediated additive effect on Uhp induction was not apparent. This is in agreement with the phosphate effect in

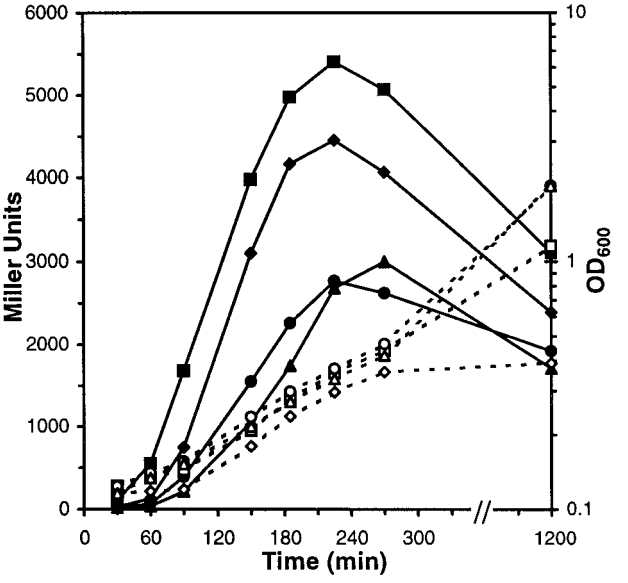

FIG. 3. Uhp induction profile in Δ*uhpBC* cells grown on pyruvate. RK1307 cells (Δ*uhpBC uhpT-lacZ*) were grown in MOPS minimal medium with the indicated Pi concentration and switched from succinate to pyruvate (40 mM) as the carbon source at time zero, as described in Materials and Methods. Pi concentrations (open symbols, OD_{600}; solid symbols, MU): ♦, ◇, 50 μM; ■, □, 200 μM; ●, ○, 2.5 mM; ▲, △, 20 mM.

Δ*uhpBC* cells as well as with the growth phase dependence of the intracellular acetyl phosphate level, as shown in Fig. 3 and previously (24).

DISCUSSION

We have investigated induction of UhpT expression (*uhpT-lacZ*) through the Uhp regulatory system in cells with a wild-type regulatory background as well as in cells with a Δ*uhpBC* background. These two strains enabled us to separate the contribution of transmembrane signaling (through UhpBC and glucose 6-phosphate) and intracellular activation (through UhpA) to the Uhp response in vivo.

Uhp induction curves ([G6P] versus UhpT expression rates) were determined in rich medium (LB) and in minimal medium (MM) with glucose or succinate as the carbon source (Fig. 1). This resulted in $K_{0.5}^{G6P}$s of 1 to 2.5 μM, in agreement with the value derived from single-time-point β-galactosidase (MU) measurements per unit of glucose 6-phosphate (27), but 10 times lower than in earlier measurements (37), due to the method previously used (based on glucose 6-phosphate uptake), as well as the phosphate concentration in the medium (8).

The difference in maximal induction rates between cells grown on succinate and cells grown on glucose reflects the known catabolite repression effect on *uhpT* transcription (8, 18, 19). This 50% reduction during growth on glucose, as shown previously (18), can be ascribed to a small amount of cyclic AMP receptor protein (the catabolite gene activator protein) available, which is required (together with UhpA) to activate *uhpT* transcription. However, glucose-grown cells were more sensitive to threshold concentrations of glucose 6-phosphate than succinate-grown cells (Fig. 1D). Apparently, at these glucose 6-phosphate concentrations, Uhp induction is not affected by catabolite repression. In rich medium (carbon source independent; LB), the catabolite repression effect was not observed (Fig. 1C), and cells were more sensitive to threshold concentrations of glucose 6-phosphate due to a lower phosphate concentration in LB medium (~1 mM; J. Tommassen, personal communication).

It has been shown previously (27) that cells grown in medium with high inorganic phosphate concentrations required higher glucose 6-phosphate-concentrations to induce UhpT expression than cells grown in medium with low inorganic phosphate, i.e., the strict extracellular effect of glucose 6-phosphate was inhibited. We quantified the inhibitory effect of phosphate on signal transfer through UhpABC in induced cells (Fig. 2), resulting in an apparent K_i of ~3 mM. Addition of a maximally inhibiting amount of phosphate to an induced culture resulted in an off-response within 3 min (presumably required to complete translation of *uhpT* mRNA). Therefore, UhpBC is quickly desensitized to the kinase off/phosphatase on state, causing phosphorylated UhpA (P-UhpA) dephosphorylation. It is likely that phosphate acts extracellularly, i.e., it is competitive with glucose 6-phosphate for the binding site in UhpC, as its inhibitory effect is rather instantaneous, and it can be mimicked to a certain extent by other anions such as sulfate (Verhamme et al., unpublished data). Furthermore, a high phosphate concentration decreases pyruvate-mediated Uhp induction in Δ*uhpBC* cells but does not block it (Fig. 3).

We observed that the Uhp system was more sensitive (than strictly Michaelis-Menten-type) for inducer concentrations above threshold levels, i.e., the dose-response curves were steeper than hyperbolic (Fig. 1). By further analysis of this phenomenon, we derived Hill coefficients (n_H) greater than 1. Apparently, this value is influenced by the carbon or energy source available to the cells for growth, resulting in an n_H of 2 in LB medium and in minimal medium with glucose and an n_H of 2.8 in minimal medium with succinate. These values imply a carbon source-dependent overall positive cooperativity ($n_H > 1$) in signaling through UhpABC to *uhpT*.

As we are dealing with a typical signal transduction system, starting with transmembrane signaling, followed by intracellular phosphoryl transfer, and leading to modulation of specific gene transcription, the contribution of each of these separate levels of signal processing will be discussed. First, cooperativity could occur at the membrane level, i.e., at the sensor components UhpC and UhpB. Glucose 6-phosphate may bind to UhpC in a cooperative manner (either positively or negatively), and this may also occur in the interaction of UhpC with UhpB to transduce the signal through the membrane. The model for induction by external glucose 6-phosphate holds that it binds to and alters the conformation of UhpC and that this information is communicated to UhpB by direct protein-protein interaction (11). There is no evidence that this occurs in higher-order clusters such as the chemoreceptor supramolecular complex (15, 16), but at least minimal lateral interaction between UhpC and UhpB molecules has to occur to achieve histidine autophosphorylation in *trans*, catalyzed through the two transmitter domains of a sensory kinase dimer (7). Future in vivo protein distribution and localization studies in membranes may reveal spatial organization. However, UhpB and the turgor sensor KdpD are the only two (functionally characterized) sensory kinases in *E. coli* with more than two transmembrane domains (36), possibly restricting spatial movement of these sensors upon signal recognition. For KdpD, it has been shown that it functions as a homodimer and that there is no change in the oligomeric state upon activation (9). Moreover, in vitro phosphorylation assays with UhpBC-enriched membranes did not reveal any apparent cooperativity in either glucose 6-phosphate concentration-dependent transmembrane signaling output (i.e., UhpB autophosphorylation) or the subsequent phosphoryl flow towards UhpA (32).

It has been shown previously that UhpA binds to the *uhpT* promoter in oligomerized form and that this occurs with positive cooperativity (6). The Hill coefficient for this effect ranged from 2.5 to 5 for unphosphorylated UhpA in vitro (22). Phosphorylated UhpA exhibits a strongly increased affinity for the *uhpT* promoter (5, 6), implying increased positive cooperativity (6), possibly approaching the upper value of this range. Our numbers indicate positive cooperativity for the overall pathway in vivo, strictly depending on phosphorylated UhpA. They do not exceed the lowest value of the 2.5 to 5 range. Therefore, the regulatory steps preceding and impairing transcriptional activation, like the recently demonstrated phosphatase activity and sequestration ability of UhpB (38), may negatively affect (via control of the level of P-UhpA) the entire signal transduction route in vivo, resulting in a more moderate positive cooperativity. This may explain why UhpT expression can be higher in the absence of UhpBC, during growth on pyruvate, when

UhpA is supposed to be phoshorylated by acetyl phosphate (Fig. 3). In addition, these data show that phosphate, besides its extracellular impact on Uhp induction in wild-type cells, also affects pyruvate-mediated Uhp induction in Δ*uhpBC* cells, possibly due to its metabolic control of the Pta-Ack pathway.

Besides further understanding of signaling through Uhp, the possibility of UhpT expression without UhpBC (and without UhpA overexpression) offers a new biotechnological opportunity to exploit glucose 6-phosphate production by *E. coli* (31). Furthermore, we measured a small additive pyruvate-mediated effect, presumably due to acetyl phosphate, on glucose 6-phosphate-triggered UhpA activation in early-exponential-phase wild-type cells, which adds to the relevance of acetyl phosphate as a physiologically significant signaling molecule (33). This should be further addressed in a Δ*pta* mutant.

Allosteric effects have been studied in detail at different levels in chemotaxis. Binding of aspartate to its chemoreceptor dimer occurs with strong negative cooperativity in vitro (2). However, in the complete transmembrane signaling step, positively cooperative interactions dominate, as shown by in vitro chemoreceptor-coupled kinase (CheA) assays (4, 14). In addition, in vivo studies on the interaction between P-CheY and the flagellar motor reveal positive cooperativity (1, 25). Also, the phosphatase CheZ interacts with P-CheY in a positively cooperative manner (3). Moreover, the overall description of chemotactic signaling has recently been changed, from a relationship between ligand binding and the output response with limited positive cooperativity (26) to a sigmoidal stimulus-response relation with strong positive cooperativity (12).

A quantitative overall description of signaling intensity has hitherto been possible only for a small minority of transmembrane signaling pathways. To find out where in a regulatory system allosteric phenomena take place and what their effect is on the final output, single pathways have to be dissected. The detailed characterization of such pathways and their mutual interactions (33) is a prerequisite for a better understanding of the integration of the complex signal transduction machinery that bacteria exploit to survive and compete.

ACKNOWLEDGMENTS

We thank R. J. Kadner for making strains available.

This work was supported by the Netherlands Organization for Scientific Research (NWO), through the division for Earth and Life Sciences (Gebied ALW).

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