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Mapping of the *Escherichia coli* Acid Glucose-1-Phosphatase Gene *agp* and Analysis of Its Expression In Vivo by Use of an *agp-phoA* Protein Fusion

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The *agp* gene of *Escherichia coli* encodes an acid glucose-1-phosphatase, one of the numerous phosphatases optimally active between pH 4 and 6 found in the periplasmic space of this bacterium. An *agp-phoA* protein fusion linked to a gene conferring kanamycin resistance was inserted into the chromosome in place of *agp* by homologous recombination and was mapped to minute 22.6. Because the activity of glucose-1-phosphatase cannot be measured accurately in whole cells, the alkaline phosphatase activity of the *agp-phoA* hybrid protein was used to monitor the expression of the chromosomal *agp* gene. The expression of *agp* was subject to catabolite repression but was unaffected by the concentration of inorganic phosphate in the growth medium. The product of the *agp* gene was required for growth on glucose-1-phosphate as the sole carbon source, a function for which alkaline phosphatase or other acid phosphatases cannot substitute.

The presence in the periplasmic space of *Escherichia coli* of an acid phosphatase able to hydrolyze glucose-1-phosphate (G-1-P) was demonstrated recently after the cloning of its structural gene (*agp*). The enzyme is a dimer with subunits of apparent M_r 44,000 and displays optimum activity between pH 4 and 5 (25). Four additional periplasmic acid phosphatase activities were also detected in the earlier study (25). Owing to this multiplicity of acid phosphatases active in the same pH range and to the overlap in their substrate specificities, it is difficult to detect mutants lacking individual phosphatases or to measure accurately the activity of each of these enzymes in a wild-type strain.

We have previously reported the isolation of plasmid-borne *agp-phoA* gene fusions using transposon Tn*phoA* (25). In this study, we transferred one of these fusions into the chromosome in place of *agp* and used it to determine the map position of *agp*. The production of alkaline phosphatase activity from this fusion was measured under different growth conditions and was used to show that expression of *agp* is subject to catabolite repression and is not controlled by the concentration of inorganic phosphate in the growth medium.

MATERIALS AND METHODS

Bacteria and plasmids. The characteristics of all the *E. coli* K-12 strains used in this study are listed in Table 1. The construction of the recombinant plasmid pEP1376 and the obtainment of the fusion plasmid pEP1390 have been described previously (25).

Media and growth conditions. The rich medium used was tryptone-yeast extract broth (TYE) (25). Plasmids derived from pBR322 were maintained in the presence of ampicillin (200 μg/ml). Other selection conditions are indicated in the text. The high-phosphate (200 mM) DM minimal medium was that of Davis and Mingioli (9). The low-phosphate triethanolamine–Bacto-Peptone (TEB) medium, the sulfur-limited medium, and the nitrogen-limited medium were as described previously (8). Anaerobic growth was obtained by overlaying 1 ml of paraffin oil on 3-ml cultures in test tubes and gently stirring the culture with a small magnet.

Genetic and recombinant DNA techniques. General mapping techniques and P1 transduction experiments were performed by the methods of Miller (23). Plasmid DNA was prepared as described by Maniatis et al. (19), and competent cells and transformants were obtained by the method of Mandel and Higa (18). Restriction mapping of plasmids and other DNA techniques were as described by Schleif and Wensink (28).

Detection and measurement of enzyme activities. Colonies on replica plates or grids were tested for the acid phosphatase expressed from the multicopy plasmid pEP1376 by overlaying the plates with 3 ml of 1 M formic acid containing 25 mM *p*-nitrophenyl phosphate (pNPP) and incubating them for 3 to 5 min at 42°C. The formic acid solution was then removed and replaced with 3 ml of concentrated ammonia solution. Positive colonies appeared bright yellow. The background hydrolysis of pNPP resulting from the expression of chromosomal acid phosphatase genes was not detected under these conditions. Alkaline phosphatase-positive colonies were visualized in the presence of 40 μg of 5-bromo-4-chloro-3-indolyl-phosphate (XP) per ml. The specific activity of alkaline phosphatase in bacteria in liquid cultures was measured as described previously (4, 22).

RESULTS

Introduction of stable labeled *agp-phoA* fusion into the chromosome in place of *agp*. The restriction map of the recombinant plasmid pEP1390 containing the *agp* gene into which a Tn*phoA* transposon (20, 21) leading to an in-frame *agp-phoA* protein fusion was inserted is given in Fig. 1. A *Sma*I-*Sma*I deletion derivative of this plasmid (pEP 1393) lacking Tn*phoA* transposase-coding sequences was introduced into strain SBS1174 [Δ*phoA pol*A(Ts)]. One transformant was grown at 42°C in the presence of kanamycin (30 μg/ml) and ampicillin at a low concentration (20 μg/ml) with serial subcultures for about 30 generations to allow integration of plasmid pEP1393 into the chromosome by homologous recombination. Bacteria harboring free recombined plasmids which no longer expressed alkaline phosphatase at

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TABLE 1. Strains of *E. coli* and plasmids used

Strain or plasmid	Genotype or relevant characteristics	Source or reference
Strains		
Mph2	F⁻ *araD139 ΔlacU169 Δ(brnQ phoA proC phoB) thi rpsL*	J. Beckwith
SBS1174	Same as Mph2 but *polA12* Tn*10-64*	This laboratory
SBS1680	Same as SBS1174 but *agp*:: *phoA*-IS*50*$_L$-*neo*	This study
SBS1254	Same as Mph2 but Δ*cya-854*	This laboratory
SBS1414	Same as SBS1254 but *agp*:: *phoA*-IS*50*$_L$-*neo*	This study
SBS1415	Same as Mph2 but *agp*::*phoA*-IS*50*$_L$-*neo*	This study
DC305	F⁻ *pyrD34 his-68 galK2 malA1 xyl-7 mtl-2 rpsL118 zcc*::Tn*10*	D. Clark via J. Wood
30SOU6	HfrH *lacZ*^{s}*43 pyrC relA*	M. Schwartz
SBS1416	Same as 30SOU6 but *appA1 zcc*::Tn*10*	This laboratory
K10	HfrC *relA pit-10 tonA22 thi*	B. Bachmann
SBS1312	Same as K10 but Δ*phoA20 pyrD34 zcc*::Tn*10*	This laboratory
SBS1422	Same as SBS1312 but *pyrD*$^+$ Tc^s (*zcc*$^+$) *agp*::*phoA*-IS*50*$_L$-*neo*	This study
SBS1554	Same as SBS1422	This study
SBS1566	Same as SBS1312 but *pyrD*$^+$	This study
SBS1568	Same as K10 but *agp*:: Δ*phoA20*-IS*50*$_L$-*neo*	This study
SBS1572	Same as SBS1422 but *agp*:: Δ*phoA20*-IS*50*$_L$-*neo*	This study
SBS815	Same as K10 but *rpsL* Δ*crp-39 srl*::Tn*10*	This laboratory
SBS816	Same as SBS815 but *crp*$^+$	This laboratory
SBS1494	Same as SBS815 but *agp*:: *phoA*-IS*50*$_L$-*neo*	This study
SBS1495	Same as SBS1494 but *crp*$^+$	This study
G19	F⁻ *putA19 gltA his purE rpsL*	B. Bachmann
SBS1520	Same as G19 but *agp*::*phoA*-IS*50*$_L$-*neo*	This study
Plasmids		
pBR322	Ap^r Tc^r	J. Beckwith
pEP1376	Ap^r Tc^s::*agp*	25
pEP1390	Ap^r Tc^s Km^r::*agp*::Tn*phoA*	25
pEP1393	Ap^r Tc^s Km^r::*agp*::*phoA*-IS*50*$_L$-*neo*	This study

a high level were selected at 30°C in the presence of kanamycin (30 μg/ml), ampicillin (200 μg/ml), and XP as light blue colonies compared with the deep blue initial population. The acid phosphatase activity in such segregants (measured at pH 4.5) was as high as in bacteria with the original (*agp*$^+$) plasmid pEP 1376 (1,900 U/mg of protein, compared with 65 to 70 U/mg of protein in the strain with pEP1393 or without a plasmid), indicating the presence of a wild-type *agp* gene on a recombined plasmid population. Stocks of bacteriophage P1 grown on some of these clones were used to transduce the Km^r marker integrated into the *agp* region of the chromosome into strains HfrH, HfrC, and HfrKL16 and into several F⁻ Δ*phoA* recipients (Table 1). The colonies of all Km^r Ap^s transductants tested (about 30% of all Km^r colonies scored, the remainder containing free or integrated plasmids) were stained blue in the presence of XP on rich medium plates (TYE), showing that the whole fusion had been transferred to the chromosomes of recipients. Compared to the wild-type bacteria, their ability to hydrolyze pNPP at pH 4 was only slightly decreased, presumably owing to the presence of the other acid phosphatases. Transductants grew as well as their parent strain on minimal medium containing glucose, glycerol, or sodium succinate as carbon source or on rich media including a low-phosphate medium. *agp* is thus not essential for the growth under these conditions.

Mapping of *agp* on the chromosome. Conjugation experiments were first performed with derivatives of strains HfrH and HfrKL16 harboring the stable labeled (Km^r) *agp-phoA* fusion. Analysis of the exconjugants by the transfer gradient method in a preliminary experiment indicated in all cases the close proximity of Km^r with the genes *pyrD* and *pyrC* (data not shown). The results of P1 transductions involving crosses between several markers of the 22- to 23-minute region of the chromosome indicated the map position shown in Fig. 2.

Expression of *agp-phoA* fusion according to growth conditions. Examination of the rate of alkaline phosphatase synthesis during growth of one of the *agp-phoA* transductants in TYE medium indicated that the enzyme was synthesized during the exponential phase and continued to accumulate in the early stationary phase (Fig. 3). The maximal specific activity was reached within a few hours after the cessation of growth and remained constant for about 24 h. Consequently, most determinations could be made on bacteria from overnight cultures.

According to the blue staining by XP of colonies on solid semisynthetic medium, the expression of the fusion was apparently influenced by the nature of the carbon source, showing a reduced activity for glucose compared with that for sodium succinate (data not shown). This suggested a possible control of *agp* transcription by cyclic AMP (cAMP) and its receptor, the CAP protein. Introducing the fusion by P1 transduction into isogenic Δ*crp* and *crp*$^+$ strains showed that the CAP protein was required for a high expression of *agp-phoA* (Table 2). The background levels of pNPP hydrolysis measured at pH 8.5 in isogenic Δ*crp agp*$^+$ or *crp*$^+$ *agp*$^+$ strains, however, were far from negligible and were not due to the expression of the wild-type *phoA* gene. About half of this remnant activity was itself under the positive control of CAP and cAMP and could be attributed to the product of the wild-type *agp* gene, acid glucose-1-phosphatase, previously shown to display a residual activity at pH 8.5 (25). The expression of the *agp-phoA* fusion in an adenylate cyclase-deficient (Cya⁻) strain was restored in the presence of cAMP (Table 2). Its dependence on exogenously added cAMP in a Cya⁻ strain showed, like most cAMP positively controlled systems, an inflexion point near 1 mM cAMP (Fig. 4), compared with about 0.1 mM in negatively controlled systems such as *appA* (33).

We have previously reported the influence of the allelic state of a new gene, *appR*, located at minute 59 of the linkage map (32, 33) on the expression of the alkaline phosphatase (*phoA*) and of the pH 2.5 acid phosphatase (*appA*). No significant change in the level of alkaline phosphatase originating from the *agp-phoA* fusion was observed in isogenic *appR* and *appR*$^+$ strains (data not shown).

Growth of strain SBS1415 in a phosphate-limited medium with a nonrepressible carbon source similarly did not significantly change this level, showing that unlike *phoA* or *appA*, *agp* is not induced by inorganic phosphate starvation (Table 3). The use of a nitrogen-limited or sulfur-limited medium also had little effect on the expression of the fusion, but growth under anaerobic conditions with glucose resulted in a four- to fivefold stimulation compared with aerobic condi-

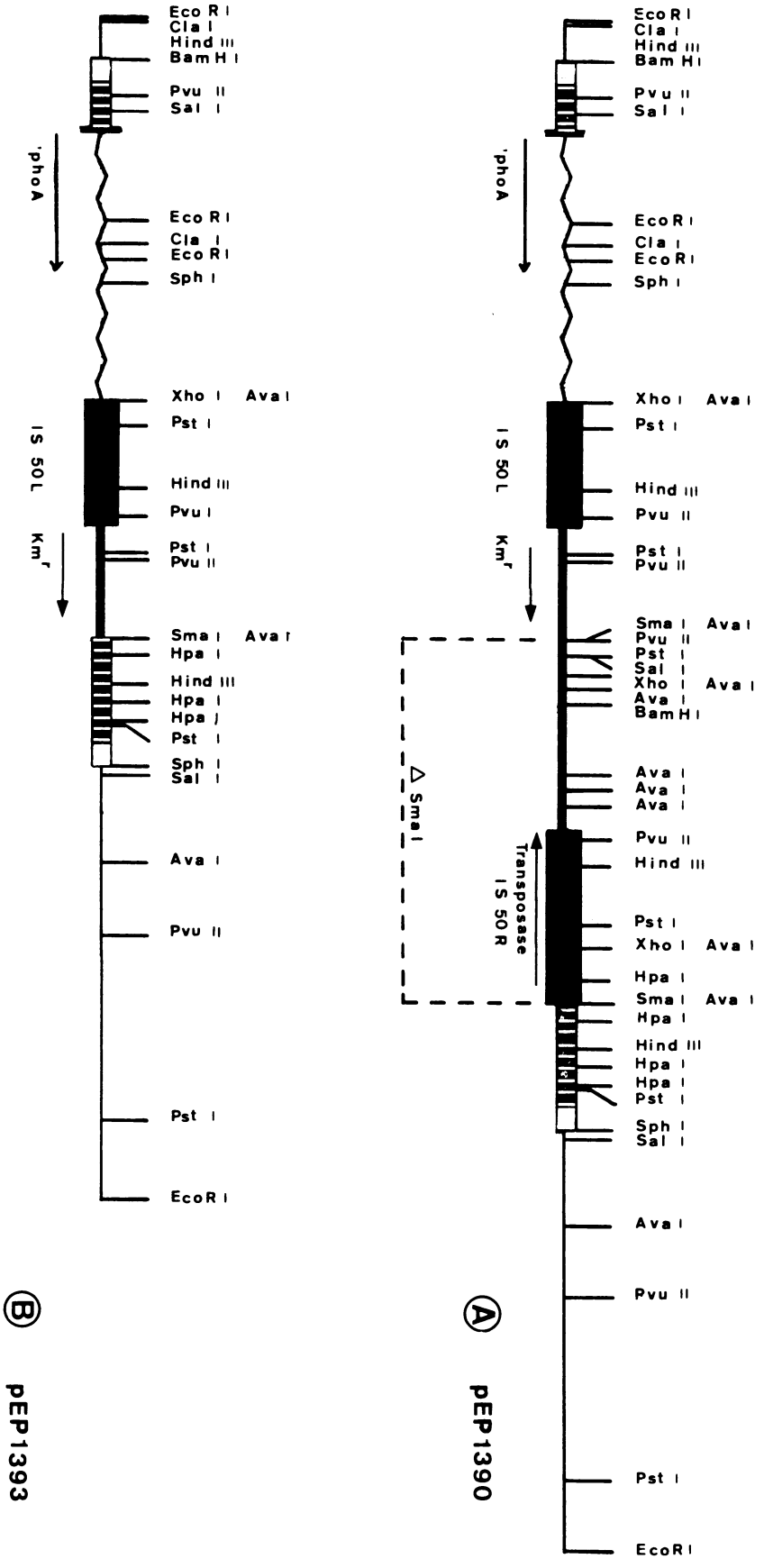

FIG. 1. Restriction maps of plasmids pEP1390 and pEP1393. (A) Map showing the position of the inserted Tn*phoA* leading to an in-frame *agp-phoA* fusion. The direction of transcription is indicated by an arrow. (B) Stabilization of the fusion by a *Sma*I-*Sma*I deletion removing $IS50_R$ and the gene for transposase. The thin line indicates pBR322 DNA, the heavy line is recombinant DNA with gene *agp* as a hatched area, the squiggled line is the *phoA* region of Tn*phoA*, and the dark area originates from Tn*5*. kb, Kilobase.

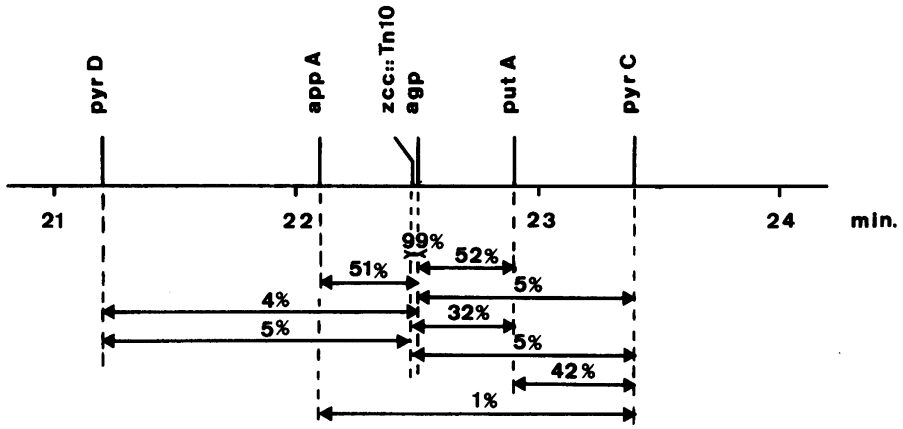

FIG. 2. Position of *agp* on the *E. coli* linkage map. The data refer to the results of the P1 transduction experiments reported in the text. The numbers indicate the percentages of cotransduction between the markers. The distances are calculated according to Wu (39).

tions (data not shown). It is not certain, however, whether this effect was or was not caused by the known elevation of the cellular concentration of cAMP in such conditions (A. Ullman, personal communication).

Effects of inactivation of *agp* gene in a Δ*phoA* strain. The introduction of an *agp-phoA* fusion in place of the original *agp* gene abolished the activity of glucose-1-phosphatase tested in acidic conditions, but since G-1-P could also be hydrolyzed by the alkaline phosphatase activity of the hybrid protein, it was necessary to inactivate the genes for both enzymes to know whether glucose-1-phosphatase was required for growth on G-1-P as the sole phosphate or carbon source.

Such a strain lacking both *phoA* and *agp* functions was obtained from SBS1554 (Δ*phoA agp-phoA*) by recombining the mutant gene Δ*phoA20* (5, 27) into the *agp-phoA* fusion. Intrachromosomal recombinants were detected on low-phosphate-medium plates containing XP as deep blue colonies (about 1%), compared with the lighter staining of the original population. This phenotype resulted from the induction of a wild-type *phoA* gene by phosphate starvation. Transduction analysis with one such recombinant, SBS1568, indeed verified the presence of an intact *phoA* gene at minute 8.8 and the absence of functional *agp-phoA* fusion linked to the *neo* (Km^r) gene at minute 22. This region was cloned by homologous recombination with plasmid pEP1376 (agp^+) in the *polA*(Ts) strain SBS1680 (resistance to high kanamycin levels), and the presence of the *agp*-Δ*phoA20*-$IS50_R$-*neo* construction was verified by restriction analysis.

The four isogenic strains SBS1554 (Δ*phoA20 agp-phoA*$^+$), SBS1566 (Δ*phoA20 agp*$^+$), SBS1568 (*phoA*$^+$ *agp*-Δ*phoA20*), and SBS1572 (Δ*phoA20 agp*-Δ*phoA20*), constructed by P1 transduction, were compared for their ability to use G-1-P as the sole phosphate or carbon source. In the presence of glucose (28 mM) as the carbon source, the four strains were

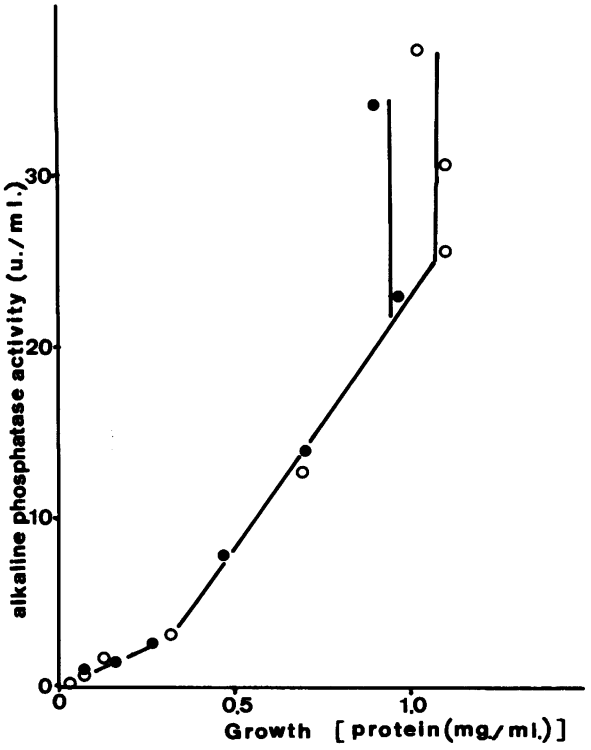

FIG. 3. Expression of an *agp-phoA* protein fusion according to the growth phases. The differential rate of alkaline phosphatase synthesis was measured over a 24-h growth period in TYE medium in two strains harboring the same *agp-phoA* fusion (described in Fig. 1) but differing in their genetic background (Table 1). Symbols: ○, SBS1415 (a derivative of MC4100); ●, SBS1422 (a derivative of K10).

TABLE 2. Analysis of the regulation of *agp* in vivo by measurements of the alkaline phosphatase activity expressed from a chromosomal *agp-phoA* fusion: dependence on cAMP and CAP[a]

Strain	Relevant genotype or phenotype	nmol of pNPP hydrolyzed/min/mg of protein	
		−cAMP	+ cAMP (1 mM)
SBS815	Δ*crp* (*phoA*$^+$) *agp*$^+$	5.4	5.2
SBS1494	Δ*crp* (*phoA*$^+$) *agp-phoA*	14.6	11
SBS816	*crp*$^+$ (*phoA*$^+$) *agp*$^+$	14.4	12
SBS1495	*crp*$^+$ (*phoA*$^+$) *agp-phoA*	57.6	36
SBS1254	Δ*cya* Δ*phoA agp*$^+$	1.9	13.2
SBS1414	Δ*cya* Δ*phoA agp-phoA*	8.0	52.4
SBS1415	*cya*$^+$ Δ*phoA agp-phoA*	56	69
Mph2	*cya*$^+$ Δ*phoA agp*$^+$	15.7	15.5

[a] Growth was in TYE medium, which contains enough inorganic phosphate (>1 mM) to repress *phoA*.

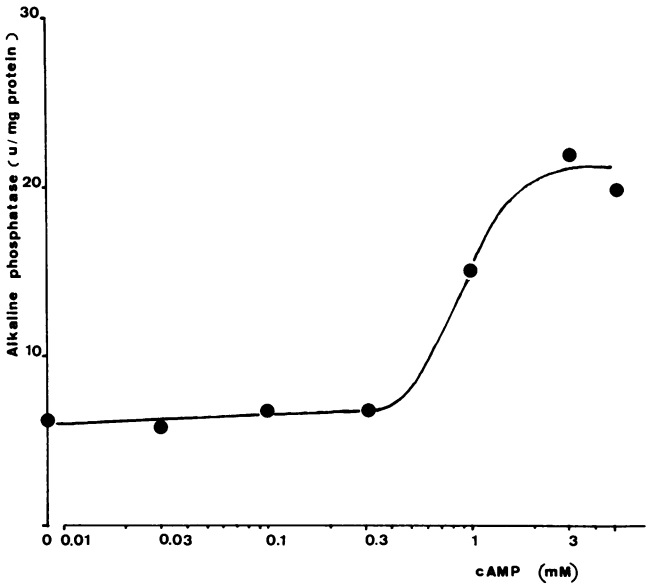

FIG. 4. Dependence on exogenously supplied cAMP of the expression of an *agp-phoA* chromosomal protein fusion in a Cya$^-$ strain. Bacteria from strain SBS1414 were grown overnight in TYE medium containing various amounts of cAMP, and the specific activity of alkaline phosphatase was determined as indicated in Materials and Methods.

able to use G-1-P as their sole phosphate source, even at low concentrations of this substrate (0.1 mM), through the activity of other, less efficient phosphatases possibly induced by inorganic phosphate starvation and providing enough phosphate for growth. Strains SBS1566 and SBS1568, however, grew better on this medium than strains SBS1572 and SBS1554, showing a more efficient dephosphorylation by the wild-type *phoA* or *agp* gene product (Fig. 5A). In the high-phosphate DM minimal medium, only strain SBS1566 (*agp*$^+$) could grow with G-1-P as the sole carbon source (Fig. 5B). In such conditions, the expression of *phoA* was repressed in SBS1568, while in SBS1554, the alkaline phosphatase activity originating from the hybrid protein was apparently insufficiently expressed to provide enough glucose to allow growth.

TABLE 3. Influence of inorganic phosphate in the growth medium on the expression of the *agp-phoA* chromosomal fusion[a]

Strain	Relevant genotype or phenotype	nmol of pNPP hydrolyzed/min/mg of protein	
		Low P_i (0.05 mM)	High P_i (10 mM)
SBS1422	Δ*phoA agp-phoA*	6.2	12
SBS1312	Δ*phoA agp*$^+$	3.7	6.5

[a] Growth was in low-phosphate TEB medium (8) supplemented or not with sodium phosphate.

DISCUSSION

The presence of an acid phosphatase active near pH 5 in *E. coli* was first reported concomitantly with alkaline phosphatase (31). Further studies on enzymatic activities in the periplasmic space showed that several distinct acid phosphatases coexisted in this extracellular compartment (2, 14, 26, 34). Biochemical fractionation of some of these enzymes in wild-type *E. coli* extracts led to the conclusion that at least four distinct species were able to hydrolyze the synthetic substrate of the phosphomonoesterase pNPP between pH 4 and 6 (27). Two of them were shown to behave as an acid hexose phosphatase and as a nonspecific acid phosphatase, respectively (14, 34).

We have recently reported the cloning on multicopy plasmids of genes promoting the overexpression of five different periplasmic acid phosphatases including *agp*, the structural gene for a glucose-1-phosphatase (25). The diffi-

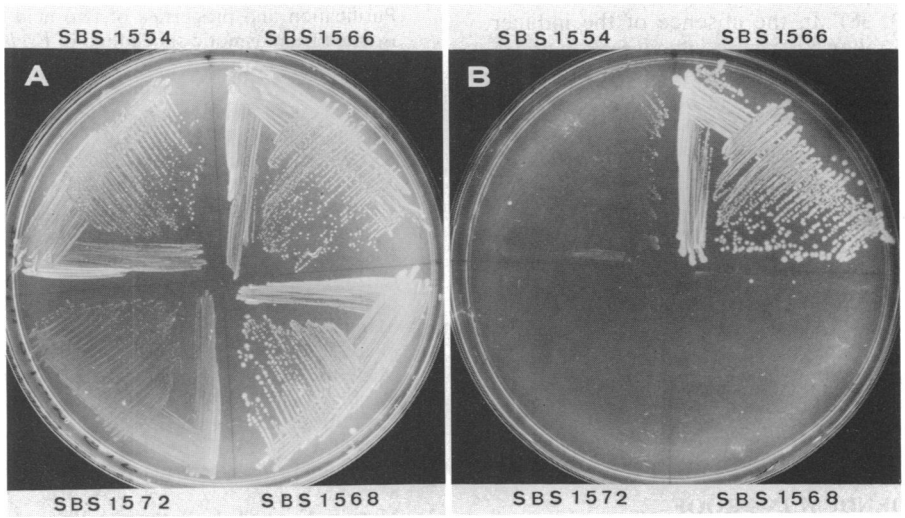

FIG. 5. Utilization of G-1-P as the sole phosphate (A) or carbon (B) source by isogenic strains differing by the activity of genes *phoA* and *agp*. (A) Bacteria from the four isogenic strains SBS1554 (Δ*phoA20 agp-phoA*), SBS1566 (Δ*phoA20 agp*$^+$), SBS1568 (*phoA*$^+$ *agp*-Δ*phoA20*), and SBS1572 (Δ*phoA20 agp*-Δ*phoA20*) were streaked on plates containing inorganic phosphate-free triethanolamine-HCl-buffered (TE) minimal medium (8) with glucose (28 mM) as the carbon source and G-1-P (0.1 mM) as the sole phosphate source. Plates were incubated for 30 h at 37°C. (B) Bacteria from the same strains were streaked on DM minimal medium (200 mM inorganic phosphate) with G-1-P (10 mM) as the sole carbon source and incubated for 30 h at 37°C.

culty in measuring the activity of this enzyme in cells, resulting from the expression of several other acid phosphatases, was overcome by the construction of a stable *agp-phoA* labeled fusion which was introduced into the chromosome in place of *agp*. We showed here that *agp* lies near minute 22.5, close to and clockwise from *appA*, the structural gene for another acid phosphatase, the pH 2.5 acid phosphatase (4, 7, 8).

The regulatory characteristics of *agp*, studied in vivo by means of an *agp-phoA* gene fusion, indicate that the conditions for *agp* expression are different from those for *appA* (8, 33). First, in contrast to *appA*, *agp* is positively controlled by CAP and cAMP and is not affected by mutations in a separate regulatory locus (*appR*) (32; Pradel, unpublished results). Second, unlike *appA* (or *phoA*), it is not influenced by the concentration of inorganic phosphate in the growth medium, suggesting that glucose-1-phosphatase is not a phosphate-scavenging enzyme. In agreement with such observations, the ability of bacteria deprived of both alkaline phosphatase and acid glucose-1-phosphatase (SBS1572) to grow on a medium containing G-1-P as the sole phosphate source (Fig. 5A) showed that under such conditions another unidentified phosphatase(s) can provide enough phosphate from this substrate for growth. The unspecific alkaline phosphatase can also hydrolyze G-1-P in vivo (cf. strain SBS1568 in Fig. 5B), but only if this enzyme is induced by inorganic phosphate starvation. The unique capacity of agp^+ strains to use G-1-P as their sole carbon source in a high-phosphate medium, by contrast, strongly suggests a role for the *agp* product in scavenging glucose from G-1-P. Such G-1-P may be present in one of the natural habitats of *E. coli*, the intestine of mammals, where it could originate from glycogen degradation by mucosal cells and leak out in appreciable proportions. Alternatively, glucose-1-phosphatase might function in connection with the *E. coli* surface enzyme 5′-nucleotidase, the product of gene *ushA* (3, 6), which cleaves UDP-glucose to G-1-P.

In *E. coli*, exogenously supplied hexose phosphates are transported through a single system (1, 10, 24, 37) encoded by gene *uhpT* in the *uhp* operon (16, 17, 29, 30, 35, 36) which is specifically induced by glucose-6-phosphate or its 2-deoxy derivative (11, 12, 29, 38). In the absence of the inducer, G-1-P is known to be taken up as free glucose after hydrolysis by a putative specific phosphatase of the cell surface (10). However, only an unspecific acid hexose phosphatase has presently been described in the periplasmic space of *E. coli* (26). The affinity of the phosphatase encoded by gene *agp* for G-1-P, its mode of regulation, and the growth characteristics of Agp^- strains indicate that this enzyme is probably not involved in phosphate economy but is absolutely required for the utilization of G-1-P as a sole carbon source whenever the concentration of inorganic phosphate is not growth limiting.

ACKNOWLEDGMENT

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ADDENDUM IN PROOF

As pointed out by M. H. Saier, Jr., the *E. coli agp* gene product probably is homologous to the *Salmonella typhimurium* acid hexose phosphatase described by Rephaeli et al. (A. W. Rephaeli, I. R. Artenstein, and M. J. Saier, Jr., J. Bacteriol. **141**:1474–1477, 1980).

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