JOURNAL OF BACTERIOLOGY, Oct. 1988, p. 4916–4923
0021-9193/88/104916-08$02.00/0

Vol. 170, No. 10

Acid Phosphatases of *Escherichia coli*: Molecular Cloning and Analysis of *agp*, the Structural Gene for a Periplasmic Acid Glucose Phosphatase

ELIZABETH PRADEL AND PAUL L. BOQUET*

Service de Biochimie, Département de Biologie, Centre d'Etudes Nucléaires de Saclay, 91191 Gif-sur Yvette Cedex, France

Received 11 April 1988/Accepted 19 July 1988

Several unknown *Escherichia coli* genes for different species of acid phosphatase were cloned in vivo with the phasmid Mu dII4042. When present in a multicopy state, each gene promoted hydrolysis of *p*-nitrophenylphosphate at acidic pH. Among seven recombinant clones that encoded periplasmic acid phosphatase activities, five different genes could be distinguished by the pH optimum and substrate preference for the enzyme and by the restriction enzyme pattern. A 1.7-kilobase recombinant DNA fragment, common to two clones, was inserted into plasmid pBR322 and shown to contain a new gene, *agp*, which leads to the overexpression of the periplasmic acid glucose-1-phosphatase, a dimer of a 44-kilodalton polypeptide. Fusions of *agp* to gene *phoA* deprived of its own signal sequence conferred an alkaline phosphatase-positive phenotype to bacteria, showing the presence of an export signal on *agp*. The resulting hybrid proteins were characterized by immunoprecipitation with an antiserum directed against purified acid phosphatase or against alkaline phosphatase, showing that *agp* is the structural gene of the acid phosphatase. The beginning, the orientation, and the end of gene *agp* on the cloned DNA fragment were determined by the characteristics of such hybrid proteins.

Several acid phosphatases in *Escherichia coli* have been described (27), but the exact number of distinct enzyme species, their physiological role, and their mode of regulation are not known precisely. Enzymatic activities promoting the hydrolysis of the synthetic substrate *p*-nitrophenylphosphate (pNPP) in acidic conditions have been found in osmotic shock fluids (4, 16, 26). Three such genes have been identified: (i) gene *ush* specifies UDP glucose hydrolase (25) and lies in the 11-min region of the chromosome (6); (ii) *appA*, the structural gene for an acid phosphoanhydride phosphohydrolase, which also cleaves pNPP (14), maps at min 22 (13); and (iii) gene *cpdB* encodes the 2′-3′ cyclic phosphodiesterase (1–3), which is also active against pNPP (16) and is located at min 96 (5). The three genes have now been cloned, their restriction maps are known, and their nucleotide sequences have been partly or entirely elucidated (9, 11, 22, 29).

At least two other pNPP-hydrolyzing enzymes with pH optima ranging from 4.0 to 6.0 have been described and partially purified, namely an acid hexose phosphatase and a nonspecific acid phosphatase (16, 27, 31). The latter accounts for 25 to 35% of the pNPP-splitting activity in an osmotic shock fluid prepared from a wild-type strain, but it may correspond to several enzyme species (16). The expression of acid phosphatases in general appears to be sensitive to growth conditions (14, 16, 28, 30, 31), and some additional activities could have escaped detection if poorly expressed in a wild-type strain under usual growth conditions.

With the aim of identifying the genes for the two remaining acid phosphatases or any new enzyme, we have used the in vivo shotgun cloning technique developed in the laboratory of Casadaban (19) to amplify directly in a wild-type strain those genes promoting high levels of pNPP hydrolysis under various acidic assay conditions. We describe here some properties of different periplasmic acid phosphatases activities overexpressed in clones harboring such genes in multicopies. We report the identification among them of a new gene, *agp*, which we show to be the structural gene for an acid glucose-1-phosphatase.

MATERIALS AND METHODS

Bacterial strains, bacteriophages, plasmids, and media. Strains, phages, and plasmids used and constructed in this study are listed in Table 1. Plate and liquid cultures were grown in the rich medium TYE. Phasmid Mu dII4042 was maintained by adding chloramphenicol (25 μg/ml), and ampicillin (200 μg/ml) was used with recombinant plasmids derived from pBR322. A minimal DM medium (15) containing all amino acids (50 μg/ml each) except methionine was used to grow bacteria of the minicell-forming strain and to label the minicells.

Cloning of the genes promoting the overexpression of acid phosphatases. The general cloning procedure with phasmid Mu dII4042 was used by the method of Groisman et al. (19), as previously reported for the cloning of gene *appA* (8). For the detection of acid phosphatase-positive colonies, each of three sets of replica plates grown for 24 h at 30°C with about 200 Cm^r colonies per plate was overlaid with 3 ml of pNPP (25 mM) in 1.5 M formic acid (for pH 2.4), 1 M formic acid (for pH 3.0), or 0.15 M potassium phthalate-HCl buffer (for pH 4.5). Plates were incubated at 42°C for 5 min, and the overlaying solution was removed and replaced by 1 ml of concentrated ammonia. Colonies stained in yellow above the background level were picked from the master plates, purified, reassayed, and submitted to further analysis.

Determination of enzyme activities and purification procedures. Periplasmic extracts were made either by an osmotic shock procedure (16) or chloroformic shock (17) and were used as a source of enzyme. For each acid phosphatase-positive clone, the pH dependence for pNPP hydrolysis (25 mM) was measured in toluenized cells and periplasmic extracts in 0.5 ml of 0.15 M glycine hydrochloride, potas-

* Corresponding author.

TABLE 1. Characteristics of strains, phages, and plasmids

Strain, phage, or plasmid	Genotype phenotype or description	Source, comments or reference
E. coli strains		
SBS1073	HfrC *relA pit-10 tonA21 rpsL* (Mu *c*ts)	Str^r transductant of strain K10 made lysogen for Mu *c*ts
SBS1295	Same as SBS1073 but *appA1 zcc*::Tn*10* Δ*phoA20*	This laboratory
CC118	F$^-$ Δ(*ara-leu*)*7679 araD139* Δ*lacX74 galE galK* Δ*phoA20 thi rpsE rpoB argE*(Am) *recA1*	C. Manoil
CC102	F42 *lacI3 zzf*::Tn*phoA1*/CC118	
SBS1405	Same as CC118 but Δ*appA*::(*neo*-IS*50*$_L$)	This laboratory (30)
B1685	F$^-$ *thr lacY proC* T6^r T3^r *minB purE his rpsL ilv xyl cycA cycB met zcf*-117:Tn*10*	R. d'Ari
Bacteriophages		
Mu *c*ts	Mu with temperature-sensitive repressor *c*ts62	M. Casadaban
Mu dII4042	Mu *c*ts62 *A*$^+$ *B*$^+$ Cm^r *repF15A lac*(*ZYA*)*913*	M. Casadaban (19)
Plasmids		
pEG109	Mu dII4042::*phoA proC*	M. Casadaban (19)
pEP1336		
pEP1337	Mu dII4042 bracketing in each plasmid a determinant causing overexpression of a pNPP-hydrolyzing activity in acid conditions	This study
pEP1341		
pEP1344		
pEP1345		
pMK1271	Mu dII4042 bracketing a 7-kb region of the chromosome upstream gene *appA* with no genes for phosphatases	This laboratory
pBR322	Tc^r Ap^r	7
pEP1348	Tc^s Ap^r::*agp* (*Bam*HI-*Bam*HI insert into *Bam*HI site of pBR322)	This study
pEP1376	Tc^s Ap^r::*agp* (*Bam*HI-*Sph*I insert into *Bam*HI-*Sph*I sites of pBR322)	
pEP1390	Tc^s Ap^r Km^r::*agp*::Tn*phoA*	This study

sium phthalate-HCl and Tris hydrochloride buffers. One milliliter of 1 M NaOH was added to stop the reaction, and the A_{410} was measured after centrifugation to remove the cells, when necessary. The release of inorganic phosphate was measured by the method of Chen et al. (10). The capacity of colonies to hydrolyze the chromogenic substrate XP (5-bromo-4-chloro-3-indolyl phosphate) was tested in the presence of 40 μg of this compound per ml in TYE medium. Glucose-1-phosphatase was purified from the osmotic shock fluid of a 1-liter culture of strain SBS1405(pEP1376) grown to early stationary phase in TYE medium plus ampicillin. Low-molecular-weight proteins were removed, and the enzyme was concentrated with an Amicon concentrator equipped with an UM20 membrane. The concentrated preparation was chromatographed on a fast protein liquid chromatography Mono-Q column (Pharmacia) in 5 mM Tris hydrochloride buffer (pH 7.8) with a linear gradient of NaCl (0 to 500 mM). One unit of enzyme corresponds to 1 nmol of pNPP hydrolyzed per min at 37°C in 50 mM potassium phthalate-HCl buffer (pH 4.5).

Fusions of alkaline phosphatase to acid phosphatase. Transposon Tn*phoA*, constructed by Manoil and Beckwith (24), was introduced into strain SBS1405(pEP1376) by mating it with CC102, and transpositions of Tn*phoA* onto the recombinant plasmid were selected as colonies able to grow on TYE medium containing 300 μg of kanamycin per ml. Single colonies were spread in a grid on the same medium with or without XP, and clones showing alkaline phosphatase (strong XP$^+$ phenotype) but no acid phosphatase activity were saved for plasmid restriction analysis.

General techniques for the analysis of recombinant DNA and of its expression. The preparation of plasmid DNA, restriction analysis, and transformation techniques were as reported previously (8). Minicells were prepared from strain B1685(pBR322) or B1685(pEP1376) grown in methionineless DM synthetic medium by the method of Frazer and Curtiss (18) and labeled in the same medium for 1 h with 150 kBq of [^{35}S]methionine per ml. Proteins were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (10% acrylamide) by the method of Laemmli (21) and stained with Coomassie blue. Radioactive bands were detected by autoradiography at −60°C of gels previously soaked for 20 min in an Amersham Amplifier and dried. Immunoprecipitation of extracts from cells labeled for 2 min with [^{35}S] methionine (75 kBq/ml) was performed by the method of Ito et al. (20). Other techniques were as described by Maniatis et al. (23).

RESULTS

Molecular cloning of some determinants promoting the overexpression of pNPP-hydrolyzing activities under acidic conditions. The in vivo cloning technique with phasmid Mu dII4042 (19) was previously used to clone the gene *appA*, the structural gene for the pH 2.5 acid phosphatase (8). The same technique was used here to clone genes for other enzymes that split pNPP under acidic conditions. Since such phasmids usually show a copy number of 25 to 50, this allows the detection of clones overexpressing a single activity encoded by a gene on the recombinant phasmid in a wild-type chromosomal background. Phage Mu dII4042 was propagated by thermoinduction of SBS1073 (a Mu *c*ts derivative of strain K10) transformed with plasmid pEG109 (19), and the resulting phage stock was used to infect bacteria of

TABLE 2. Periplasmic localization and some characteristics of acid phosphatase activities overexpressed in five of the isolated Mu dII recombinant clones

Recombinant plasmid	pH optimum with pNPP	Activity at optimal pH[a]		Phosphate released from substrates[b]				Staining by XP in vivo[c]
		Whole cells	Shock fluids	PEA	β-Gly-P	AP	Glu-1-P	
pEP1336	4.5	130	2,300	<1	<1	14	115	−
pEP1337	3.5	425	1,060	<1	<1	217	320	−
pEP1339	4.5	433	2,280	29	23	22	1,008	+
pEP1340	5.0	138	2,470	ND	ND	ND	ND	−
pEP1341	4.5	410	1,900	30	26	35	751	+
pEP1344	3.0	580	1,110	<1		19	580	−
pEP1345	3.0	355	1,130	<1	8	39	424	−
pMK1271[d]	4.8	42	357	<1	<1	8	37	−
pEG109[e]	8.8	1,220	7,650	612	ND	1,614	1,915	++

[a] With 25 mM pNPP as the substrate and expressed in nanomoles of PNPOH formed per minute per milligram of protein at 37°C.
[b] Determined on whole toluenized cells by the method of Chen et al. (10) and expressed in nanomoles of PO_4^{3-} formed per minute per milligram of protein at 37°C. PEA, Phosphorylethanolamine (0.5 mM); β-Gly-P, β-glycerophosphate (5 mM); AP, acetyl phosphate (0.5 mM); Glu-1-P, glucose-1-phosphate (0.1 mM). ND, Not determined.
[c] Colonies were grown on minimal plates plus glycerol as the carbon source. −, No staining; +, weak staining; ++, intense staining.
[d] Control; negative clone containing a known chromosomal region around min 22.
[e] Original *phoA*$^+$ recombinant clone (19).

strain SBS1295, an *appA1* Δ*phoA* Mu *cts* derivative of strain K10 (Table 1). Chloramphenicol-resistant transductants were replicated on a series of rich-medium plates, and colonies were tested for the hydrolysis of pNPP at pH 2.4, pH 3.0, and pH 4.5. Colonies that showed a high staining intensity within a short reaction time appeared with a frequency of ca. 0.4% and were purified from the master plates. Bacteria with 16 such clones were grown to early stationary phase in rich medium, and their periplasmic extracts were prepared by a chloroformic shock.

The ability of the shock fluids and toluenized cells to hydrolyze pNPP according to pH was compared. For seven clones, the specific activity of the phosphatase was much higher in the shock fluid fraction than in whole cells, suggesting that the corresponding plasmids may contain genes for periplasmic acid phosphatases (Table 2). Toluenized bacteria from these seven clones were assayed for their ability to split phosphate from several phosphorylated compounds. The results show five distinct types of activity with respect to substrate preference and pH optimum (Table 2). Also, analysis of the restriction enzyme patterns of the recombinant plasmids showed that all other genes were different from each other and different from reported acid phosphatase genes (data not shown).

Phasmids pEP1339 and pEP1341 were chosen for this first study.

Restriction analysis and subcloning of the gene responsible for acid phosphatase overexpression in plasmids pEP1339 and pEP1341. Restriction analysis of plasmids pEP1339 and pEP1341 showed that they contained a 5-kilobase-pair (kb)-long piece of chromosomal DNA in common. The restriction map of this fragment (Fig. 1a) was entirely different from those reported for genes *cpdB*, *ush*, and *appA*.

A 4.3-kb *Bam*HI-*Bam*HI fragment corresponding to most of this cloned piece of DNA was further subcloned into the *Bam*HI site (Tc^r) of pBR322. The resulting plasmid, pEP1348, itself conferred the high acid phosphatase level to transformed bacteria (Fig. 1A). Several deletions made on this recombinant plasmid caused a complete loss of the acid phosphatase activity, showing that the responsible gene lies in a 1.5-kb-long region on one side of this fragment (Fig. 1B). The 1.7-kb *Bam*HI-*Sph*I fragment in pEP1348 was further subcloned, in a reverse orientation with respect to the vector, between the *Bam*HI and *Sph*I sites of pBR322, resulting in plasmid pEP1376. This plasmid produced the high acid phosphatase level in transformants. Deletion analysis showed that the responsible gene extended at least beyond the unique *Sal*I and *Nru*I sites of the subcloned fragment (Fig. 1C).

Characterization of the acid phosphatase overproduced in the presence of plasmid pEP1376. Bacteria from strain SBS1405 (deleted for the pH 2.5 acid phosphatase structural gene *appA*) harboring plasmid pEP1376 or pBR322 were grown in rich medium to early stationary phase and subjected to an osmotic shock. The shock fluid obtained from SBS1405(pEP1376) contained most of the total pNPP-splitting activity with an optimum in the pH 4 to pH 5 region. The specific activity corresponding to SBS1405(pBR322) was less than 2% of that in SBS1405(pEP1376). Filtration of the shock fluid on a Biogel P150 column under nondenaturing conditions showed that the activity eluted with an apparent M_r of about 95,000. Purification was further achieved by fast protein liquid chromatography on a Pharmacia Mono-Q column, with a linear gradient of NaCl (0 to 500 mM). The enzyme eluted as a single peak near 250 mM NaCl (not shown) with a specific activity about 10 times higher than in crude extracts (Table 3). However, a substantial loss of total activity was observed with this procedure. SDS-polyacrylamide gel electrophoresis analysis of the shock fluid of strain SBS1405(pEP1376) showed a major polypeptide band with an apparent M_r of 44,000 (about 50% of the total proteins of this fraction as determined by staining intensity). The enzyme in the pooled active fractions eluted from the Mono-Q column was homogeneous, with the same M_r of 44,000 under such denaturing conditions (Fig. 2).

TABLE 3. Enzymatic specific activities of the acid phosphatase at different steps in the purification procedure

Step	Sp act[a]	
	SBS1405(pEP1376)	SBS1405(pBR322)
Crude extract	1,015	58
Shock fluid	5,180	85
FPLC–Mono-Q (pooled active fractions)	10,550	

[a] Arbitrary units per milligram of protein (see Materials and Methods).

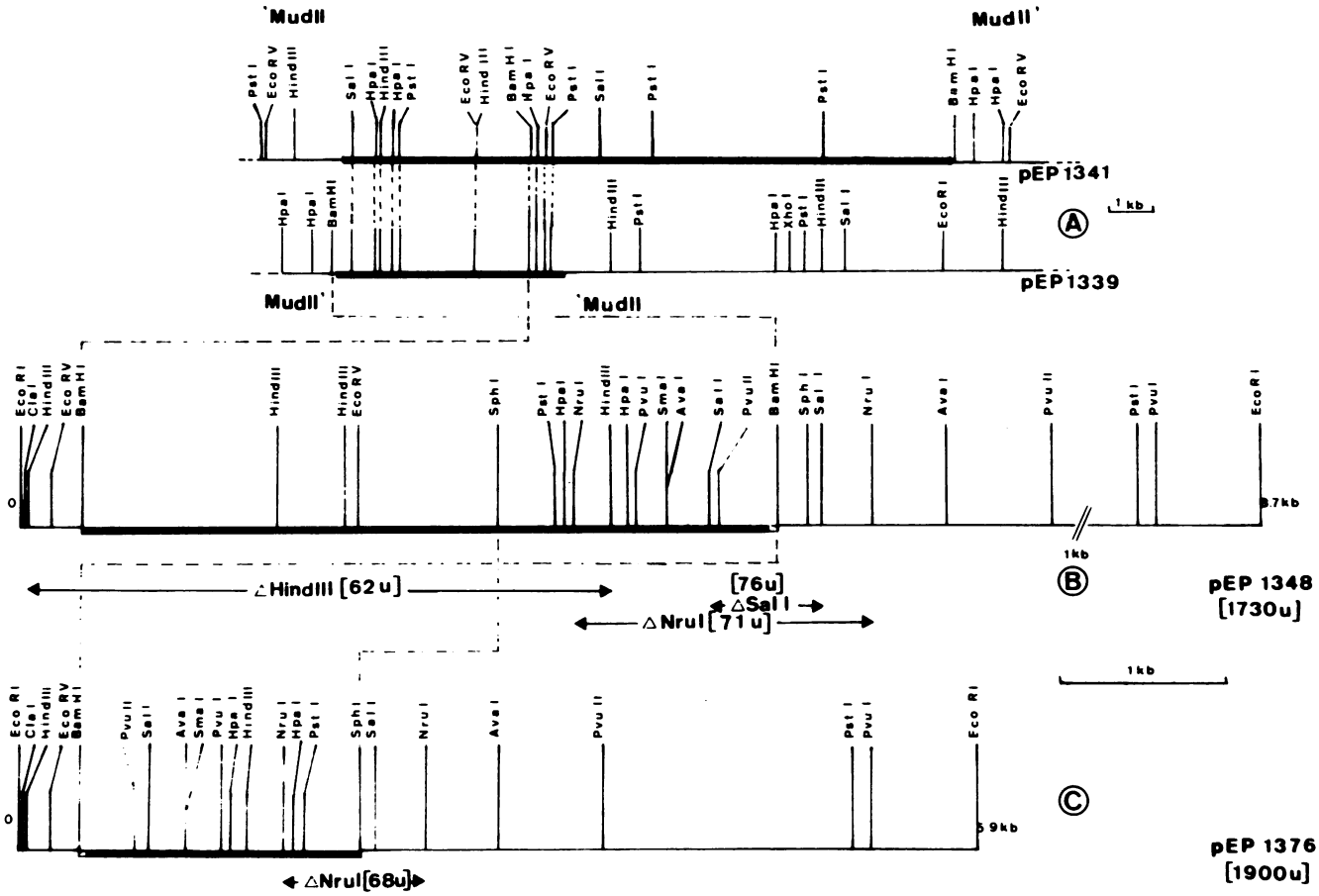

FIG. 1. Restriction analysis and subcloning of the chromosomal region involved in acid phosphatase overexpression in phasmids pEP1339 and pEP1341. (A) Restriction map of the fragments cloned in vivo in pEP1339 and pEP1341 (thick line). The Mu dII DNA is indicated by a thin line. (B) Detailed restriction map of the 4.3-kb *Bam*HI-*Bam*HI fragment (thick line) originating from pEP1339 and subcloned into the *Bam*HI site of pBR322 (thin line). (C) Structure of a smaller subclone containing the 1.7-kb *Bam*HI-*Sph*I fragment specifying the high acid phosphatase level. Symbols are the same as in part B. The extent of deletions made on the different plasmids are indicated by double-headed arrows, and the corresponding enzymatic specific activity (measured with 25 mM pNPP at pH 4.5) in strain CC118 harboring each of the plasmids derived from pBR322 is indicated in brackets (in units per milligram of protein). The enzymatic specific activity in strain CC118 harboring plasmid pBR322 (resulting from the expression of various acid phosphatase genes on the chromosome) was 70 U/mg of protein.

Analysis of the pH dependence of pNPP hydrolysis by the enzyme in potassium-phthalate buffers showed a complex shape with a nonnegligible residual activity over the alkaline region (Fig. 3). Addition of EDTA up to 10 mM did not cause any significant inhibition, showing that divalent cations are probably not required for activity. The K_ms of the enzyme for several phosphorylated compounds are indicated in Table 4. Clearly, glucose-1-phosphate showed the highest affinity of all substrates tested. A rabbit antiserum directed against purified enzyme was prepared.

Experiments with minicells and fusions with Tn*phoA* show that the cloned gene is the structural gene of the phosphatase. Minicells prepared from strain B1685 (Table 1) containing plasmid pEP1376 and labeled with [^{35}S]methionine expressed a single polypeptide of M_r 44,000 which was absent with the vector pBR322 (Fig. 4).

Transposon Tn*phoA* was introduced into strain CC118, harboring either plasmid pEP1348 or plasmid pEP1376, by mating with strain CC102, and transpositions of Tn*phoA* into pEP1348 and pEP1376 were selected as colonies with a high level of resistance to kanamycin and loss of the acid phosphatase activity. Some colonies of this type showed a strong alkaline phosphatase-positive phenotype, showing that in-frame fusions were formed. The position and orientation of three such fusions are indicated in Fig. 5. The alkaline phosphatase-positive fusions show that the cloned gene encodes an exported protein, which is transcribed from the *Bam*HI end towards the *Sph*I end. SDS-polyacrylamide gel electrophoresis analysis of an osmotic shock fluid from bacteria containing the fusion plasmid pEP1390 revealed the disappearance of the 44-kilodalton polypeptide band (Fig. 6A), which is therefore encoded by this gene. Immunoprecipitation of ^{35}S-labeled proteins from bacteria harboring either pEP1376 or pEP1390 by a serum directed against the purified acid phosphatase or against alkaline phosphatase showed that the gene fusion on pEP1390 resulted in the expression of a hybrid protein of M_r 58,500 (Fig. 6B). According to the position of the fusion joint and assuming the existence on the gene of a putative signal sequence of ca. 60 base pairs, the origin and extremity of this gene could be localized on the restriction map (Fig. 5). This position was confirmed by analysis of the M_r of hybrid proteins resulting from the two other alkaline phosphatase-positive fusions available (data not shown). Consequently, the cloned gene is

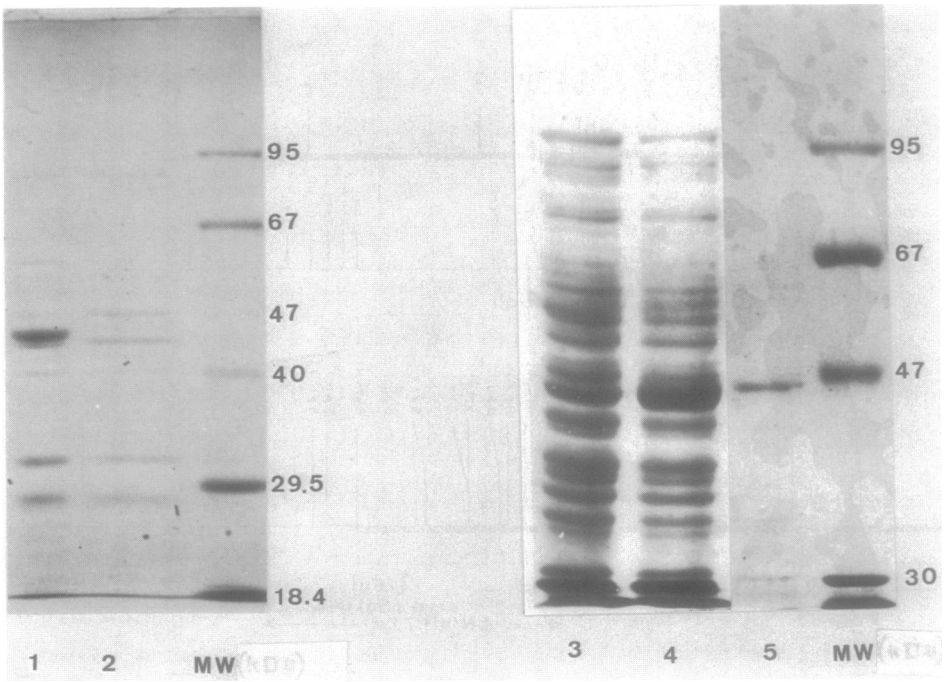

FIG. 2. SDS-polyacrylamide gel electrophoresis of crude extracts, shock fluids, and purified enzyme. Homologous extracts from strain SBS1405 harboring plasmid pEP1376 or pBR322 are compared. Pure enzyme was prepared from strain SBS1405(pEP1376) as described in the text. The gel was in 10% acrylamide, and staining was with Coomassie blue. Lanes: 1, pEP1376, shock fluid; 2, pBR322, shock fluid; 3, pBR322, crude extract; 4, pEP1376, crude extract; 5, purified enzyme; MW, 10^3 molecular weight markers.

very likely the structural gene of this periplasmic acid phosphatase which shows a higher affinity for glucose-1-phosphate. We propose the denomination *agp* (acid glucose phosphatase) for this gene.

DISCUSSION

Acid phosphatases are widely distributed in the microbial kingdom, and *E. coli* makes several such enzymes that are in the periplasmic space (26). The chromogenic substrate for phosphomonoesterases pNPP is hydrolyzed by intact *E. coli* cells or shock fluids within a broad pH range (from pH 2.0 to pH 8.0), even in strains lacking the gene for alkaline phosphatase or in bacteria grown in high-phosphate media. Consequently, a classical selection of mutants altered in the expression of a given acid phosphatase in the pH 3.0 to pH 6.0 range, by assays with this substrate, is hampered by the presence of the other overlapping activities. Moreover, the substrate specificities for these enzymes are poorly known, and some were already shown to lack specificity (16, 27). Accordingly, the isolation of a strain showing a reduced pNPP-hydrolyzing activity, between pH 3 and pH 4, led to a highly pleiotropic mutation interfering with the expression of several exported proteins (12). The ability of phasmids derived from a mini-Mu bacteriophage and containing the origin of replication of a multicopy plasmid to clone in vivo and amplify in one step 5- to 15-kb-long segments of the chromosome was used here to identify unknown determinants promoting high levels of pNPP hydrolysis. Each of seven individual clones overexpressed a pNPP-hydrolytic activity located in the periplasmic space. In five clones, the type of acid phosphatase made was different (by pH optimum and efficiency on different substrates), and accordingly the cloned fragments showed different restriction maps.

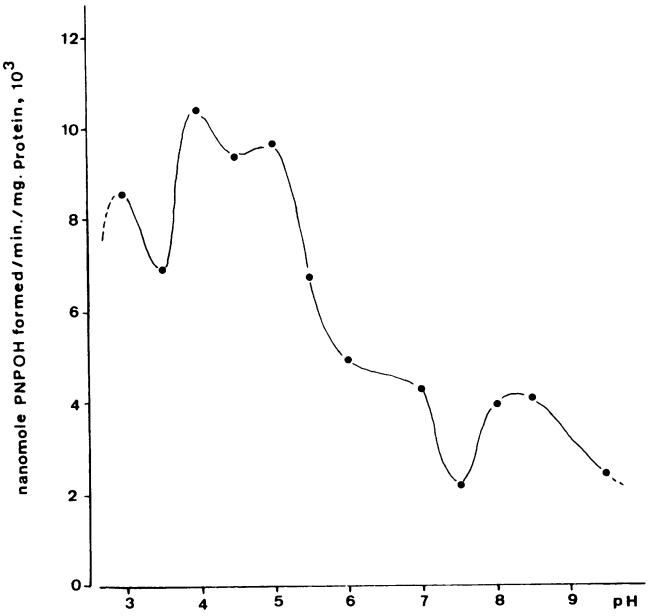

FIG. 3. pH dependence of the acid phosphatase. Enzyme assays were performed with pNPP as the substrate (25 mM) in potassium phthalate buffers as described in Materials and Methods and with 0.5 μg of enzyme.

TABLE 4. Affinity of the purified acid phosphatase for some phosphorylated compounds

Substrate[a]	K_m (mM)
pNPP	30
Glucose-1-P	0.5
Glucose-6-P	15
Fructose-1-P	50
Fructose-6-P	25
Ribose-5-P	30
β-Glycero-P	100
α-Glycero-P	>100

[a] P, Phosphate.

Colonies of bacteria from the two related clones pEP1339 and pEP1341 were noticeably stained by the chromogenic substrate XP, even when grown on a high-phosphate medium, suggesting that the enzyme specified could contribute to the known background staining of wild-type strains or Δ*phoA* mutants by XP. The 1.7-kb-long *Bam*HI-*Sph*I chromosomal DNA fragment common to these two clones and subcloned into pBR322 promoted the overexpression of a periplasmic polypeptide of M_r 44,000 and of a periplasmic phosphatase activity optimally active between pH 4 and pH 5 which showed a higher affinity for glucose-1-phosphate than for other phosphorylated substrates. This phosphatase had an apparent M_r of 95,000 in the native state and migrated as a 44,000-M_r polypeptide in SDS-polyacrylamide gels. That the two proteins are the same is shown by the combination of results from experiments with minicells and *agp*-Tn*phoA* fusions. Such fusions made an active alkaline phosphatase, showing that *agp* contains an export signal in the N-terminal region. The Tn*phoA* insertions simultaneously led to the disappearance of the original 44,000-M_r polypeptide and to a loss of the acid phosphatase activity, with the formation of a hybrid protein precipitating with an antiserum directed against purified acid phosphatase or alkaline phosphatase. The hybrid protein, however, was not detected by Coomassie staining of gels run with the corresponding shock fluid, but this could be explained if the fusion protein is extremely unstable, as shown in some other cases (8, 24).

These results indicate that the cloned gene is very likely the structural gene of the periplasmic acid phosphatase. Restriction analysis shows that this gene is different from *cpdB*, *ush*, or *appA* (8, 9, 11, 22). Comparison of our results with previous data from Dvorak et al. (16) suggests that the product of gene *agp* could be different from the acid hexose phosphatase purified earlier from wild-type cells and also reported to be active as a dimer. An interpretation of the

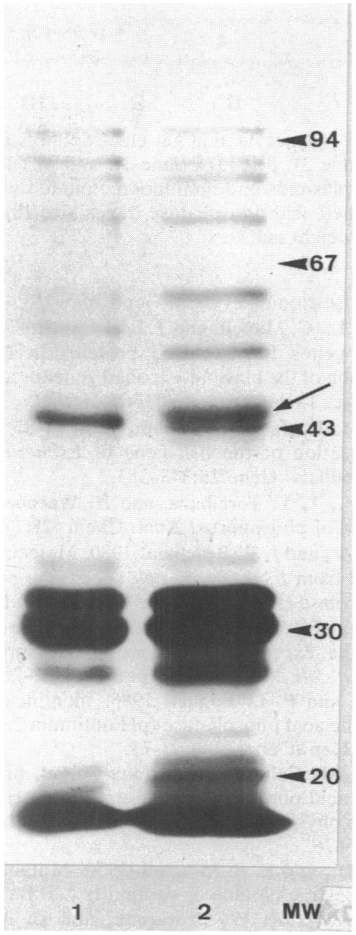

FIG. 4. Identification of the product encoded by the gene cloned on pEP1376. Autoradiogram of an SDS-polyacrylamide gel electrophoresis of lysates prepared from ^{35}S-labeled minicells obtained from strain B 1685 harboring plasmid pBR322 (lane 1) or pEP1376 (lane 2). Lane MW, 10^3 molecular weight markers. The arrow indicates the 44,000-M_r polypeptide specifically encoded by pEP 1376.

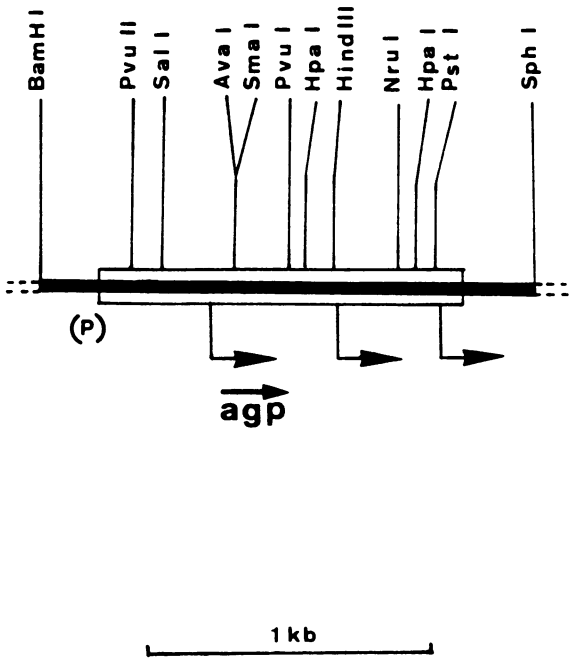

FIG. 5. Position of the fusion joints in three alkaline phosphatase-positive Tn*phoA* fusions and localization of gene *agp*. Data are derived from restriction analysis of the plasmids pEP1376 and pEP1348 harboring different *agp-phoA* fusions. The position of the fusion joints and the direction of transcription (arrows) were derived from restriction analysis of the corresponding plasmids (with that in pEP1390 at left). The origin of gene *agp* (thick open bar) was mapped in relation to the apparent M_r of the hybrid proteins and to the position of the fusion joints, assuming the existence of a 60-base-pair signal sequence in the 5'-terminal part of the gene. Its extremity was positioned with 44,000 as the M_r of the protein. A promoter region (P) is contained within the cloned *Bam*HI-*Sph*I fragment.

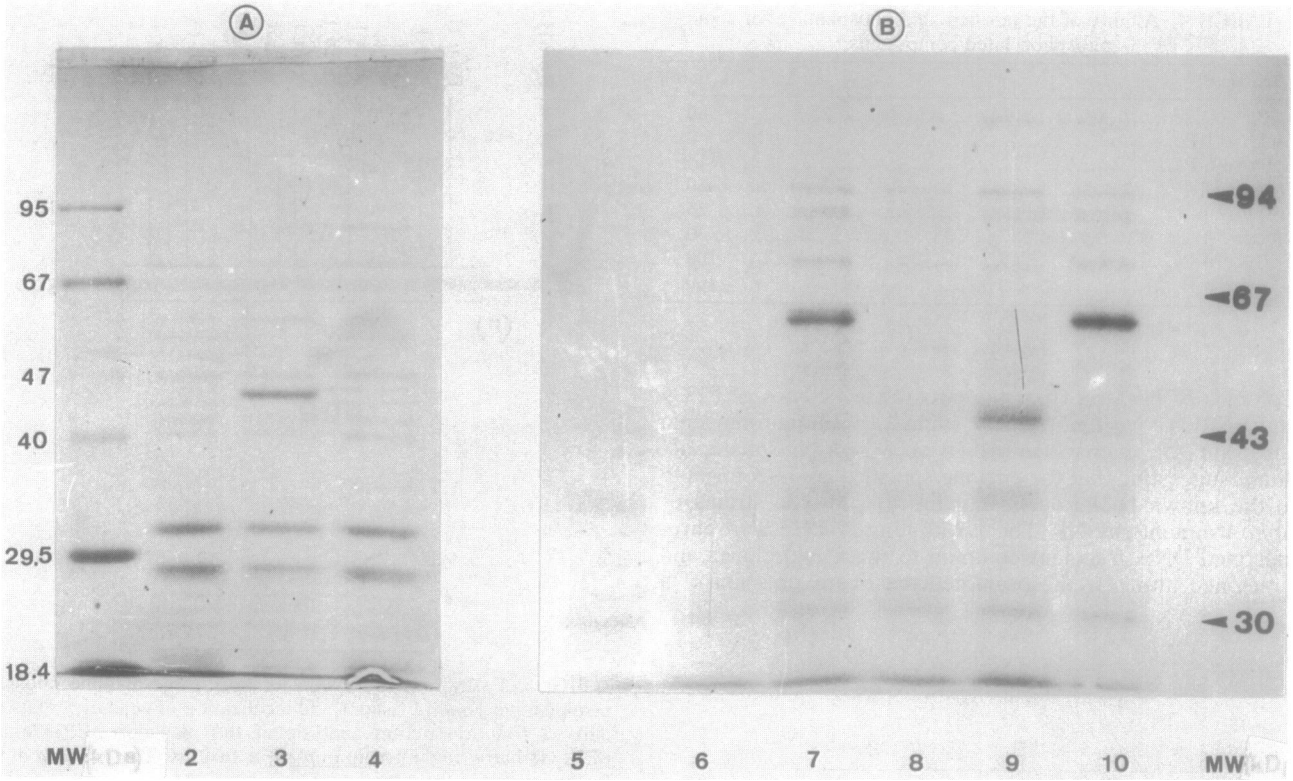

FIG. 6. Analysis of the product encoded by the fusion plasmid pEP1390. (A) SDS-polyacrylamide gel electrophoresis and Coomassie staining of shock fluids extracted from strain SBS1405 harboring plasmids pBR322 (lane 2), pEP1376 (lane 3), and pEP1390 (lane 4). (B) Autoradiogram of ^{35}S-labeled lysates corresponding to the same strain with the same plasmids and immunoprecipitated with an antiserum directed against alkaline phosphatase (lanes 5 to 7) or with antiserum against the purified acid phosphatase (lanes 8 to 10). Lanes 5 and 8, pBR322; lanes 6 and 9, pEP1376; lanes 7 and 10, pEP1390; lanes MW, 10^3 molecular weight markers.

biological significance of a glucose-1-phosphatase in the periplasmic space requires information about its regulation and about the effects of an *agp* deletion. Such studies are currently in progress. Further characterization of the enzymes overexpressed in the other clones reported here may also help determine the number of acid phosphatases present in *E. coli*.

LITERATURE CITED

1. **Anraku, Y.** 1964. A new cyclic phosphodiesterase having a 3′ nucleotidase activity from *Escherichia coli* B. I. Purification and properties of the enzyme. J. Biol. Chem. **239**:3412–3418.
2. **Anraku, Y.** 1964. A new cyclic phosphodiesterase having a 3′ nucleotidase activity from *Escherichia coli* B. II. Further studies on substrate specificity and mode of action of the enzyme. J. Biol. Chem. **239**:3420–3424.
3. **Anraku, Y.** 1966. Cyclic phosphodiesterase of *Escherichia coli*, p. 130–149. *In* D. Cantoni and B. Davis (ed.), Procedures in nucleic acid research. Harper & Row, Publishers, Inc., New York.
4. **Beacham, I. R.** 1979. Periplasmic enzymes in gram-negative bacteria. Int. J. Biochem. **10**:877–883.
5. **Beacham, I. R., and S. Garrett.** 1980. Isolation of *Escherichia coli* mutants (*cpdB*) deficient in periplasmic 2′:3′-cyclic phosphodiesterase and genetic mapping of the *cpdB* locus. J. Gen. Microbiol. **119**:31–34.
6. **Beacham, I. R., and E. Yagil.** 1976. Genetic location of the gene (*ush*) specifying periplasmic uridine 5′-diphosphate glucose hydrolase (5′-nucleotidase) in *Escherichia coli* K-12. J. Bacteriol. **128**:487–489.
7. **Bolivar, F., R. L. Rodriguez, P. J. Green, H. L. Betlach, H. L. Heynecker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene **2**:95–113.
8. **Boquet, P. L., C. Manoil, and J. Beckwith.** 1987. Use of Tn*phoA* to detect genes for exported proteins in *Escherichia coli*: identification of the plasmid-encoded gene for a periplasmic acid phosphatase. J. Bacteriol. **169**:1663–1669.
9. **Burns, D. M., L. J. Abraham, and I. R. Beacham.** 1983. Characterisation of the *ush* gene of *Escherichia coli* and its protein products. Gene **25**:343–353.
10. **Chen, P. S., T. Y. Torribara, and H. Warner.** 1956. Microdetermination of phosphorus. Anal. Chem. **28**:1756–1759.
11. **Cowman, A., and I. R. Beacham.** 1980. Molecular cloning of the gene (*ush*) from *Escherichia coli* specifying periplasmic UDP-sugar hydrolase (5′-nucleotidase). Gene **12**:281–286.
12. **Dassa, E., and P. L. Boquet.** 1981. *expA*, a conditional mutation affecting the expression of a group of exported proteins in *Escherichia coli*. Mol. Gen. Genet. **181**:192–200.
13. **Dassa, E., and P. L. Boquet.** 1985. Identification of the gene *appA* for the acid phosphatase (pH optimum 2.5) of *Escherichia coli*. Mol. Gen. Genet. **200**:68–73.
14. **Dassa, E., M. Cahu, B. Desjoyaux-Cherel, and P. L. Boquet.** 1982. The acid phosphatase with optimum pH of 2.5 of *Escherichia coli*: physiological and biochemical study. J. Biol. Chem. **257**:6669–6676.
15. **Davis, B. D., and E. S. Mingioli.** 1950. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. **60**:17–28.
16. **Dvorak, H. F., R. W. Brockman, and L. A. Heppel.** 1967. Purification and properties of two acid phosphatase fractions isolated from osmotic shock fluid of *Escherichia coli*. Biochemistry **6**:1743–1751.
17. **Ferro-Luzzi Ames, G., C. Prody, and S. Kustu.** 1984. Simple, rapid, and quantitative release of periplasmic proteins by chloroform. J. Bacteriol. **160**:1181–1183.
18. **Frazer, A. C., and R. Curtiss.** 1975. Production, properties and

utility of bacterial minicells. Curr. Top. Microbiol. Immunol. **69**:1–84.
19. **Groisman, E. A., B. A. Castilho, and M. J. Casadaban.** 1984. *In vivo* DNA cloning and adjacent gene fusing with a mini-Mu-lac bacteriophage containing a plasmid replicon. Proc. Natl. Acad. Sci. USA **81**:1480–1483.
20. **Ito, K., P. Bassford, and J. Beckwith.** 1981. Protein localization in *Escherichia coli*: is there a common step in the secretion of periplasmic and outer membrane proteins? Cell **24**:707–717.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Liu, J., D. M. Burns, and I. Beacham.** 1986. Isolation and sequence analysis of the gene (*cpdB*) encoding periplasmic 2′-3′-cyclic phosphodiesterase. J. Bacteriol. **165**:1002–1010.
23. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
24. **Manoil, C., and J. Beckwith.** 1985. Tn*phoA*: a transposon probe for protein export signals. Proc. Natl. Acad. Sci. USA **82**:8129–8133.
25. **Neu, H. C.** 1967. The 5′ nucleotidase of *Escherichia coli*. I. Purification and properties. J. Biol. Chem. **242**:3896–3904.
26. **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.
27. **Rogers, D., and F. J. Reithel.** 1960. Acid phosphatases of *Escherichia coli*. Arch. Biochem. Biophys. **89**:97–104.
28. **Torriani, A.** 1960. Influence of inorganic phosphate in the formation of phosphatases by *Escherichia coli*. Biochim. Biophys. Acta **38**:460–479.
29. **Touati, E., and A. Danchin.** 1987. The structure of the promoter and amino terminal region of the pH 2.5 acid phosphatase structural gene (*appA*) of *E. coli*: a negative control of transcription mediated by cyclic AMP. Biochimie **69**:215–221.
30. **Touati, E., E. Dassa, J. Dassa, and P. L. Boquet.** 1987. Acid phosphatase (pH 2.5) of *Escherichia coli*: regulatory characteristics, p. 31–40. *In* A. Torriani-Gorini, F. G. Rothman, S. Silver, A. Wright, and E. Yagil (ed.), Phosphate metabolism and cellular regulation in microorganisms. American Society for Microbiology, Washington, D.C.
31. **von Hofsten, B., and J. Porath.** 1962. Purification and some properties of an acid phosphatase from *Escherichia coli*. Biochim. Biophys. Acta **64**:1–12.