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Co-Regulation in *Escherichia coli* of a Novel Transport System for *sn*-Glycerol-3-Phosphate and Outer Membrane Protein Ic (e, E) with Alkaline Phosphatase and Phosphate-Binding Protein

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Mutants constitutive for the novel outer membrane protein Ic (e or E) contained a recently discovered binding protein for *sn*-glycerol-3-phosphate. The corresponding parental strains missing the outer membrane protein Ic (e, E) were negative or strongly reduced in the synthesis of the binding protein. In addition, strains that were previously isolated as mutants constitutive for the *sn*-glycerol-3-phosphate transport system (*ugp*$^+$ mutants) and that produced the novel periplasmic proteins GP1 to GP4 also synthesized a new outer membrane protein with the same electrophoretic mobility on sodium dodecyl sulfate-polyacrylamide gels as protein Ic. Screening of different *ugp*$^+$ mutants revealed the existence of three types in respect to the four novel periplasmic proteins GP1, -2, -3, and -4: (i) one containing all four proteins; (ii) one containing only proteins GP1, -2, and -3; (iii) one containing only proteins GP1, -2, and -4. In confirmation of the data presented in the accompanying paper by Tommassen and Lugtenberg (J. Bacteriol. **143**:151-157, 1980), we found that purified GP1 is identical to alkaline phosphatase, whereas purified GP3 has binding activity of inorganic phosphate and is identical to the phosphate-binding protein. Moreover, growth conditions that lead in a wild-type strain to the derepression of alkaline phosphatase synthesis also derepressed the synthesis of the *sn*-glycerol-3-phosphate-binding protein as well as the corresponding transport system. Thus, the new *sn*-glycerol-3-phosphate transport system is part of the alkaline phosphatase regulatory system.

Recently, we found a new transport system for *sn*-glycerol-3-phosphate (G3P) in mutants (*ugp*$^+$ mutants) that arose as G3P$^+$ suppressors in strains carrying a defective transport system for G3P coded for by *glpT* (3) at 48 min on the *Escherichia coli* chromosome (4). These *ugp*$^+$ mutants mapped outside *glpT* and synthesized the new periplasmic proteins (GP1, -2, and -3). One of these proteins (GP2) was identified as a high-affinity binding protein for G3P (2).

Along other lines, we were interested in the pore-forming activity of osmotic shock fluid in black lipid films due to soluble outer membrane proteins Ia and Ib (6). In this respect, we were interested whether or not the newly discovered outer membrane protein Ic (18) also exhibited pore-forming activity when shock fluids of this mutant were used in the black lipid pore assay. Although this was indeed the case (R. Benz and U. Henning, manuscript in preparation) we noticed that the periplasmic proteins of the Ic-carrying strain exhibited a polyacrylamide gel pattern very similar to those obtained from shock fluids of our G3P$^+$ *ugp*$^+$ mutants.

Strain W620Ic, which contained the new outer membrane protein Ic, had been isolated after a mutational event that released mutants missing outer membrane proteins Ia and Ib from their general permeability problems of the outer membrane. Similar new outer membrane proteins had been isolated by van Alphen et al. (38) and by Foulds and Chai, (11) who called them proteins e and E, respectively. All of these new outer membrane proteins were found to be identical (24).

The present manuscript demonstrates the close relationship of the synthesis of the outer membrane protein Ic (e, E) to the novel G3P transport system. During our attempt to collect the relevant strains from different laboratories, we learned that Lugtenberg and co-workers had come to similar conclusions (35). While searching for growth conditions that would induce or derepress the synthesis of outer membrane protein e, they discovered that limitation of P_i was such a condition (N. Overbeeke and B. Lugtenberg, FEBS Lett., in press). This observation connects to the regulation of alkaline phospha-

tase (P1) as well as three or four other periplasmic proteins, P2, P3, and P4 (27). Although P4 had previously been identified as the phosphate-binding protein (42, 44) the function of P2 and P3 were not known.

Thus, it became obvious to test our purified periplasmic proteins GP1 and GP3 (2) for enzymatic activity of alkaline phosphatase and phosphate-binding activity, respectively.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The strains used are listed in Table 1. Phage TC45 specific for protein Ic (e, E) (10) was obtained from U. Henning. The strains were grown under aeration in minimal medium A (26) containing 0.2% glucose as a carbon source. To derepress the formation of alkaline phosphatase and the G3P-binding protein, the cells were first grown to an optical density (578 nm) of 1.0 (Eppendorf photometer 1101, 1-cm path length cuvettes) in minimal medium A containing 0.2% glucose as carbon source. They were harvested, washed twice in Tris medium (13) lacking P_i, and resuspended to an optical density (578 nm) of 0.1 in the same medium containing 0.2% glucose and 60 μM P_i (low P_i medium). Incubation was continued at 30 or 37°C overnight. Bacteriophage sensitivity and absorption were tested as described elsewhere (9).

Transport assays. To measure *ugp*$^+$-dependent G3P transport, cells were grown logarithmically to an optical density (578 nm) of 0.5 in minimal medium A containing 0.2% glucose as a carbon source. They were washed twice and resuspended in the same medium to the same optical density. Before the addition of [^{14}C]G3P (120 mCi/mmol, New England Nuclear Corp.; final concentration 0.3 μM), glyceraldehyde-3-phosphate (1 mM final concentration) and glycerol (1 mM final concentration) were added. Aliquots of 100 μl were filtered through a membrane filter (0.65-μm pore size; Millipore Corp.) at different time intervals and washed with 10 ml of minimal medium A. All operations were done at room temperature. The filters were dried and counted in toluene-based scintillation fluid.

Preparation of osmotic shock proteins. To isolate the periplasmic shock fluid, the cells were grown overnight at 30 or 37°C in 500 ml of DYT medium (26). The osmotic shock procedure was done by the method of Neu and Heppel (28) with modifications as described previously (32). Routinely, about 10 mg of total shock proteins was obtained from a 500-ml culture. The purification of GP1, the G3P-binding protein, and GP3 was done as previously described (2).

Immunodiffusion assay for the G3P-binding protein. To test for the presence of G3P-binding protein, crude periplasmic shock proteins of the different strains were transferred to the outer wells of an immunodiffusion plate (Immunoplate, Hyland Division, Travenol Laboratories) and compared to purified G3P-binding protein. Rabbit antiserum against purified binding protein was placed in the center well. After incubation for 2 h at 37°C, the immunodiffusion plates were washed in 500 ml of 2% sodium chloride overnight, stained with Coomassie brilliant blue, and destained overnight.

Preparation of outer membrane proteins. To isolate outer membrane proteins, various strains were grown overnight at 30 or 37°C in 500 ml of DYT medium (26). The cells were harvested by centrifugation and broken by two passages through a French

TABLE 1. *Bacterial strains (E. coli K-12 derivatives)*

Strains	Parent	Known markers	Known relevant phenotype	Source/ reference
LA3432 }	LA3430x	F$^-$ *rpsL nalA glpT ugp*$^+$	GLPT$^-$ (GP1,2,3)$^+$	3
LA3433 }	LA108	F$^-$ *rpsL nalA glpT ugp*^{oa}	GLPT$^-$ (GP1,2,3)^o	3
TS100	MC4100	F$^-$ *araD139 lacU169 thi relA rpsL glpR*	GLPT$^+$ (GP1,2,3,4)^o	3
LA5001	TS101	*glpT*::Mu *cts nalA*	GLPT$^-$ (GP1,2,3,4)^o	3
LA5301	LA5001	*glpT*::Mu *cts nalA ugp*$^+$	GLPT$^-$ (GP1,2,3)$^+$	3
LA5137	LA5037	*glpT*::Mu *cts nalA ugp*$^+$	GLPT$^-$ (GP1,2,3,4)$^+$	3
LA5337	LA5037	*glpT*::Mu *cts nalA ugp*$^+$	GLPT$^-$ (GP1,2,4)$^+$	3
W620		*thi pyrD gltA galK rpsL trp his*	Ia$^+$ Ib$^+$ Ic^o	18
W620Ic	W620/18	*thi pyrD gltA galK rpsL trp his ompB? nmpA*	*TuIa*^{r} *TuIb*^{r} *Ic*$^+$	*18*
W620/18	*W620*	*thi pyrD gltA galK rpsL trp his ompB?*	TuIa^r TuIb^r Ic^o	18
PC0479	PC0417	*thr leu thi pyrF thyA argG ilvA his codA lacY tonA tsx rpsL deoC supE uvrB*	b$^+$ c$^+$ e^o	24
CE1107	PC0479	*thr leu thi pyrF thyA argG ilvA his codA lacY tonA tsx rpsL deoC supE uvrB ompB*	b$^-$ c$^-$ e^o	24
CE1108	CE1107	*thr leu thi pyrF thyA argG ilvA his codA lacY tonA tsx rpsL deoC supE uvrB ompB nmpA*	b$^-$ c$^-$ e$^+$	24
JF568		*aroA ilv metB his purE cyc xyl lac rpsL tsx proC*	Ia$^+$ Ib$^+$ E^o	11
JF694	JF703 JF568	*par tolF ilv metB his purE cyc xyl lac rpsL tsx proC nmpA*	Ia$^-$ Ib$^-$ E$^+$	11

[a] The symbol o reflects the repressed state of the system in contrast to the constitutive state, designated +.

pressure cell, and the membrane fractions were obtained as described elsewhere (21). Solubilization of outer membrane proteins was accomplished by first extracting membrane fractions with 10 mM HEPES (*N*-2-hydroxethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, pH 7.3, containing 2% Triton X-100, followed by the extraction of the Triton-insoluble material with the same buffer containing 2% Triton-X-100 and 5 mM EDTA, as described by Schnaitman (31). The Triton-EDTA-solubilized proteins were precipitated with cold ethanol and dissolved in 10 mM Tris-hydrochloride (pH 7.3) containing 1% sodium dodecyl sulfate (SDS) and 1% dithiothreitol.

Analytical techniques. Two-dimensional polyacrylamide gel electrophoresis of periplasmic proteins was performed as described elsewhere (21) with further modification (32); 12.5% polyacrylamide slab gel electrophoresis was done by the method of Laemmli (23). Electrophoresis buffer consisted of 0.24 M Tris, 0.19 M glycine (pH 8.3), and 0.1% SDS. To demonstrate the characteristic behavior of GP1 in SDS, protein samples were applied on the gel after the addition of 1% SDS–1% dithiothreitol with or without heating at 100°C, for 10 min. Electrophoresis was performed for 16 h at 75 V and at a final amperage of 5 mA. Gels were stained with Coomassie brilliant blue for 2 h and destained overnight.

Binding assay. To determine the K_D for the P_i-binding protein, a variation of the dialysis technique was employed based on the retention phenomenon of binding proteins (33). A small Visking tubing open on one end was tightly fit with its open end onto a bluntly cut plastic pipetting tip. A 500-μl amount of pure binding protein (0.87 mg/ml) was introduced, and the set-up was suspended at 4°C overnight in 10 ml of 10 mM Tris-hydrochloride (pH 7.3) containing 1 mM azide and 0.11 pM $^{32}P_i$. The dialysis bag was immersed into the surrounding buffer, so that both surface levels were equal, while the outside fluid was gently stirred. The dialysis bag was then immersed in a similar fashion on top of an Erlenmeyer flask containing 1 liter of the same buffer without labeled P_i. Aliquots of 5 μl were removed at different time intervals from the bag and counted in 5 ml of Bray scintillation fluid. The rate of decrease of substrate from the dialysis bag in comparison to the rate of decrease in the absence of binding protein is a function of binding protein concentration and its K_D (33).

Alkaline phosphatase activity. Enzymatic hydrolysis of *p*-nitrophenylphosphate was followed at room temperature by measuring the increase of absorbance at 400 nm. Assay conditions were 1 ml of 1 M Tris-hydrochloride (pH 8.0), 10 μl of enzyme solution, and 20 μl of *p*-nitrophenylphosphate (50 mM).

RESULTS

***ugp*$^+$ mutants contain an outer membrane protein similar to Ic.** Figure 1 shows the SDS slab gel electrophoretic pattern of outer membrane proteins isolated from strain LA3432 (*ugp*$^+$), LA3433 (*ugp*^{o}), W620/18 (Ia$^-$ Ib$^-$ Ic^o) and W620Ic (Ia$^-$ Ib$^-$ Ic$^+$). As can be seen, the *ugp*$^+$ mutant contains a protein that runs identically to protein Ic. This protein is either absent in the *ugp*^{o} strain or present in a much smaller amount. *ugp*$^+$ mutants that had been isolated as G3P$^+$ suppressor strains, such as LA5301, LA5137, and LA5337, were highly sensitive against phage TC45, which uses protein Ic (e, E) as a receptor (10). This indicates that the outer membrane protein synthesized in the *ugp*$^+$ mutant is in fact protein Ic (e, E). However, some *ugp*$^+$ mutants which had been constructed by an Hfr cross, such as strain LA3432, formed 10^7 times less plaques with phage TC45 than did strain W620Ic. Apparently, protein Ic (e, E) is essential but not sufficient for infection by phage TC45.

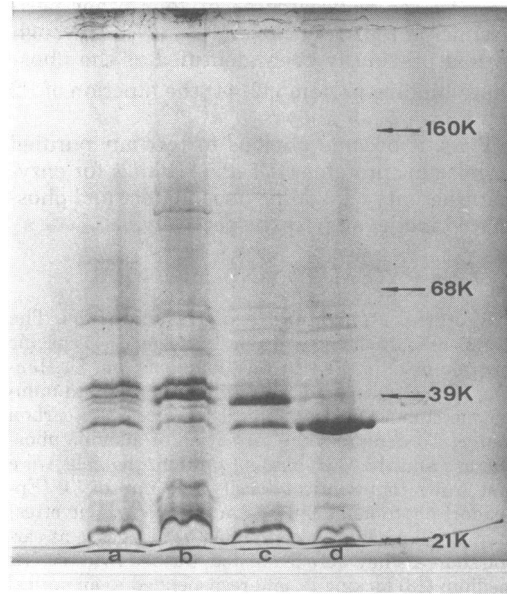

FIG. 1. *SDS-polyacrylamide gel electrophoresis of outer membrane proteins from the following strains: a, LA3433 (ugp°); b, LA3432 (ugp+); c, W620Ic (Ia- Ib- Ic+); d, W620/18 (Ia- Ib- Ic°). Before electrophoresis, samples were made 1% with respect to SDS and 1 mM with respect to dithiothreitol and heated to 100°C for 10 min. Molecular weight standards: trypsin inhibitor (21,000); E. coli RNA polymerase α-subunit (39,000); β-subunit (155,000); β'-subunit (160,000); bovine serum albumin (68,000).*

Periplasmic proteins of strains carrying the outer membrane protein Ic. Figure 2 shows a set of two-dimensional polyacrylamide gel electrophoretic patterns of periplasmic proteins of strain W620Ic carrying the outer membrane protein Ic (Fig. 2F) as well as the proteins of its isogenic Ic° parent (Fig. 2E). For comparison the pattern of the periplasmic proteins of three *ugp*$^+$ mutants (3) are shown. These mutants always contain the proteins GP1 and GP2,

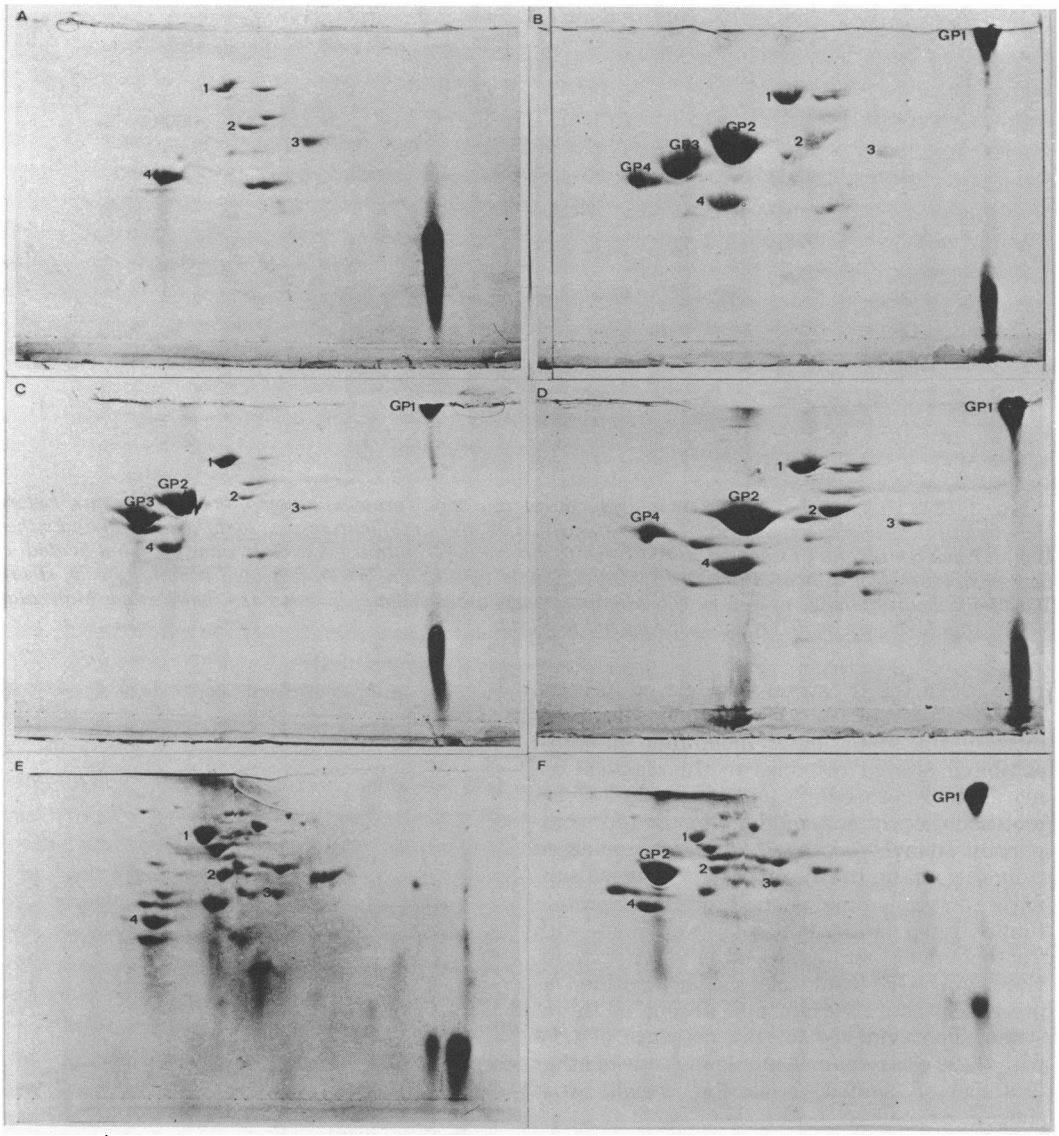

FIG. 2. *Two-dimensional polyacrylamide gel electrophoretic analysis of osmotic shock proteins of ugp$^+$ and ugp^o strains. A, ugp^o strain LA5001; B, ugp$^+$ mutant LA5137; C, ugp$^+$ mutant LA5301; D, ugp$^+$ mutant LA5337; E, wild-type strain W620; F, strain W620Ic. The spots designated 1, 2, 3, and 4 are marked for orientation purpose only. The first dimension (left to right) consists of electrophoresis in 8 M urea followed by electrophoresis (top to bottom) in SDS. A 300-μg amount of crude shock proteins was applied.*

but may or may not contain two other periplasmic proteins GP3 and GP4. As can be seen, strain W620Ic, in contrast to its parent W620, contains GP1 and GP2, whereas GP3 and GP4 are absent. The same results can be seen in Fig. 3. Here, the proteins were separated on SDS-polyacrylamide slab gel electrophoresis. Before the application on the gel, the samples were or were not boiled in SDS containing dithiothreitol. In this system, GP1 and GP2 can be recognized clearly. GP1 is dissociated after treatment with dithiothreitol and migrates identically to *E. coli* alkaline phosphatase. GP3 and GP4 migrate distinctly from GP1 and GP2, but do not separate from each other. Again, it can be seen that the strain W620Ic carrying the outer membrane protein Ic contains GP1 and GP2 in the periplasm, whereas its parent W620 does not. As parental strain to W620Ic, only the proteins of strain W620 (Ia$^+$ Ib$^+$) are shown. This is not the strain

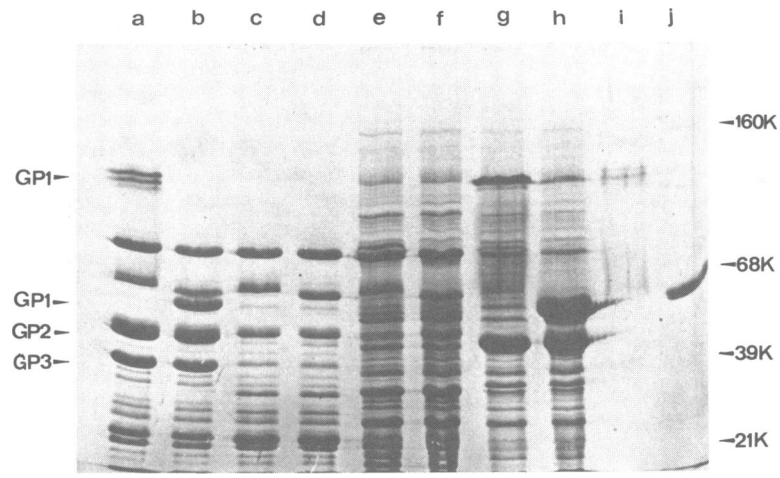

FIG. 3. *SDS slab gel electrophoretic analysis of osmotic shock proteins of ugp$^+$ and ugp$^-$ strains. Before electrophoresis, samples were made 1% with respect to SDS and 1 mM with respect to dithiothreitol and either kept at room temperature or heated to 100°C for 10 min. a, ugp$^+$ mutant LA3432; b, same as a, but heated; c, ugp$^-$ strain LA3433; d, heated preparation of c; e, wild-type strain W620; f, heated sample of e; g, strain W620Ic; h, heated sample of g; i, E. coli alkaline phosphatase (Sigma); j, same as i, but heated. Molecular weight standards are as described in the legend to Fig. 1.*

from which the Ic$^+$ strain was derived directly. The direct parent (W620/18), which is devoid of most of the major outer membrane proteins, exhibited altered response to the classical osmotic shock procedure, and its pattern of osmotic shock proteins could not be used for comparison. In fact, very few proteins were released from this strain. In this respect, it behaved similarly to strains missing the murein lipoprotein (unpublished data). Similar gels were made with Lugtenberg's e$^+$ strains as well as with its parent. Here, only GP1 could clearly be identified as present in the e$^+$ strain and absent in the e$^-$ strain. The evidence for the presence of GP3 with these gels remained ambiguous due to other proteins of similar molecular weight (not shown).

Cross-reactivity to G3P-binding protein in shock fluids derived from strains carrying the outer membrane proteins Ic (e, E). Rabbit antisera against pure G3P-binding protein were used to identify cross-reactive material in shock fluids of strains carrying the outer membrane proteins Ic (e, E). As can be seen in Fig. 4A and B, cross-reactivity is present only in shock fluids of strains that carry Ic or e. The corresponding parental strains contain it in much smaller amounts that are visible clearly only when the concentration of the sample is increased fivefold (Fig. 4, wells 3 and 8). Also, the e$^+$ strain of Lugtenberg contains considerably less G3P-binding protein than the Ic$^+$ strain

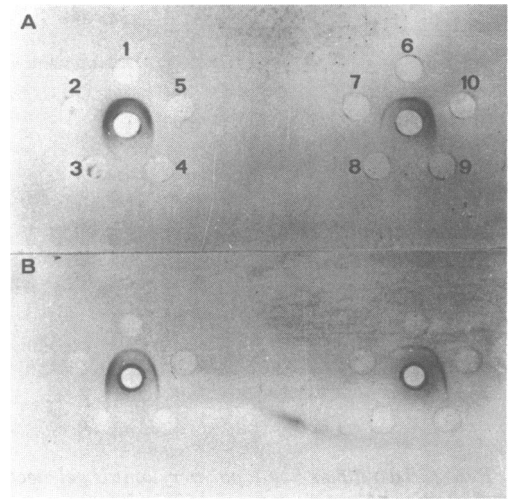

FIG. 4. *Cross-reactivity in osmotic shock fluids against the G3P-binding protein in strains containing protein e or Ic. The outer wells 1 and 6 contained purified G3P-binding protein (5 μg); the other outer wells contained shock proteins of the following strains: 2, W620Ic, 15 μg; 3, W620, 14 μg; 4 and 9, ugp$^-$ strain LA3433, 20 μg; 5 and 10, ugp$^+$ mutant LA3432, 15 μg; 7, strain CE1108, 15 μg; 8, PC0479, 15 μg. The center well contained 7 μl of antiserum against purified G3P-binding protein. In (A) the outer wells contained 7 μl of undiluted shock fluid or pure G3P-binding protein. In (B) the outer wells contained 7 μl of a fivefold dilution of shock fluid or pure G3P-binding protein.*

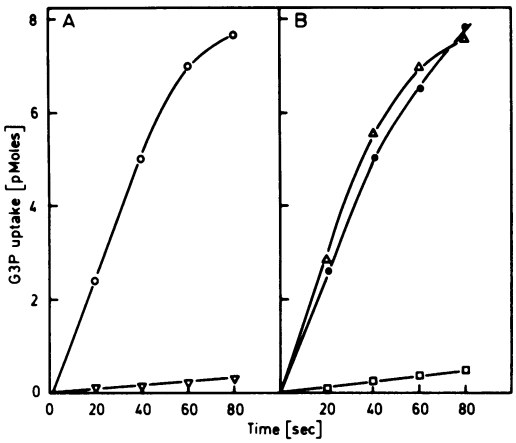

FIG. 5. *Comparison of ugp⁺-dependent G3P uptake in wild-type, Ic⁺, ugp⁺, and ugp° strains. Cells were grown in minimal medium containing glucose as sole carbon source. The results are given as amount of G3P taken up per 100 μl of cell suspension of optical density 0.5 at 578 nm. A, ugp⁺ mutant LA3432 (○), ugp° strain LA3433 (▽); B, wild-type strain W620 (□), strain W620Ic (△). For phosphate limitation (●) logarithmically growing cells of strain W620 were transferred to Tris medium containing 60 μM phosphate and incubated overnight.*

W620Ic of Henning or our *ugp*⁺ mutant strains. The E⁺ strain of Foulds also exhibited cross-reactivity similarly to the Ic⁺ strain, in contrast to its E° parent (data not shown).

ugp⁺-dependent transport activity of strains that carry the outer membrane protein Ic. Figure 5 shows the transport activity for G3P of strains carrying Ic and their corresponding parental strains. The transport assay was performed in the presence of 1 mM glyceraldehyde-3-phosphate and 10 mM glycerol. This prevents any uptake of radioactively labeled G3P via the *glpT*-dependent transport system. In addition, no uptake of [^{14}C]glycerol would be measured that might arise from the hydrolytic activity of alkaline phosphatase. As can be seen, strain W620Ic showed transport activity for G3P similar to our *ugp*⁺ mutants. In contrast, strain W620 devoid of Ic did not. The same results were obtained with the E⁺ strain of Foulds. Again, only the E⁺ strain exhibited high G3P transport activity, whereas the E° parent did not. However, under the same conditions the e⁺ strain obtained from Lugtenberg exhibits only residual transport activity (data not shown), even though it synthesized the G3P-binding protein in small amounts.

Phosphate limitation leads to the derepression of the *ugp*⁺-dependent transport system for G3P. As shown in Fig. 5B, wild-type strain W620 exhibited no *ugp*⁺-dependent transport activity unless grown under phosphate limitation. Simultaneously, cross-reactivity against anti-G3P-binding protein antibodies appears in the shock fluid of this strain after phosphate limitation (not shown). The same results were found with other wild-type strains, for instance TS 100. Moreover, strains defective in the *glpT*-dependent transport system, such as strain LA5001, also could be derepressed for the *ugp*⁺-dependent transport system by phosphate limitation.

Nature of GP1 and GP3. The pH optimum for the hydrolysis of *p*-nitrophenylphosphate for GP1 and alkaline phosphatase in 1 M Tris at different pH values at 21°C was measured. In both cases pH 8.2 gave the highest activity. The turnover number for *p*-nitrophenylphosphate hydrolysis at pH 8.0 (using a molecular weight of 86,000 for GP1) was found to be 4,000/s. This compares favorably with the value of 2,700/s as determined for alkaline phosphatase (13). This, together with the observation that GP1 migrates on SDS gels identically as purified alkaline phosphatase (Fig. 2), clearly demonstrates the identity of GP1 with alkaline phosphatase.

GP3 was tested for binding activity towards P_i. This was done by measuring the retention phenomenon of binding proteins for their substrate when dialyzed against a substrate-free buffer. Figure 6 shows the release of $^{32}P_i$ from a dialysis bag containing GP3 at 0.87 mg/ml in comparison to the release in the absence of GP3. The time to release half of the substrate can be used to determine the K_D according to the following formula: $T_{1/2}$ (with GP3) = $T_{1/2}$ (without GP3)·(1 + [P]/K_D), where [P] is the concentration of binding sites.

With a molecular weight for GP3 of 37,000 (2) and one binding site per molecule, a K_D for phosphate binding of 1.6 μM was determined. This value agrees reasonably well with 0.8 μM described for the phosphate-binding protein (25). To identify GP3 on two-dimensional gels (Fig. 2), purified GP3 was added to shock fluids of *ugp*⁺ mutant strains that missed either of the two spots later identified as GP3 and GP4. These samples were then subjected to two-dimensional polyacrylamide gel electrophoresis. In this manner, only one gel showed an additional spot that was identical to GP3. Thus, GP3 is the phosphate-binding protein. By using normal slab gel electrophoresis in the presence of SDS (Fig. 3), GP3 and GP4 could not be separated. They were probably identical to the protein bands P4 as named by Morris et al. (27) and P4a and P4b as named later by Willsky and Malamy (42).

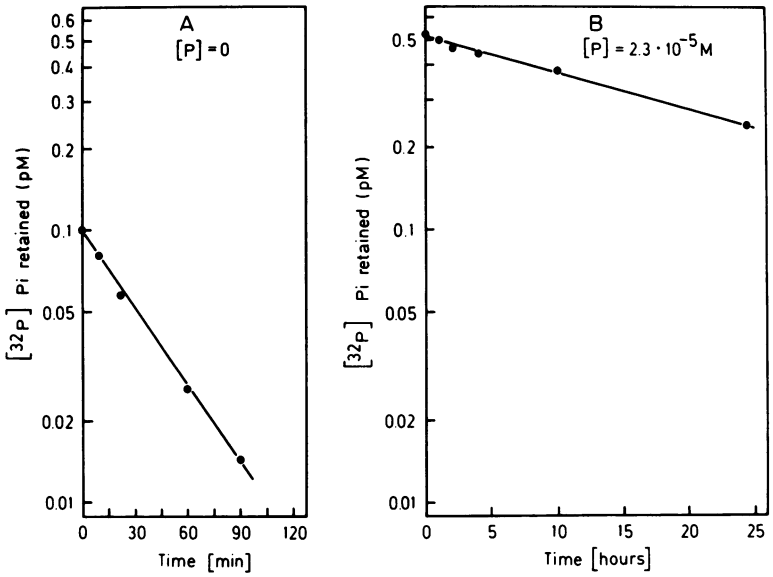

FIG. 6. *Retention of [^{32}P]phosphate by the periplasmic phosphate-binding protein. Dialysis bags were filled with 500 μl of phosphate-binding protein (0.87 mg/ml) in buffer (10 mM Tris-hydrochloride, pH 7.3) or 500 μl of buffer without protein. The bags were equilibrated for 48 h against 10 ml of Tris-hydrochloride, 1 mM sodium azide, 1.1 × 10^{-13} M [^{32}P]phosphate (285 Ci/mg of phosphate), pH 7.3, at 4°C. They were then transferred in 1 liter of buffer and gently stirred at 4°C. After different times of incubation, aliquots of 5 μl were removed from the bag and counted. A, control; B, 0.87 mg of phosphate-binding protein per ml.*

DISCUSSION

In this paper we demonstrated that the synthesis of a novel G3P transport system (*ugp*$^+$-dependent system) as well as the appearance of the periplasmic proteins GP1, -2, -3, and -4 (3) is co-regulated with the synthesis of the outer membrane protein Ic (e, E). In addition, and in confirmation of data of Tommassen and Lugtenberg (35), we could show that the periplasmic protein GP1 is identical to alkaline phosphatase (30), whereas GP3 is identical to the phosphate-binding protein (25). This connects the regulation of the *ugp*$^+$-dependent G3P transport system as well as protein Ic (e, E) to the complicated regulatory system of alkaline phosphatase and several other periplasmic proteins discovered and named P1 to P4 by Morris et al. (27).

The regulation of alkaline phosphatase and proteins P1–P4 may be summarized as follows. Phosphate limitation leads to derepression (19, 36). Constitutive synthesis occurs by mutations in the *phoR* gene (12, 37), whereas mutations in *phoB* abolish the alkaline phosphatase synthesis (7, 8, 27). In addition, mutations in *phoS* (1, 14), the structural gene for the phosphate-binding protein (15), as well as in *phoT* (41), which affect transport of P_i (PST system), lead to derepression.

Difficulties in the understanding of alkaline phosphatase regulation arise on several levels. Physiological conditions in a wild-type strain that maintain high levels of intracellular P_i but change nucleotide levels lead to the derepression of alkaline phosphatase (40). This indicates that P_i itself is not the co-repressor. A mutation in *phoR*Ic distinct from *phoB* turns off all of the P1 to P4 proteins, except part of the P4 band later named P4a, the phosphate-binding protein (42). Even though the *phoS* gene product, the phosphate-binding protein, is part of the PST-phosphate transport system (16, 41), the absence of this protein in *phoS* mutants apparently only alters but does not abolish phosphate transport (43). In addition, *phoT* mutants that turn off the PST-phosphate transport system still synthesize the phosphate-binding protein (protein P4a) in large amounts (42, 44). Clearly, a better understanding of the role of phosphate transport in regulation of the "alkaline phosphatase system" is needed.

Comparing the SDS slab gel electrophoretic analysis of samples containing the periplasmic protein P1 to P4 (P4a, P4b) published by Morris et al. (27), Yagil et al. (44), and Willsky and Malamy (42) with our slab gels (Fig. 3) and two-dimensional gels (Fig. 2) containing the periplasmic proteins GP1 to GP4 the following correlations can be made: P1 = GP1 = alkaline phosphatase; P2 = GP2 = G3P-binding protein;

P4a = GP3 = phosphate-binding protein; P4b = GP4 = protein of unknown function. Thus, our *ugp*$^+$ mutant strains are missing P3.

The derepression of the *ugp*$^+$ transport system as defined by the appearance of the G3P transport activity and the G3P-binding protein can apparently be accomplished by different mutations that give rise to different phenotypes (Fig. 2): (i) those that result in the constitutive synthesis of GP1, -2, -3, and -4; (ii) those that synthesize GP1, -2, and -4 constitutively but lack GP3, the phosphate-binding protein; (iii) those that synthesize GP1, -2, and -3 but lack GP4. In addition, mutant W620Ic isolated for the constitutive synthesis of outer membrane protein Ic that simultaneously acquire *ugp*$^+$ properties synthesize GP1 and GP2 but lack both GP3 and GP4. A detailed genetic analysis of these mutants will be necessary to identify the genes that lead to these different phenotypes and may be related to the known genes involved in derepression of alkaline phosphatase and in the derepression of new outer membrane proteins (5, 17, 29). In retrospect it is obvious that our selection procedure to obtain G3P$^+$ suppressor mutants (3) would favor the appearance of strains that are constitutive for alkaline phosphatase. In a background that is G3P$^-$, we selected strains that grew on plates containing G3P but low concentrations of P_i (1 mM). Thus, constitutive synthesis of alkaline phosphatase alone would yield strains that grow on G3P, due to the hydrolysis of G3P to glycerol. In fact, our *ugp*$^+$ mutant strains that exhibit high transport activity for G3P were unable to grow on G3P in media containing 100 mM phosphate. Studies are in progress to elucidate the role of alkaline phosphatase and the *ugp*$^+$ transport system in the ability of these strains to grow on G3P at low but not at high concentrations of P_i.

The most intriguing part of these studies is the observation that a particular outer membrane protein Ic (e, E) is connected to the phosphatase regulon. By in vivo permeability tests, protein e was found to prefer to some extent certain nucleotides (38). Also, in vitro studies with this protein by conductivity tests in black lipid films (6) have shown that this pore protein exhibits general permeability properties but in addition anion specificity (R. Benz and U. Henning, manuscript in preparation). Despite this anion specificity, a priori it appears unlikely that protein Ic (e, E) has the specific function to overcome a permeability barrier of the outer membrane for P_i or G3P. Particularly for the latter, it seems superfluous since G3P normally is transported very well by the *glpT*-dependent transport system in the absence of Ic (e, E). In analogy to the maltose-maltodextrin transport system, where the λ receptor of the outer membrane is essential for maltodextrin but not for maltose transport (34, 39), it is tempting to speculate that protein Ic (e, E) is essential to overcome the permeability barrier for a class of compounds that contains phosphate or glycerolphosphate in polymeric form. Such components might be oligonucleotides, teichoic acid (suggestion of E. C. C. Lin), or polyphosphate. Indeed, it has been found that *E. coli* contains a periplasmic protein that hydrolyzes polyphosphate. This enzyme is distinct from alkaline phosphatase, but is also derepressed under conditions of phosphate limitation (22). Thus, it will be necessary to study the ability of *E. coli* to use different polymeric phosphates as sole source of phosphate in relation to the amount of protein Ic (e, E) in the outer membrane.

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LITERATURE CITED

1. **Aono, H., and N. Otsuiji.** 1968. Genetic mapping of regulator gene *phoS* for alkaline phosphatase in *Escherichia coli*. J. Bacteriol. **95**:1182–1183.
2. **Argast, M., and W. Boos.** 1979. Purification and properties of the sn-glycerol-3-phosphate-binding protein of *Escherichia coli*. J. Biol. Chem. **254**:10931–10935.
3. **Argast, M., D. Ludtke, T. J. Silhavy, and W. Boos.** 1978. A second transport system for *sn*-glycerol-3-phosphate in *Escherichia coli*. J. Bacteriol. **136**:1070–1083.
4. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
5. **Bavoil, P., H. Nikaido, and K. von Meyenburg.** 1977. Pleiotropic transport mutants of *Escherichia coli* lack porin, a major outer membrane protein. Mol. Gen. Genet. **158**:23–33.
6. **Benz, R., B. A. Boehler-Kohler, R. Dieterle, and W. Boos.** 1978. Porin activity in the osmotic shock fluid of *Escherichia coli*. J. Bacteriol. **135**:1080–1090.
7. **Bracha, M., and E. Yagil.** 1973. A new type of alkaline phosphatase negative mutant in *E. coli* K12. Mol. Gen. Genet. **122**:53–60.
8. **Brickman, E., and J. Beckwith.** 1975. Analysis of the regulation of *Escherichia coli* alkaline phosphatase synthesis using deletions and ø80 transducing phages. J. Mol. Biol. **96**:307–316.
9. **Chai, T. J., and J. Foulds.** 1978. Two bacteriophages which utilize a new *Escherichia coli* major outer membrane protein as part of their receptor. J. Bacteriol. **135**:164–170.
10. **Chai, T. J., and J. Foulds.** 1979. Inactivation of bacteriophages by protein E, a new major outer membrane protein isolated from an *Escherichia coli* mutant. J. Bacteriol. **137**:226–233.
11. **Foulds, J., and T. J. Chai.** 1978. A new outer membrane protein found in *Escherichia coli tolF* mutant resistant to phage TuIb. J. Bacteriol. **133**:1478–1483.

12. **Garen, A., and H. Echols.** 1962. Genetic control of induction of alkaline phosphatase synthesis in *E. coli*. Proc. Natl. Acad. Sci. U.S.A. **48**:398–402.
13. **Garen, A., and C. Levinthal.** 1960. A fine structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. Biochim. Biophys. Acta **38**:470–483.
14. **Garen, A., and N. Otsuji.** 1964. Isolation of a protein specified by a regulator gene. J. Mol. Biol. **8**:841–852.
15. **Gerdes, R. G., and H. Rosenberg.** 1974. The relationship between the phosphate-binding protein and a regulatory gene product from *Escherichia coli*. Biochim. Biophys. Acta **351**:77–86.
16. **Gerdes, R. G., K. P. Strickland, and H. Rosenberg.** 1977. Restoration of phosphate transport by the phosphate-binding protein in spheroplasts of *Escherichia coli*. J. Bacteriol. **131**:512–518.
17. **Hazumi, N., H. Yamada, and S. Mizushima.** 1978. Two new peptidoglycan-associated proteins in the outer membrane of *Escherichia coli* K12. FEMS Microbiol. Lett. **4**:275–277.
18. **Henning, U., W. Schmidmayr, and J. Hindenach.** 1977. Major proteins of the outer cell envelope membrane of *Escherichia coli* K-12: multiple species of protein I. Mol. Gen. Genet. **154**:293–298.
19. **Horiuchi, T., S. Horiuchi, and D. Mizumo.** 1959. A possible negative feedback phenomenon controlling formation of alkaline phosphatase in *E. coli*. Nature (London) **183**:1529–1531.
20. **Ichira, S., and S. Mizushima.** 1979. Arrangement of proteins 0-8 and 0-9 in outer membrane of *Escherichia coli* K12. Existence of homotrimers and heterotrimers. Eur. J. Biochem. **100**:321–328.
21. **Johnson, W. C., T. J. Silhavy, and W. Boos.** 1975. Two-dimensional polyacrylamide gel electrophoresis of envelope proteins of *Escherichia coli*. Appl. Microbiol. **29**:405–413.
22. **Kulaev, J. S.** 1975. Biochemistry of inorganic polyphosphates. Rev. Physiol. Biochem. Pharmacol. **73**:131–158.
23. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
24. **Lugtenberg, B., R. van Boxtel, C. Verhoef, and W. van Alphen.** 1978. Pore proteins of the outer membrane of *Escherichia coli* K12. FEBS Lett. **96**:99–105.
25. **Medveczky, N., and H. Rosenberg.** 1970. The phosphate binding protein of *Escherichia coli*. Biochim. Biophys. Acta **211**:158–168.
26. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
27. **Morris, H., M. J. Schlesinger, M. Bracha, and E. Yagil.** 1974. Pleiotropic effects involved in the regulation of *Escherichia coli* K-12 alkaline phosphatase. J. Bacteriol. **119**:583–592.
28. **Neu, H. C., and L. A. Heppel.** 1965. The release of enzymes from *Escherichia coli* by osmotic shock and during the formation of spheroplasts. J. Biol. Chem. **240**:3685–3692.
29. **Pugsley, P. A., and C. A. Schnaitman.** 1978. Identification of three genes controlling production of new outer membrane pore proteins in *Escherichia coli* K-12. J. Bacteriol. **135**:1118–1129.
30. **Reid, T. W., and I. B. Wilson.** 1971. *In* (P. D. Boyer (ed.), The enzymes, 3rd ed., vol. IV, Academic Press, Inc., New York.
31. **Schnaitman, C. A.** 1974. Outer membrane proteins of *Escherichia coli*. III. Evidence that the major protein of *Escherichia coli* 0111 outer membrane consists of four distinct polypeptide species. J. Bacteriol. **118**:442–453.
32. **Silhavy, T. J., I. Hartig-Beecken, and W. Boos.** 1976. Periplasmic protein related to the *sn*-glycerol-3-phosphate transport system of *Escherichia coli*. J. Bacteriol. **126**:951–958.
33. **Silhavy, T. J., S. Szmelcman, W. Boos, and M. Schwartz.** 1975. On the significance of the retention of ligand by protein. Proc. Natl. Acad. Sci. U.S.A. **72**: 2120–2124.
34. **Szmelcman, S., M. Schwartz, T. J. Silhavy, and W. Boos.** 1976. Maltose transport in *Escherichia coli* K-12. A comparison of transport kinetics in wild-type and λ resistant mutants with the dissociation constants of the maltose binding protein as measured by fluorescence quenching. Eur. J. Biochem. **65**:13–19.
35. **Tommassen, J., and B. Lugtenberg.** 1980. Outer membrane protein e of *Escherichia coli* is co-regulated with alkaline phosphatase. J. Bacteriol. **143**:151–157.
36. **Torriani, A.** 1960. Influence of inorganic phosphate in the formation of phosphatases by *E. coli*. Biochim. Biophys. Acta **38**:460–470.
37. **Torriani, A., and F. Rothman.** 1961. Mutants of *Escherichia coli* constitutive for alkaline phosphatase. J. Bacteriol. **81**:835–836.
38. **van Alphen, W., N. van Selm, and B. Lugtenberg.** 1978. Pores in the outer membrane of *Escherichia coli* K12; involvement of proteins b and e in the functioning of pores for nucleotides. Mol. Gen. Genet. **159**:75–83.
39. **Wandersman, C., M. Schwartz, and T. Ferenci.** 1979. *Escherichia coli* mutants impaired in maltodextrin transport. J. Bacteriol. **140**:1–13.
40. **Wilkins, A.** 1972. Physiological factors in the regulation of alkaline phosphatase synthesis in *Escherichia coli*. J. Bacteriol. **110**:616–623.
41. **Willsky, G. R., R. L. Bennet, and M. H. Malamy.** 1973. Inorganic phosphate transport in *Escherichia coli*: involvement of two genes which play a role in alkaline phosphatase regulation. J. Bacteriol. **113**:529–539.
42. **Willsky, G. R., and M. H. Malamy.** 1976. Control of the synthesis of alkaline phosphatase and the phosphate-binding protein in *Escherichia coli*. J. Bacteriol. **127**: 595–609.
43. **Willsky, G. R., and M. H. Malamy.** 1974. The loss of *phoS* periplasmic protein leads to change in the specificity of a constitutive inorganic phosphate transport system in *E. coli*. Biochem. Biophys. Res. Commun. **60**: 226–233.
44. **Yagil, E., N. Silberstein, and R. C. Gerdes.** 1976. Coregulation of the phosphate-binding protein and alkaline phosphatase synthesis in *Escherichia coli*. J. Bacteriol. **127**:653–659.