Regulation of *Escherichia coli* adenylate cyclase activity during hexose phosphate transport

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In *Escherichia coli*, cAMP levels vary with the carbon source used in the culture medium. These levels are dependent on the cellular concentration of phosphorylated EnzymeIIA^{glc}, a component of the glucose-phosphotransferase system, which activates adenylate cyclase (AC). When cells are grown on glucose 6-phosphate (Glc6P), the cAMP level is particularly low. In this study, we investigated the mechanism leading to the low cAMP level when Glc6P is used as the carbon source, i.e. the mechanism preventing the activation of AC by phosphorylated EnzymeIIA^{glc}. Glc6P is transported via the Uhp system which is inducible by extracellular Glc6P. The Uhp system comprises a permease UhpT and three proteins UhpA, UhpB and UhpC which are necessary for *uhpT* gene transcription. Controlled expression of UhpT in the absence of the regulatory proteins (UhpA, UhpB and UhpC) allowed us to demonstrate that (i) the Uhp regulatory proteins do not prevent the activation of AC by direct interaction with EnzymeIIA^{glc} and (ii) an increase in the amount of UhpT synthesized (corresponding to an increase in the amount of Glc6P transported) correlates with a decrease in the cAMP level. We present data indicating that Glc6P *per se* or its degradation is unlikely to be responsible for the low cAMP level. It is concluded that the level of cAMP in the cell is determined by the flux of Glc6P through UhpT.

Keywords: *Escherichia coli*, adenylate cyclase, cyclic AMP, phosphotransferase system, glucose 6-phosphate transport

INTRODUCTION

In *Escherichia coli*, intracellular cAMP concentration is dependent on the carbon source used in the culture medium (Epstein *et al.*, 1975; Joseph *et al.*, 1982). The lowest concentration is obtained when cells are grown on glucose or glucose 6-phosphate (Glc6P). A model has been proposed which explains the low level of cAMP observed when cells grow with glucose as the carbon source. This model involves the phosphotransferase system (PTS) which regulates adenylate cyclase (AC) (Saier & Feucht, 1975; Postma *et al.*, 1993). EnzymeIIA^{glc}, a glucose-specific component of the PTS is phosphorylated via a phosphorylation cascade including

EnzymeI and HPr, the general components of the PTS. When phosphorylated, EnzymeIIA^{glc} activates AC. During glucose transport, the intracellular concentration of phosphorylated EnzymeIIA^{glc} decreases causing a decrease in intracellular cAMP concentration (Feucht & Saier, 1980; Postma *et al.*, 1981). This model is supported by the fact that total cAMP in Δcrr strains (lacking EnzymeIIA^{glc}) is low and close to that of a wild-type strain grown on glucose. However, the mechanism leading to the activation of AC by EnzymeIIA^{glc} remains to be explained.

Glc6P is transported into the cell via the Uhp system which is inducible by extracellular Glc6P (Dietz & Heppel, 1971a). The Uhp system consists of a permease UhpT (encoded by *uhpT*) which is transcriptionally regulated by three regulatory proteins, UhpA, UhpB and UhpC (encoded by *uhpA*, *uhpB* and *uhpC*, respectively) (Weston & Kadner, 1987). In the presence of external Glc6P, the membrane proteins UhpB and UhpC probably phosphorylate UhpA, promoting the transcription of the *uhpT* gene. UhpA and UhpB belong to the family of two

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Abbreviations: AC, adenylate cyclase; Fru6P/Fru1P, fructose 6-phosphate/1-phosphate; Glc6P/Glc1P, glucose 6-phosphate/1-phosphate; Man6P, mannose 6-phosphate; PTS, phosphotransferase system.

Table 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Genotype or description	Origin
TP2503	F ⁻ <i>xyl-5 argH1 ilvA</i>	De Reuse & Danchin (1988)
TP2504	F ⁻ <i>xyl-5 argH1 ilvA ptsG22 xcf-229::Tn10</i>	De Reuse & Danchin (1988)
TP2100	F ⁻ <i>xyl-5 argH1 ilvA ΔlacX74</i>	Roy <i>et al.</i> (1983)
TP2862	F ⁻ <i>xyl-5 argH1 aroB ilvA Δcrr::kan</i>	Lévy <i>et al.</i> (1990)
TP9517	TP2503 <i>uhpA::miniTn10tet</i>	Dumay & Crasnier (1994)
RK4981	F ⁻ <i>ΔuhpT2050 pyrE41 metE70 rbsR22 relA1 deoC1 gltS014 rpsL150 gyrA219 ptsF25 λ⁻non-9 fliD5301 Δ(argF-lac)169[araD139]</i>	Shattuck-Eidens & Kadner (1983) via B. Bachmann (<i>E. coli</i> Genetic Stock Center)
AE304-3	F ⁻ <i>tna::Tn10 ΔlacX74 tbi tsx T6^r bglR11 bglB3(Am)</i>	A. Wright
TP9522	RK4981 <i>tna::Tn10</i>	P1/AE304-3 × RK4981
TP9523	TP2503 <i>tna::Tn10 ΔuhpT2050</i>	P1/TP9522 × TP2503
TP9524	RK4981 <i>uhpA::miniTn10tet</i>	P1/TP9517 × RK4981
TP9525	TP9524 <i>Δ(uhpABCT)</i>	Tn10 excision of TP9524
TP9526	TP9525 <i>tna::Tn10</i>	P1/AE304-3 × TP9525
TP9527	TP2100 <i>tna::Tn10 Δ(uhpABCT)</i>	P1/TP9526 × TP2100
AT2243-11 ^c	Hfr <i>uhp-1 tonA22 ompF627 relA1 spoT1 metB1 pyrE42 T2^r</i>	Ferenci <i>et al.</i> (1971)
TP9528	AT2243-11 ^c <i>tna::Tn10</i>	P1/AE304-3 × AT2243-11 ^c
TP9529	TP2503 <i>tna::Tn10 uhp-1</i>	P1/TP9528 × TP2503
TP9530	TP9529 <i>uhp-1</i>	Tn10 excision of TP9529
DF40	Hfr <i>pgi-2 ompF627 relA1 spoT1 pit-10 mcrB1 phoM510 fadL701 fhuA22 garB10</i>	Fraenkel & Levisohn (1967)
TST6	F ⁻ <i>malF3089::Tn10 [araD139] ptsF25 deoC1 Δ(argF-lac)169 rpsL150 relA1 fliB5301</i>	T. Silhavy (Princeton, NJ)
TP9531	DF40 <i>malF3089::Tn10</i>	P1/TST6 × DF40
TP9532	TP9530 <i>malF3089::Tn10 pgi-2</i>	P1/TP9531 × TP9530
FB8	F ⁻ prototrophic	Bruni <i>et al.</i> (1977)
FB8Δpts	FB8 <i>Δ(ptsH ptsI crr)::kan</i>	Biville <i>et al.</i> (1991)
TP9503	TP2503 <i>Δcrr::kan</i>	P1/TP2862 × TP2503
TP9533	TP9503 <i>tna::Tn10 uhp-1</i>	P1/TP9528 × TP9503
DF1671 DZ1	F ⁻ <i>thr-1 leuB6 hisG1 str-115 pgi-2 rpsL Δ(edd-xwf)15</i>	Fraenkel & Banerjee (1972) via B. Bachmann
CAG12074	Wild-type <i>zea-3068::Tn10</i>	Singer <i>et al.</i> (1989)
TP9534	DF1671 DZ1 <i>zea-3068::Tn10</i>	P1/CAG12074 × DF1671 DZ1
TP9535	TP2503 <i>zea-3068::Tn10 Δ(eda-xwf)15</i>	P1/TP9534 × TP2503
Plasmids		
pJF118HE	ColE1 <i>lacI^a tacP rrnB Ap^r</i>	Fürste <i>et al.</i> (1986)
pDIA187	Derivative of pBR322 containing the <i>uhpT</i> gene, Ap ^r	This work
pDIA188	Derivative of pJF118HE containing the <i>uhpT</i> gene, Ap ^r	This work
pDIA4705	Derivative of pBR322 containing the <i>crr</i> gene, Ap ^r	Zeng <i>et al.</i> (1992)

component regulatory systems (Weston & Kadner, 1988) and UhpC shows sequence similarity with UhpT (Island *et al.*, 1992). It has been suggested that UhpB and UhpC interact during induction (Island & Kadner, 1993).

Glc6P does not inhibit AC activity when measured in toluenized cells (Harwood & Peterkofsky, 1975) or with purified AC (Yang & Epstein, 1983). The mechanism leading to a low level of cAMP when Glc6P is used as the carbon source is not yet understood. Expression of glucose-PTS proteins is not diminished in strains grown on Glc6P as shown by fusion experiments (De Reuse & Danchin, 1988), which indicates that the low cAMP level is not related to a low level of expression of EnzymeIIA^{glc}. In this study, we analyse the effect of various hexose

phosphates on cAMP production. This level is shown to be dependent on the presence of phosphorylated EnzymeIIA^{glc}. We then investigate the activity of phosphorylated EnzymeIIA^{glc} during growth on Glc6P. We show that activation of AC by phosphorylated EnzymeIIA^{glc} is prevented when Glc6P is transported into the cell.

METHODS

Bacterial strains and plasmids. The strains and plasmids used in this work are listed in Table 1. The growth medium was either LB or M63 minimal medium (Miller, 1992) supplemented with the required amino acids (1 mM), thiamine (5 µg ml⁻¹) and a carbon source (0.4%). When required, ampicillin and tetra-

cycline were added at $100 \mu\text{g ml}^{-1}$ and $10 \mu\text{g ml}^{-1}$, respectively. P1 transductions were done as described by Miller (1992). Uhp-constitutive strains were isolated on M63 plates supplemented with fructose 1-phosphate (Fru1P) (Ferenci *et al.*, 1971). Strains lacking phosphoglucose isomerase (*pgi*⁻) produced pink colonies on glucose MacConkey plates and showed slow growth on glucose M63 plates. Strain TP9535 lacking Glc6P dehydrogenase (*zwf*⁻) was checked for the lack of Glc6P dehydrogenase activity according to the method of Fraenkel & Levisohn (1967). Strain TP9525 which contains a deletion of the *uhp* genes ($\Delta\text{uhp}ABCT$) was obtained as follows. The Tn10 transposon of strain TP9524 (which contains a deletion in the *uhpT* gene, Table 1) was removed from the chromosome according to the method of Bochner *et al.* (1980) as modified by Maloy & Nunn (1981). Tet^r clones were analysed by PCR for the presence of the *uhpC* gene. One of the clones showing a deletion of the *uhpC* gene was further analysed by Southern blot hybridization (Southern, 1975).

Determination of total cAMP concentrations. In *E. coli*, more than 99% of the cAMP synthesized is excreted in the culture medium (Matin & Matin, 1982). In addition, under our experimental conditions, the excretion rate of cAMP is a linear function of the intracellular concentration (Epstein *et al.*, 1975; Crasnier *et al.*, 1994). For these reasons, total cAMP was measured. A radioimmunological assay was performed as described by Guidi-Rontani *et al.* (1981) with exponential phase cells grown in M63. The amount of cAMP was expressed as pmol (mg dry wt bacteria)⁻¹. Total cAMP (mg dry wt bacteria)⁻¹ was constant during the exponential phase of growth. Values were calculated as the means of three determinations.

Measurements of cAMP synthesis. *In vivo* measurements of cAMP synthesis were performed as described by Harman & Botsford (1979). Bacteria were grown in M63 to mid-exponential phase. Cells were centrifuged and resuspended in fresh M63 medium supplemented with 0.1% casamino-acids and chloramphenicol at $100 \mu\text{g ml}^{-1}$. The cell suspension was aerated at 37 °C, samples were taken at zero time and after 5, 10, 20 and 30 min. Total cAMP was determined as described above and rates of cAMP synthesis were calculated from the slopes of plots.

Cloning of the *uhpT* gene. A promoterless *uhpT* gene was obtained by PCR using chromosomal DNA from strain TP2503. The primers were chosen according to the sequence of the *uhpT* gene (Island *et al.*, 1992). The upstream primer (CCATCGATTACAATGCATGCCCTCACGC) was located 41 nucleotides upstream of the ribosome-binding site. The downstream primer (CGGAATTCGTTTATGCCACTGTCAACTG) covered the translation termination codon of *uhpT*. The amplified 1.5 kb fragment was digested with *Cla*I and *Eco*RI (whose restriction sites were introduced in the primers) and cloned into pBR322 to give pDIA187. This plasmid placed the *uhpT* gene under the control of the anti-tet promoter. Ap^r transformants of strain TP9523 ($\Delta\text{uhp}T$) were obtained on LB plates but failed to grow with Glc6P or fructose 6-phosphate (Fru6P) in agreement with the fact that overproduction of the *uhpT* gene has been shown to be deleterious to the cells (Kadner *et al.*, 1992). Therefore, the *uhpT* gene was further subcloned as an *Eco*RI–*Hind*III fragment of pDIA187 into pJF118HE. The plasmid thus obtained, pDIA188, carries the *uhpT* gene under the control of the *tac* promoter (Fig. 1).

Measurements of transport and inducer exclusion by the PTS. Uptake of α -[methyl-¹⁴C]glucoside or [¹⁴C]mannose by intact cells was measured according to the method of Bouvet & Grimont (1987). Cultures were grown in MM63 supplemented

Fig. 1. Plasmid pDIA188. Plasmid pDIA188 carries the *uhpT* gene under the control of the *tac* promoter.

with 0.4% pyruvate plus 1 mM glucose or 1 mM mannose. Exponential phase cells were washed twice in M63 containing chloramphenicol at $40 \mu\text{g ml}^{-1}$. The samples were shaken for 15 min at 25 °C before starting the experiment at zero time by adding pyruvate (10 mM final concentration) and α -[methyl-¹⁴C]glucoside ($50 \mu\text{M}$, $14.8 \times 10^7 \text{ Bq mmol}^{-1}$) or [¹⁴C]mannose ($100 \mu\text{M}$, $14.8 \times 10^7 \text{ Bq mmol}^{-1}$). When needed, hexose phosphates at 1 mM final concentration were added at zero time.

Inducer exclusion can be analysed on M63 plates supplemented with 1% (w/v) glucose, 0.2% lactose and $40 \mu\text{g X-Gal ml}^{-1}$ (Tyler *et al.*, 1969). Under these conditions, dephosphorylated EnzymeIIA^{stc} prevents lactose entrance by interacting with the lactose permease. Consequently, wild-type strains grow as white colonies. Replacement of glucose by Glc6P in these plates made it possible to analyse the ability of this carbon source to cause inducer exclusion.

RESULTS

Effect of hexose phosphates on cAMP levels

Glc6P is transported into the cell by the UhpT permease which also mediates the entry of a large variety of hexose phosphates (Winkler, 1966). We investigated cAMP production by the wild-type strain TP2503 growing with different hexose phosphates. With Glc6P, total cAMP was threefold lower than that obtained with pyruvate and twofold lower than that obtained with glucose (Table 2), in agreement with previous data (Epstein *et al.*, 1975). Growth on Fru6P or mannose 6-phosphate (Man6P) resulted in an increased level of cAMP as compared to that obtained with Glc6P, although strain TP2503 grew on Fru6P, Man6P or Glc6P with the same doubling time. However Fru6P and Man6P, which are transported into the cell by UhpT, have been shown to be poor inducers of the Uhp system. This prompted us to analyse the effect of the hexose phosphates on cAMP production in a Uhp-constitutive derivative of strain TP2503. The *uhp*^c mu-

Table 2. cAMP levels in strains grown on different hexose phosphates

Values are means of three determinations $\pm$ SEM. ND, not determined; NG, no growth.

Carbon source	Total cAMP [pmol (mg dry wt) ⁻¹]		
	TP2503 (wild-type)	TP9529 (<i>uhp^c</i>)	TP9503 (Δ <i>crr</i>)
Glucose	300 $\pm$ 100	250 $\pm$ 50	230 $\pm$ 30
Glc6P	150 $\pm$ 20	150 $\pm$ 20	80 $\pm$ 20
Fru6P	800 $\pm$ 150	150 $\pm$ 10	100 $\pm$ 10
Man6P	1000 $\pm$ 200	220 $\pm$ 20	100 $\pm$ 10
Glc1P	3200 $\pm$ 1000	320 $\pm$ 30	ND
Fru1P	NG	500 $\pm$ 50	NG
Pyruvate	1000 $\pm$ 200	1100 $\pm$ 200	150 $\pm$ 50

tation (*uhp-1*) from strain AT2243-11^c (Table 1) was transduced into strain TP2503 to give strain TP9529. Whatever the hexose phosphate used for growth (Fru6P or Man6P), TP9529 showed a low level of cAMP very similar to that obtained with strain TP2503 grown on Glc6P (Table 2). The *uhp^c* mutation did not affect cAMP production of strain TP9529 grown either on glucose or pyruvate. Therefore, different substrates of the UhpT permease were able to decrease the cAMP level provided that UhpT was expressed. In agreement with this observation, growth of the wild-type strain TP2503 with Fru6P in the presence of 0.01% Glc6P as an inducer (Shattuck-Eidens & Kadner, 1981) resulted in a fourfold decrease in the cAMP level as compared to that obtained in the absence of induction [200 versus 800 pmol (mg dry wt)⁻¹].

Glucose 1-phosphate (Glc1P) is a non-inducing UhpT substrate whose glucose moiety can enter the cell via the PTS after periplasmic dephosphorylation (Pradel & Boquet, 1989; Dietz & Heppel, 1971b). With wild-type strain TP2503, growth on Glc1P gave a cAMP level 10-fold higher than on glucose (Table 2), analogous to the high level of cAMP in cells grown in a limiting concentration of glucose (Dumay & Crasnier, 1994). However, with the *uhp^c* strain, Glc1P growth resulted in a low level of cAMP (Table 2).

Another non-inducing substrate for UhpT is Fru1P (Pogell *et al.*, 1966; Ferenci *et al.*, 1971). When the *uhp^c* strain TP9529 was grown on Fru1P, the cAMP level was slightly increased as compared to that of the same strain grown on the other hexose phosphates (Table 2, see Discussion).

Taken together, these results indicated that a decrease in the cAMP level might correlate with an increase in transport via UhpT, whatever the hexose phosphate transported. Table 2 also shows that the cAMP level of a Δ *crr* strain (TP9503) grown on Glc6P, Fru6P or Man6P was decreased as compared to that of the wild-type strain grown on the same carbon source. This result indicates that high cAMP levels require the presence of EnzymeIIA^{glc}.

Effect of Glc6P metabolism on cAMP levels

A *zwf⁻* strain lacking Glc6P dehydrogenase (TP9535, Table 1) showed a low level of cAMP when grown on Glc6P [220 pmol (mg dry wt)⁻¹]. As a *pgi⁻* strain lacking phosphoglucose isomerase (TP9532, Table 1) which, as previously reported (Fraenkel & Levisohn, 1967), has increased doubling time when grown on Glc6P as compared to wild-type strains, also has a relatively low cAMP level [400 pmol (mg dry wt)⁻¹]. These relatively low cAMP levels in cells grown on Glc6P might be related to the concentration of Glc6P in the cell. Therefore, we measured the cAMP in a *pgi⁻* strain grown on Fru6P. In this strain the reversible transformation of Fru6P into Glc6P does not occur. Strains TP9529 (*uhp^c* *pgi⁺*) and TP9532 (*uhp^c* *pgi⁻*) grown on Fru6P showed the same growth rate and had similar low quantities of cAMP, i.e. 200 pmol (mg dry wt)⁻¹ with TP9532 versus 150 pmol (mg dry wt)⁻¹ with TP9529. These results suggest that Glc6P *per se* is not causing a decrease in the cAMP level. Addition of 0.1% deoxyglucose 6-phosphate (a non-metabolizable analogue of Glc6P) to cells of strain TP9529 (*uhp^c*) growing on pyruvate decreased the cAMP level from 1000 to 300 pmol (mg dry wt)⁻¹. In these conditions, the addition of deoxyglucose 6-phosphate did not affect growth of strain TP9529.

Controlled expression of UhpT

To further analyse the role of UhpT in the regulation of the cAMP level, a plasmid was constructed containing the hexose phosphate permease gene (*uhpT*) under the control of the *tac* promoter (pDIA188, Fig. 1). Due to the presence of the *lacI^q* repressor gene on the plasmid, the expression of *uhpT* was dependent on the amount of IPTG in the culture medium. A strain containing a deletion of the entire *uhp* operon was constructed as described in Methods (TP9525, Table 1). This deletion was introduced into strain TP2100 (a Δ *lac* derivative of TP2503). The resulting strain TP9527 (Δ *uhp*.ABCT Δ *lac*) transformed with pDIA188 (Fig. 1) was grown in Glc6P M63 with different concentrations of IPTG. The cAMP levels and the doubling times of growth as a function of

Fig. 2. Variation of cAMP levels and growth rate with IPTG concentration. Strain TP9527 ($\Delta uhpABC7$) containing pDIA188 was grown in minimal medium M63 supplemented with 0.4% Glc6P (a) or 0.4% Fru1P (b). The total cAMP (□) and the doubling time (■) were measured as a function of the IPTG concentration in the growth medium.

the IPTG concentration are shown in Fig. 2. In the absence of IPTG, strain TP9527(pDIA188) grew with a doubling time of 3 h and produced an elevated amount of cAMP [about 3000 to 4000 pmol (mg dry wt)⁻¹, Fig. 2a]. Increasing the IPTG concentration from 0 to 20 μM resulted in a decrease of the doubling time which correlated with a decrease of the cAMP level (Fig. 2a). The doubling time and the cAMP level of the wild-type strain were obtained when an IPTG concentration of 20 μM IPTG was used. Above 20 μM IPTG, the growth stopped after two doubling times (see Discussion). With glucose instead of Glc6P, there was no variation in the doubling time or the cAMP levels as a function of the IPTG concentration, even above 20 μM IPTG.

With TP9527(pDIA488) grown on Fru1P, 100 μM IPTG was required to obtain a growth rate and a cAMP level

Fig. 3. cAMP synthesis in different strains after growth on Glc6P or Glc. cAMP synthesis was measured as described in Methods with strain TP2503 (wild-type) grown on Glc6P (□) or glucose (■). cAMP synthesis was not observed with strain TP9503 (Δcrr) grown on Glc6P (○). AC from strain TP2503 grown on Glc6P was inhibited when Glc6P or Glc was present in the assay medium (●).

comparable to those of the *uhp^c* strain TP9529 grown on Glc6P (Fig. 2b).

Activity of EnzymeIIA^{glc} during hexose phosphate transport

AC activity measured in resting cell suspensions incubated with casamino-acids (Methods) was not detectable in a Δcrr strain (TP9503) grown on Glu6P while, in a wild-type strain (TP2503) grown on Glc6P or glucose, activity was 130 pmol cAMP min⁻¹ (mg dry wt)⁻¹ (Fig. 3). These data, and the fact that β -galactosidase activities with a *cya-lacZ* protein fusion [encoded by pDIA483 (Crasnier *et al.*, 1994)] were similar in strains grown on glucose or Glc6P (data not shown), suggest that the amounts of AC and phosphorylated EnzymeIIA^{glc} available for AC activation are the same in resting cell suspensions obtained from cells grown either on glucose or on Glc6P. Addition of Glc (which is known to cause EnzymeIIA^{glc} dephosphorylation) or Glc6P in the assay medium resulted in inhibition of activity (Fig. 3).

The ability of Glc6P to cause inducer exclusion through dephosphorylation of EnzymeIIA^{glc} was analysed on M63 plates supplemented with Glc6P (see Methods). In contrast to that which was observed with glucose, both wild-type strain FB8 and FB8 Δpts exhibited a blue phenotype on these plates, i.e. no inducer exclusion as in the wild-type strain, Glc6P does not act on AC via dephosphorylation of EnzymeIIA^{glc}. As a control, inducer exclusion was observed (white phenotype) with strain FB8 Δpts transformed with plasmid pDIA4705 which carries the *crr* gene.

Fig. 4. Inhibition of α -[methyl- 14 C]glucoside transport by Fru6P in strains TP9529 (*uhp^c pgi⁺*) and TP9532 (*uhp^c pgi⁻*). Uptake of α -[methyl- 14 C]glucoside by strain TP9529 (■) and strain TP9532 (□) was measured as described in Methods. Zero time corresponds to the addition of α -[methyl- 14 C]glucoside to the reaction mixture. In the presence of 1 mM Fru6P, α -[methyl- 14 C]glucoside uptake was inhibited in both strain TP9529 (●) and strain TP9532 (○).

Fig. 5. Inhibition of [14 C]mannose transport by Glc6P in strain TP9533 (*uhp^c Δ crr*). Uptake of [14 C]mannose by strain TP9533 (□) was measured as described in Methods. Addition of 1 mM Glc6P resulted in an inhibition of the uptake (■).

Effect of hexose phosphates on PTS-sugar transport

Glc6P has been shown to inhibit PTS-mediated transport (Kornberg, 1973; Lengeler & Steinberger, 1978). We analysed the effect of Glc6P on the uptake of α -[methyl- 14 C]glucoside, a non-metabolizable analogue of glucose. As reported by Lengeler & Steinberger (1978), an inhibitory effect of Glc6P on the uptake of α -[methyl- 14 C]glucoside was observed only when the Uhp system was induced. The *uhp^c* strain TP9529 exhibited the same inhibition of α -methylglucoside uptake in the presence of Glc6P. Addition of Fru6P, Man6P or deoxyglucose 6-phosphate also resulted in an inhibition of α -[methyl- 14 C]glucoside transport in strain TP9529 (data not shown), indicating that Glc6P was not the only sugar phosphate that could cause the decreased α -[methyl- 14 C]glucoside transport rate. The same experiments were carried out with strain TP9532, a *pgi⁻* derivative of strain TP9529 (in this strain, Glc6P cannot be synthesized from Fru6P). The inhibitory effect of Fru6P on α -methylglucoside transport is shown in Fig. 4. The same inhibition was observed with both *pgi⁺* and *pgi⁻* strains, suggesting that Glc6P *per se* was not responsible for the decreased α -[methyl- 14 C]glucoside transport rate.

We then investigated the effect of Glc6P transport on [14 C]mannose uptake in a Δ crr strain (lacking EnzymeIIA^{glc}). A total deletion of the *crr* gene was transduced into the *uhp^c* strain TP9529. The resulting strain TP9533 was able to transport mannose through its specific PTS. As shown in Fig. 5, mannose uptake was also inhibited by the addition of 1 mM Glc6P. This result indicated that Glc6P transport inhibited the PTS-de-

pendent transport of mannose in the absence of EnzymeIIA^{glc}.

DISCUSSION

Strains growing in the presence of Glc6P as sole carbon source show low levels of cAMP but the regulatory mechanism leading to the low level of cAMP is not known. We have shown here that Glc6P degradation is not directly involved in the regulation of the cAMP level. Transport of a non-metabolizable analogue of Glc6P (deoxyglucose 6-phosphate) decreased the cAMP level when added to cells growing on pyruvate cells. Strains lacking either Glc6P dehydrogenase (*zwf*) or phosphoglucose isomerase (*pgi*) had a low level of cAMP when grown on Glc6P, close to that of a wild-type strain grown on Glc6P. In addition, Glc6P *per se* was not involved in the regulation of the cAMP level as a *pgi⁻* strain showed low cAMP levels when grown in the presence of Fru6P. The fact that the cAMP level in a *pgi⁻* strain grown on Glc6P was increased as compared to the same strain grown on Fru6P [400 versus 200 pmol (mg dry wt)⁻¹] was most likely due to the slow growth observed with Glc6P as compared to Fru6P.

We also showed that a low level of cAMP was obtained only when UhpT, the Glc6P permease, is induced and Glc6P (or a UhpT substrate) transported as shown by the following results: (1) in the absence of Glc6P transport, a Uhp-constitutive strain grown on pyruvate showed a high level of cAMP (Table 2); (2) different substrates of the UhpT permease were able to decrease the cAMP level provided that the UhpT permease was expressed; and (3) increasing the amount of UhpT synthesized, by using a vector carrying the *uhpT* gene under the *tac* promoter (IPTG-inducible), led to a decrease in the cAMP level

when the hexose phosphates transported by UhpT were added. In the absence of IPTG, slow growth was observed and an elevated amount of cAMP was measured as compared to a wild-type strain grown on Glc6P. In agreement with this data, Yang *et al.* (1979) reported that Glc6P-limited growth in a chemostat also resulted in a high cAMP level. With Glc6P, both growth rate and cAMP levels characteristic of a wild-type strain were obtained for 20 μ M IPTG. Fürste *et al.* (1986) also showed that, with a similar expression vector, induction by IPTG is detectable in the range 10–100 μ M. Above 20 μ M IPTG, the growth stopped, as demonstrated previously by Kadner *et al.* (1992), showing that amplified UhpT transport inhibits growth. With Fru1P, the same response to IPTG was observed with a shift in the curve as compared to that obtained with Glc6P (five times more IPTG required to obtain both the same growth rate and cAMP level). In addition, the cAMP level of the *uhp^c* strain grown on Fru1P was slightly increased as compared to that of the same strain grown on the other hexose phosphates (Table 2). These data could be related to the low affinity of Fru1P for UhpT as compared to that of Glc6P (Pogell *et al.*, 1966; Maloney *et al.*, 1990). Taken together, these results suggest that UhpA, UhpB and UhpC did not play any role in decreasing the cAMP level in cells growing in the presence of hexose phosphates, although, in wild-type cells, these proteins may have an indirect effect on the cAMP level by regulating the synthesis of UhpT. Thus the level of cAMP is dependent on the amount of Glc6P (or any other hexose phosphates) transported by the UhpT permease.

We propose that the regulation of the cAMP level when Glc6P was used as carbon source occurred during Glc6P transport. The UhpT transport system has been proposed to function in two modes (Maloney *et al.*, 1990). In one mode, two Glc6P⁻ anions move into the cell in exchange for the export of one Glc6P²⁻. This exchange is equivalent to the net entry of one Glc6P²⁻ and two H⁺ and is thermodynamically equivalent to a 2H⁺:Glc6P²⁻ symport with Δ pH acting as the sole driving force. It has been reported that a collapse of the proton electrochemical gradient leads to a decrease of AC activity (Peterkofsky & Gazdar, 1979). However, under our experimental conditions, a collapse of the gradient by transport via UhpT is unlikely to occur as proton pumping under aerobic steady-state conditions is too rapid for a collapse of the gradient to take place. The exchange system by UhpT can also operate by moving two H₂PO₄⁻ anions out and one Glc6P²⁻ in. Although it has been shown that phosphate is necessary for the activation of the AC-PTS complex (Peterkofsky, 1988), it is unlikely that Glc6P transport leads to a depletion of the phosphate pool which is tightly maintained in *E. coli*. However, the possibility remains that local phosphate concentrations at the membrane level are decreased during Glc6P transport, affecting the activation of AC by EnzymeIIA^{g1c}.

Both functions of the PTS, i.e. glucose transport and activation of AC, are inhibited during Glc6P transport. To explain the inhibitory effect of Glc6P on PTS transport, it has been proposed that the PTS permeases,

EnzymeIICBA^{mtl} or EnzymeIICB^{gut}, were inhibited by intracellular hexose phosphates (Lengeler & Steinberger, 1978). However, attempts to reproduce the inhibitory effect of Glc6P on the PTS permeases *in vitro* have failed (Jacobson *et al.*, 1983). In strain TP2504 (lacking EnzymeIICB^{g1c}) grown on Glc6P, a low cAMP level was measured [200 pmol (mg dry wt)⁻¹], indicating that transport of Glc6P remained effective in maintaining a low cAMP level in the absence of the glucose permease. Therefore an inhibition of EnzymeIICB^{g1c} by hexose phosphate (which could have affected EnzymeIIA^{g1c} regulation of AC) could not be related to the low cAMP level. Finally, the fact that EnzymeIIA^{g1c} was present in strains grown on Glc6P (as shown by measurement of AC activity) and was probably phosphorylated (as shown by the inducer exclusion experiment) suggested that AC cannot be activated by phosphorylated EnzymeIIA^{g1c} during Glc6P transport. Thus it is worth noting that the mechanism of 'catabolite repression' by hexose phosphate may be different from the one occurring with glucose. It must be emphasized that regulation of AC activity during Glc6P transport probably occurs at the membrane level. This is supported by data indicating that the soluble enzymes of the PTS exist as peripheral membrane constituents associated with the integral membrane Enzyme II complex (Saier *et al.*, 1982) or with data showing a localization of Enzyme I to the inner surface of the cytoplasmic membrane (Ghosh *et al.*, 1989).

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