JOURNAL OF BACTERIOLOGY, Apr. 1978, p. 287-294
0021-9193/78/0134-0287$02.00/0

Vol. 134, No. 1
Printed in U.S.A.

Escherichia coli Mutants Deficient in the Production of Alkaline Phosphatase Isozymes

ATSUO NAKATA,* MARIKO YAMAGUCHI, KAZUKO IZUTANI, AND MITSUKO AMEMURA

Department of Experimental Chemotherapy, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka, Japan 565

Received for publication 29 September 1977

Escherichia coli K-12 mutants showing an altered isozyme pattern of alkaline phosphatase were isolated. Whereas wild-type strains synthesized all three isozymes in a synthetic medium supplemented with Casamino Acids or arginine but synthesized only isozyme 3 in a medium without supplement, the mutant strains synthesized isozyme 1 and a small amount (if any) of isozyme 2, but no isozyme 3, under all growth conditions. The mutation responsible for the altered isozyme pattern, designated *iap*, was mapped by P1 transduction in the interval between *cysC* and *srl* (at about 58.5 min on the *E. coli* genetic map). It was cotransducible with *cysC* and *srl* at frequencies of 0.54 and 0.08, respectively. The order of the genes in this region was *srl-iap-cysC-argA-thyA-lysA*. Three more independent mutations were also mapped in the same locus. We purified isozymes 1′ and 3′ from *iap* and *iap*$^+$ strains and analyzed the sequences of four amino acids from the amino terminus of each polypeptide. They were Arg-Thr-Pro-Glu (or Gln) in isozyme 1′ and Thr-Pro-Glu (or Gln)-Met in isozyme 3′, which were identical with those of corresponding isozymes produced by the wild-type *phoA*$^+$ strain (P. M. Kelley, P. A. Neumann, K. Schriefer, F. Cancedda, M. J. Schlesinger, and R. A. Bradshaw, Biochemistry **12**:3499-3503, 1973; M. J. Schlesinger, W. Bloch, and P. M. Kelley, p. 333-342, in *Isozymes*, Academic Press Inc., 1975). These results indicate that the different mobilities of isozymes 1, 2, and 3 are determined by the presence or absence of amino-terminal arginine residues in polypeptides.

It has been reported that the enzymatically active alkaline phosphatase (EC 3.1.3.1) of *Escherichia coli* is composed of two identical polypeptides (20), coded by a single structural gene (5). Occasionally, several distinct isozyme bands of equal spacing are observed when the purified enzyme sample is analyzed by starch or polyacrylamide gel electrophoresis (6, 10, 11, 18, 21, 23). These isozymes are not aggregates of active enzyme molecules; their formation depends on the growth conditions (14, 18, 21, 23). Of the three most commonly observed isozymes, it has been reported that isozymes 1 and 3 are purely homologous dimers, but different from each other, and isozyme 2 is composed of one monomer of isozyme 1 and one monomer of isozyme 3 (10, 12, 19, 21). (For the number of isozymes, we followed the designation of Schlesinger. The isozyme showing the slowest mobility on electrophoresis [the least negatively charged] of the three isozymes is referred to as isozyme 1, and the faster and fastest isozymes are noted as 2 and 3, respectively.)

In a previous paper (14), we reported a mutant strain unable to convert isozyme 1 to 3. In this paper, we isolated three more mutants and mapped the locus of the mutation of the *E. coli* chromosome map. We also confirmed the amino acid sequences in the amino-terminal region of electrophoretically altered isozymes 1′ and 3′, purified separately from mutant and wild-type strains. The results support the presumption that the different mobilities of the three commonly observed isozymes are due to differences in amino-terminal amino acid residues.

MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 strains used in this study and their sources are listed in Table 1.

Buffer and culture media. TM buffer contained 0.01 M tris(hydroxymethyl)aminomethane (Tris; Trizma base, Sigma Chemical Co.) and 1 mM $MgSO_4$. The pH was adjusted to 7.2 with HCl, unless otherwise stated.

TG medium, a mineral salt solution buffered with Tris at pH 7.2 and containing 0.2% glucose (15) was supplemented with phosphate (as KH_2PO_4) either at a high (6.4×10^{-4} M) or low (6.4×10^{-5} M) concentration, referred to as HP or LP, respectively. It was also supplemented with a high (0.2%) or low (0.002%) concentration of Casamino Acids, referred to as HC or LC, respectively. For instance, TGLPHC medium was TG medium supplemented with phosphate (6.4×10^{-5}

TABLE 1. *E. coli K-12 strains*

Strain	Genotype	Source
AB311	Hfr(PO11) *thr-1 leu-6 thi-1 lacZ4 strA8* λ^{def} *supE44*	*E. coli* Genetic Stock Center strain 311
AB1917	Hfr(PO12) *thr-1 leu-6 thi-1 purF1 his-33 lacZ4 str-8* λ^- *supE44*	*E. coli* Genetic Stock Center strain 1917
C75	HfrC *phoS*	N. Otsuji
C90	HfrC *phoT*	N. Otsuji
Hfr G11	Hfr(PO124) *hisA323* λ^-	*E. coli* Genetic Stock Center strain 5398
KL16	Hfr(PO45) *thi-1 rel-1* λ^-	N. Otsuji
KL25	Hfr(PO46) *supE42*	*E. coli* Genetic Stock Center strain 4244
KL983	Hfr(PO53) *xyl-7 lacY1*	*E. coli* Genetic Stock Center strain 4240
P13	Hfr(PO104) *his-49 cys-23 gal-5 str-58* λ^- $T1^r$ $T3^r$	N. Otsuji
P72	Hfr(PO102) *metB1 relA1*	*E. coli* Genetic Stock Center strain 5050
PK191	Hfr(PO66) *thi-1* DE5 λ^- *sup-56 relA1*?	*E. coli* Genetic Stock Center strain 4316
U24R3Ua	HfrC *phoA*($U_{24}R_3A$) (reference 11)	F. G. Rothman
AN234[a]	HfrC *phoT iap*	NTG mutagenesis: C90, this paper
AN705[a]	HfrC *phoS iap*	NTG mutagenesis: C75, this paper
AN788[a]	HfrC *phoS iap*	NTG mutagenesis: C75, this paper
AN798[a]	HfrC *phoS iap*	NTG mutagenesis: C75, this paper
ANP13[a]	Hfr(PO104) *his cys gal* str^r *thyA*	Trimethoprim: P13
AT713	F^- *thi-1? argA21 cysC43 lysA22 mtl-2 xyl-7 malA1 strA104* λ^r λ^- *supE44*?	*E. coli* Genetic Stock Center strain 4529
JC411	F^- *argG6 metB1 his-1 leu-6 mtl-2 xyl-7 malA1 gal-6 lacY1 str-104*	*E. coli* Genetic Stock Center strain 4274
LC607	F^- *leu proC purE trp lys metE thi ara lacZ xyl* str^r *tonA tsx*	K. Matsubara
W3350-594-*srl*	F^- str^r *srl*	H. Ogawa
ANB11[a]	F^- *leu proC trp* str^r *phoT iap*	Hfr cross: AN234 × LC607
ANB12[a]	F^- *leu proC trp* str^r *phoT iap*	Hfr cross: AN234 × LC607
ANB24[a]	F^- *leu phoA*($U_{24}R_3A$) *trp* str^r *phoT iap*	P1 transduction: U24R3Ua × ANB11
ANB34[a]	F^- *leu proC purE trp* str^r	Hfr cross: AN234 × LC607
ANB35[a]	F^- *leu phoA*($U_{24}R_3A$) *purE trp* str^r	P1 transduction: U24R3Ua × ANB34
ANC7[a]	F^- *purE proC* str^r *metE iap*	Hfr cross: AN234 × LC607
ANC8[a]	F^- *leu proC* str^r *metE iap*	Hfr cross: AN234 × LC607
ANJ411[a]	F^- *leu his* str^r *met iap*	Hfr cross: AN234 × JC411

[a] For the strains constructed in our laboratory, only genotypes confirmed after purification were described.

M) and Casamino Acids (0.2%). In some experiments, arginine was added to the medium at a final concentration of 200 μg/ml to suppress the formation of isozyme 3 (18). TGHP or TGLP medium containing 1.3% agar was used for minimal plates. Any required amino acid, purine, or pyrimidine (as nucleoside) at a final concentration of 20 μg/ml, or vitamin B_1 at a final concentration of 5 μg/ml, was added to the medium, if necessary.

T broth and L broth have been described previously (15). T broth and L broth plates each contained 1.3% agar. They were also supplemented with 20 μg of purine or pyrimidine per ml, if necessary.

Bacterial conjugation and P1 transduction. The procedures for the bacterial mating and transduction with P1 phage have been described previously (15). The phage used in this study was a P1*vir* strain obtained from J. Tomizawa.

Mutagenesis and isolation of the mutant strains. Mutagenesis of bacterial cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG; Nakarai Chemical Co.) was performed by the method of Glover (7). Initially, after mutagenesis of strain C90, the cells were plated immediately, and colonies were picked into T broth to make the mutagenized stock culture. Later on, several colonies of strain C75 were picked together into T broth (mass screening technique) after mutagenesis.

A spontaneous mutant requiring a high concentration of thymine was isolated from a TGHP plate supplemented with 10 μg of trimethoprim per ml and 500 μg of thymidine per ml (13).

Electrophoresis. To examine alkaline phosphatase isozyme patterns, cells were collected by centrifugation and resuspended in a 1/5 to 1/10 volume of TM buffer. The cell suspension was treated with toluene and incubated at 37°C with shaking for 30 to 60 min. It was then heated at 90°C for 15 min to denature other proteins, and the clear supernatant was subjected to electrophoresis.

Polyacrylamide gel electrophoresis with or without 0.1% sodium dodecyl sulfate (Pierce Chemical Co.) and staining for protein with Coomassie brilliant blue R250 (Colab Laboratories Inc.) were carried out by the method of Ostrove and Maizel (17). Acrylamide, bisacrylamide, and *N,N,N',N'*-tetramethylenediamine were purchased from Wako Pure Chemical Industries, Ltd.

Preparation and purification of alkaline phosphatase isozymes. To analyze the amino acid sequences in amino-terminal regions of isozymes, isozyme 1' was prepared from strain ANB24 [*phoA*($U_{24}R_3A$)-*iap*], and isozyme 3' was prepared from strain ANB35 [*phoA*($U_{24}R_3A$)-*iap*$^+$]. We designated isozymes 1 and 3 produced by a pseudorevertant alkaline phosphatase structural gene as isozymes 1' and 3', respectively, to distinguish them from the wild-type isozymes, whose mobilities on electrophoresis were different. The bacteria cultured in TGLPLC medium supplemented with required amino acids were harvested by centrifugation, washed once, and suspended in TM buffer, pH 8.0. The cell suspension was subjected to sonic treatment. Washing and disruption of the cells were performed in the cold. The sonically disrupted cell suspension was heated at 85°C for 15 min, and the denatured bacterial mass was removed by centrifugation. The crude enzyme preparation was purified by diethylaminoethyl-cellulose (DE52; Whatman Ltd.) and Sephadex G-150 (Whatman Ltd.) column chromatography (3). The enzyme sample was then subjected to polyacrylamide gel electrophoresis with a single large comb. After electrophoresis, the prerequisite isozyme was extracted. The purity of the isozyme sample was examined by electrophoresis with and without sodium dodecyl sulfate and was found to contain a trace of contaminating protein or other isozymes.

Determination of amino-terminal amino acid and amino acid sequences in the amino-terminal region. The amino-terminal amino acid residues in alkaline phosphatase isozymes were determined by the sodium dodecyl sulfate-1-dimethylaminonaphthalene-5-sulfonyl(dansyl) method (25). The manual Edman degradation method (4) was also applied to determine the amino-terminal amino acid and amino acid sequences in the amino-terminal region of the polypeptides. The dansyl and phenylthiohydrantoin amino acids were subjected to two-dimensional thin-layer chromatography on a polyamide sheet (9). The solvent systems for dansyl amino acids were 28% aqueous ammonia-water (1:100, vol/vol) for one dimension and formic acid-*n*-butanol-*n*-heptane (1:10:10, vol/vol) for the other. For the phenylthiohydrantoin amino acids, toluene-*n*-heptane-acetic acid (60:30:16, vol/vol) was used for one dimension, and 25% acetic acid was used for the other. For the PTH arginine, ethanol-*n*-butanol-acetic acid (35:10:1, vol/vol) was used. All solvents contained 2-(4'-*tert*-butylphenyl)-5-(4'-biphenylyl)-1,3,4-oxadiazole at a final concentration of 250 μg/ml. Standard dansyl or phenylthiohydrantoin amino acids were products of Seikagaku Kogyo Co. Ltd. Chemicals for the amino acid analysis were purchased from Nakarai Chemical Co.

RESULTS

Isolation of isozyme mutants. As the variations in isozyme pattern depend on the medium in which wild-type alkaline phosphatase-synthesizing bacteria grow, we stabilized our cultural conditions to obtain a constant isozyme pattern on electrophoresis. When alkaline phosphatase was extracted from cells grown in TGLPHC medium with or without arginine, all three isozymes were observed. Only isozyme 3 was observed when the enzyme was extracted from the cells grown overnight in either TGLP or TGLPLC medium, or from cells incubated overnight in TG medium or TM buffer after synthesis of the enzyme.

After mutagenesis with NTG, individual colonies of strain C90 were picked and cultured overnight in TGLPHC and TG medium. The alkaline phosphatase isozyme pattern from each culture was then examined by electrophoresis as described in Materials and Methods. Figure 1 shows part of an electrophoretogram of alkaline phosphatase prepared from a mutagenized stock culture of C90. A slow-moving isozyme was discovered in 1 of the 494 cultures examined (Fig. 1B, comb no. 234). After the results from the stock culture for no. 234 were confirmed, a clone was picked and named AN234. Similarly, three more mutants were isolated from mutagenized cultures of strain C75, namely, AN705, AN788, and AN798. All of these mutant strains produced isozyme 1 and a small amount of isozyme 2 but no isozyme 3 in TGLPLC medium, in which the parental wild-type strains produced only isozyme 3 after overnight incubation (Fig. 2).

Characterization of the mutant strains. The conditions that would result in the formation of only isozyme 1 were examined. Since it is known that isozyme 1 is observed as the major band in the enzyme preparation extracted from bacterial cells grown in a rich medium or extracted during very early-logarithmic-phase growth, it is possible that the growth rate of the mutant strains in the medium without supplemented Casamino Acids was quite different from that of their parental wild-type strains. Comparisons of the rates of growth and of alkaline phosphatase synthesis were made among strains AN234, AN705, AN788, AN798, and C90 in TGHPLC medium. No difference was observed among the strains in duration of lag and logarithmic growth phases, nor did we detect any difference in the rate of enzyme synthesis. After 7 h of incubation, the isozyme patterns were compared. The mutant strains produced only isozyme 1, whereas strain C90 produced almost exclusively isozyme 3.

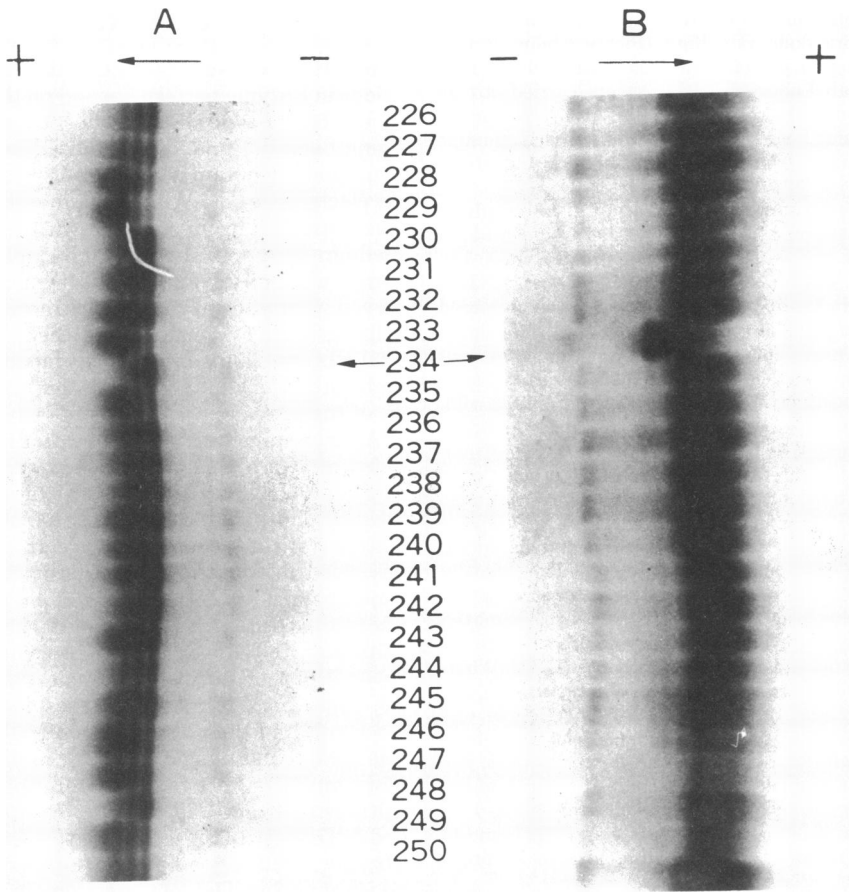

FIG. 1. *Polyacrylamide gel (7.5%) electrophoresis of alkaline phosphatase isolated from mutagenized strains of C90. Electrophoresis was carried out at a constant voltage of 300 V for 3 h at room temperature. After electrophoresis, the gel was stained with a mixture of 0.2 mg of α-naphthylacid phosphate and 0.7 mg of o-dianisidine (tetrazotized [fast blue B salt; Sigma Chemical Co.]) per ml in 0.1 M Tris-hydrochloride, pH 8.0. The mutagenized bacteria were grown in TGLPHC medium (A). Half of them were transferred into TG medium and then incubated overnight (B).*

It is possible that the change in the isozyme pattern resulted from a mutation either in the alkaline phosphatase structural gene or in an enzyme involved in isozyme conversion. A preliminary mating experiment was performed to distinguish between these two alternatives. Bacterial crosses were performed between strains AN234 and LC607. All the Pro^+-Leu^+ or Leu^+-Met^+ recombinants produced isozyme 3 in TGLPLC medium, as did the F^- recipient cells, whereas about 50% of the Met^+-Lys^+ or Lys^+-Trp^+ recombinants were unable to form isozyme 3 under the same growth conditions (Table 2). These results indicate that the mutation occurred not in the structural gene but in some other locus between *trp* and *metE*, because *phoA* is located very close to *proC* (1, 15). This was confirmed by P1 transduction experiments. Recombinant strains with the *proC* genotype that were incapable of producing isozyme 3 (ANB11) or capable of producing isozyme 3 (ANB34) were isolated after the above Hfr mating. They were then transduced to Pro^+ with phage P1 grown on strain U24R3Ua (a pseudorevertant of a negative point mutation of the structural gene producing alkaline phosphatase that has full activity but moves faster than the wild-type enzyme on electrophoresis [11]). Since the cotransduction frequencies of *proC* and *phoA* are very high (15), most of the Pro^+ transductants produced the fast-moving enzyme rather than the wild-type one. However, the isozyme patterns of these cotransductants were characteristic of the recipient strains. Strain ANB34 and its transductant ANB35 produced all three isozymes in TGLPHC medium supplemented with arginine, but the

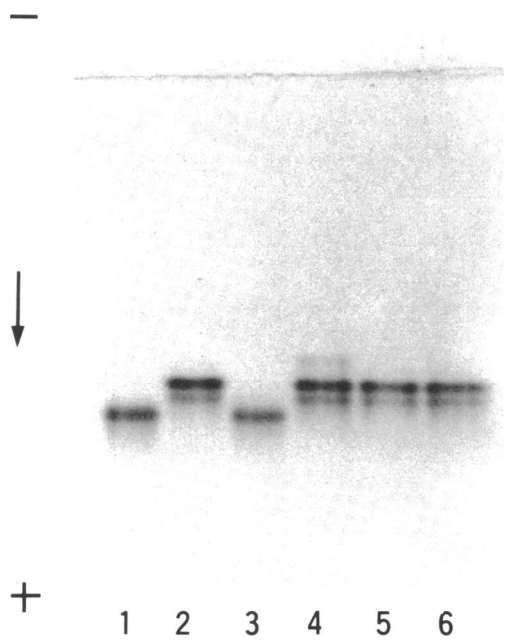

FIG. 2. *Alkaline phosphatase isozyme pattern in the mutant strains incapable of converting isozyme 1 to isozyme 3. The bacteria were inoculated in TGLPLC medium and incubated at 37°C overnight. After incubation, the enzyme samples were extracted from the cells of strains C90 (1), AN234 (2), C75 (3), AN705 (4), AN788 (5), and AN798 (6). Electrophoresis and staining of the gel were carried out as described in the legend to Fig. 1.*

TABLE 2. *Transfer of Iap⁻ character into F⁻ recipient strain by conjugation between AN234 and LC607*

Selected phenotype	No. of recombinants examined for Iap	No. of Iap⁻
Pro⁺ Leu⁺ Str^r	20	0
Leu⁺ Met⁺ Str^r	15	0
Met⁺ Lys⁺ Str^r	70	33 (47.1)[a]
Lys⁺ Trp⁺ Str^r	46	25 (54.3)[a]

[a] Numbers in parentheses represent percentages.

isozymes of ANB35 migrated to the same position as those extracted from strain U24R3Ua bacteria grown in the same medium (Fig. 3). Only isozyme 3 was observed when the enzyme samples were extracted from these bacterial cells after further incubation in TM buffer at 37°C overnight. Again, the isozyme from ANB35 bacteria migrated to the same position as the isozyme extracted from U24R3Ua bacteria after similar treatment. Only isozyme 1 (and a small amount of isozyme 2) was observed in enzyme preparations from strains ANB11 and its transductant ANB24, even after incubation in TM buffer; the isozyme prepared from the transductant strain moved to the same position as the isozyme 1 produced by the donor strain. Thus, we concluded that there is a gene (or genes) controlling the conversion of alkaline phosphatase isozyme 1 to isozyme 3 on the *E. coli* chromosome and tentatively proposed to designate this gene *iap*, an abbreviation of isozyme of alkaline phosphatase.

Mapping by P1 transduction. Conjugation experiments employing Hfr donor strains with various origins of chromosome transfer suggested that the *iap* allele should be mapped in the *thyA* region of the chromosome (Table 3). Transductions with P1 were then performed as shown in Table 4. Recipient bacteria infected with a P1 phage lysate prepared on Iap⁻ bacteria (AN234) were selected, and the isozyme patterns of transductants were examined. The *iap* mutation cotransduced with either *cysC*⁺ or *srl*⁺. Since we did not have a *cysC-srl* double-mutant strain, we could not perform three-factor crosses. A spontaneous mutation requiring a high concentration of thymine (ANP13) was isolated from the *cysC* strain and then used as a recipient strain of P1 transduction. The result suggested that the *iap* locus is outside *cysC* and *thyA*. To confirm this, a *cysC-argA-lysA* triple-mutant strain (AT713) was infected with the same P1 phage lysate, and either Cys⁺ or Arg⁺ transductants were selected and examined for unselected phenotypes. We concluded that the *iap* locus lies between *srl* and *cysC*. Three other independently isolated *iap* mutations from strain C75 also cotransduced with *cysC* at similar frequencies (Table 5). However, it was impossible to determine whether the *iap* locus lay to the left or the right of *mutS*, which is located between *srl* and *cysC* (1).

Identification of amino acid sequences in the amino-terminal region of electrophoretically altered isozymes. We confirmed the amino acid sequences in the amino-terminal region of isozyme 1′ prepared from *phoA*($U_{24}R_3A$)-*iap* (ANB24) and of isozyme 3′ prepared from *phoA*($U_{24}R_3A$)-*iap*⁺ (ANB35). Highly purified samples of isozymes 1′ and 3′ were prepared, and their amino-terminal amino acid residues were determined by the sodium dodecyl sulfate-dancyl method and by the Edman (phenylisothiocyanate) method. The amino acid sequences in the amino-terminal region of each isozyme were analyzed manually by the Edman method. The amino acid sequence in isozyme 1′ was Arg-Thr-Pro-Glu (or Gln), and that in isozyme 3′ was Thr-Pro-Glu (or Gln)-Met. These sequences were identical with those of corresponding isozymes isolated from the wild-type strain (2, 8, 22), although one of the amino-terminal amino

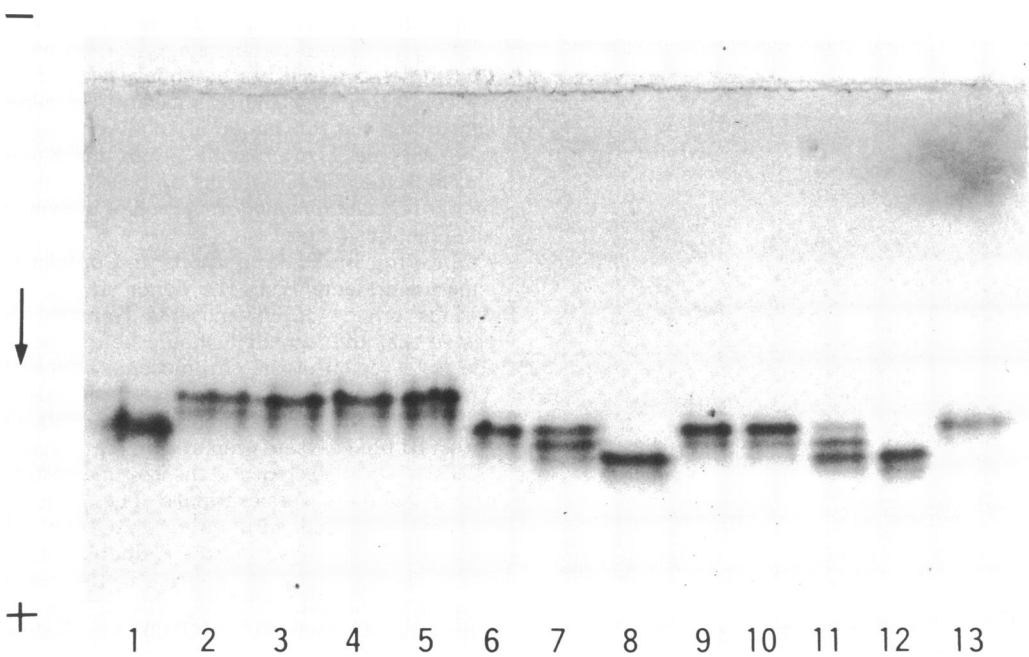

FIG. 3. *Isozyme pattern of the strains with wild-type and pseudorevertant structural genes. The exponentially grown cells in TGHPHC medium supplemented with arginine (200 μg/ml) were harvested by centrifugation and resuspended in fresh TGHC medium with arginine to allow synthesis of alkaline phosphatase. The incubation at 37°C was continued overnight. After incubation, the cells were collected by centrifugation and suspended in sterile TM buffer. The cell suspension was divided into two portions, one of which was further incubated overnight. The cells were collected by centrifugation, resuspended in TM buffer, and then disrupted by sonic treatment. (1 and 13) Isozyme 3 extracted from C90; (2) isozyme 1 extracted from AN234 (as standard); (3) enzyme sample was extracted from ANB11 before incubation in TM buffer over-night; (4) extract from ANB11 after incubation; (5) extract from ANB34 before incubation; (6) extract from ANB34 after incubation; (7) extract from U24R3Ua before incubation; (8) extract from U24R3Ua after incubation; (9) extract from ANB24 before incubation; (10) extract from ANB24 after incubation; (11) extract from ANB35 before incubation; (12) extract from ANB35 after incubation. Electrophoresis and staining of the gel were carried out as described in the legend to Fig. 1.*

TABLE 3. *Conjugation experiments between Hfr Iap⁺ strains and F⁻ Iap⁻ recipient strains*

Donor strain	Iap⁻ recipient strain	Selected phenotype[a]	No. of recombinants examined	No. of Iap⁺
PK191	ANJ411	His⁺ (Str^r)	29	21 (72.4)[b]
AB1917	ANC7	Met⁺ (Thr⁺ Leu⁺ His⁺)	20	2 (10.0)
AB1917	ANC8	Met⁺ (Thr⁺ His⁺)	38	5 (13.2)
AB1917	ANJ411	Met⁺ (Thr⁺ Pur⁺)	29	1 (3.4)
Hfr G11	ANJ411	Met⁺ (Str^r)	34	3 (8.8)
KL25	ANJ411	Met⁺ (Str^r)	30	0
P72	ANJ411	Leu⁺ (Str^r)	30	0
AB311	ANJ411	His⁺ (Thr⁺)	58	0
KL983	ANJ411	His⁺ (Str^r)	53	0
KL16	ANB11	Trp⁺ (Str^r)	40	9 (22.5)
KL16	ANB12	Trp⁺ (Str^r)	40	10 (25.0)
KL16	ANJ411	His⁺ (Str^r)	29	7 (24.1)

[a] Counterselection of Hfr donor bacteria was performed with the phenotype given in parentheses.
[b] Numbers in parentheses represent percentages.

acid residues was different from that reported by Piggot et al. (18). Thus, a difference in amino-terminal amino acid residues was observed between isozymes 1 and 3, irrespective of alteration of the isoelectric point of the enzymes as a whole, presumably determined by the substitution of

TABLE 4. *Transduction of the Iap$^+$ recipient strains to Iap$^-$ with P1 phage grown on strain AN234*

Recipient strain	Selected phenotype	No. of transductants examined	Transductant phenotype[a]			
			Iap$^-$	Cys$^+$	Arg$^+$	Lys$^+$
P13	Cys$^+$	50	25 (50.0)			
W3350-594-*srl*	Srl$^+$	50	4 (8.0)			
ANP13	Thy$^+$	250	1[b] (0.4)	7 (2.8)		
AT713	Cys$^+$	50	29 (58.0)		5 (10.0)	0
	Arg$^+$	98	5[b] (5.1)	14 (14.3)		38 (38.8)

[a] Numbers in parentheses represent percentages.
[b] All transductants showing Iap$^-$ phenotype also showed Cys$^+$ phenotype.

TABLE 5. *Transduction of iap with P1 phage*

Donor strain	Recipient strain	Selected phenotype	No. of transductants examined	No. of Iap$^-$
C75	P13	Cys$^+$	20	0
AN705	P13	Cys$^+$	30	15 (50.0)[a]
AN788	P13	Cys$^+$	30	17 (56.7)
AN798	P13	Cys$^+$	30	22 (73.3)

[a] Numbers in parentheses represent percentages.

an amino acid residue inside the polypeptide rather than the amino terminus. This indicates that the amount of arginine residue at the amino-terminal position of two homologous polypeptides constituting an active enzyme molecule determines the isozymic variations in mobility on electrophoresis.

DISCUSSION

The isozymes of alkaline phosphatase in *E. coli* K-12 are distinguishable on starch or polyacrylamide gel electrophoresis (6, 10–12, 18, 23). The occurrence of variations is considered to be a result of epigenetic modification of the enzyme molecules (19, 21).

The difference between the fingerprint patterns of the tryptic peptides of two isozymes was first observed by Schlesinger and Andersen (21). Molecular variation in the amino-terminal region was discovered in the polypeptide fragments (produced as a result of premature chain termination of protein synthesis) of alkaline phosphatase-negative nonsense mutants (16, 24). Piggot et al. (18) found differences in amino-terminal amino acid residues among isozymes. These authors identified asparagine in isozyme A, valine and threonine in isozyme B, and threonine in isozyme C. Isozyme A is the least negatively charged form, and isozymes B and C are the more and most negatively charged forms, respectively, in the enzyme preparation of Piggot et al. On the other hand, Kelley et al. (8) and Schlesinger et al. (22) reported that the amino-terminal amino acid residue in isozyme 1 (the least negatively charged form in their enzyme preparation) is arginine and that in isozyme 3 is threonine. Since isozyme 2, which migrates at a speed between that of isozyme 1 and that of isozyme 3 on electrophoresis, is known to be a heterologous dimer composed of one monomer of isozyme 1 and one monomer of isozyme 3 (10, 19, 21), its amino-terminal amino acids are thought to be arginine in one polypeptide and threonine in the other. Schlesinger and co-workers also compared the polypeptides after cleavage with cyanogen bromide but could find no difference except in the amino-terminal fragments (8, 22).

If the different mobilities of isozymes of equal spacing are determined by their different amino-terminal amino acid residues, it may be possible to isolate mutant strains incapable of isozyme formation. One such mutation might occur in an alkaline phosphatase-producing structural gene. A mutation might also occur in some other gene involved in the conversion of isozyme 1 to isozyme 3.

We isolated mutant strains that synthesized isozyme 1 and a small amount (if any) of isozyme 2 but no isozyme 3, under cultural conditions in which isozyme 3 accumulated in the wild-type strains. The mutant strains showed normal growth cycles in which the isozyme pattern varied as it did in the wild-type strains, and they synthesized alkaline phosphatase at the same rate as the parental strains did. The evidence shows that the mutation occurred not in an alkaline phosphatase structural gene but in a gene located between *srl* and *cysC* on the *E. coli* chromosome. Thus, we tentatively proposed to designate the gene *iap*, an abbreviation of isozyme of alkaline phosphatase.

We compared the amino acid sequences in the amino-terminal region of isozymes 1′ and 3′, the two isozymes being prepared from *iap* and *iap*$^+$ strains, respectively, that carry a pseudorevertant structural gene. The amino acid sequences in the amino-terminal region of isozymes 1′ and 3′ were similar to those of corresponding isozymes purified from the wild-type (*phoA*$^+$-*iap*$^+$) strain already reported by others (8, 22). The fact that the presence or absence of amino-terminal arginine residues is observed in the pseu-

dorevertant isozymes, in spite of their different isoelectric points, indicates that the different mobilities among isozymes (at least among isozymes 1, 2, and 3) are determined by the number of amino-terminal arginine residues of the two polypeptides forming active enzyme molecules.

The mode of action of the *iap*$^+$ gene product is yet to be clarified. It is likely that the mutant that does not synthesize isozyme 3 lacks a protease that removes the amino-terminal arginine residue of isozyme 1. An in vitro system of isozyme conversion is now being developed to identify the putative protease and to clarify the process of isozyme conversion.

ACKNOWLEDGMENTS

This research was supported by a grant from the Japanese Ministry of Education, Science, and Culture.

We thank J. Kawamata for his valuable discussion and encouragement throughout this work. We thank B. J. Bachmann, F. G. Rothman, N. Otsuji, K. Matsubara, H. Ogawa, and J. Tomizawa for supplying bacterial and phage strains. We are also indebted to S. Iwanaga and Y. Hokama for their supervision and the use of facilities at the Institute for Protein Research, Osaka University, during the analysis of amino acids.

LITERATURE CITED

1. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
2. **Bridgen, J., and D. S. Secher.** 1973. Molecular heterogeneity of alkaline phosphatase. FEBS Lett. **29**:55–57.
3. **Csopak, H., G. Garellick, and B. Hallberg.** 1972. Purification of *Escherichia coli* alkaline phosphatase. Improved growth conditions for the bacteria, modified methods of preparation of the enzyme. Acta Chem. Scand. **26**:2401–2411.
4. **Edman, P.** 1970. Sequence determination, p. 211–255. *In* S. B. Needleman (ed.), Protein sequence determination. Springer-Verlag, Berlin.
5. **Garen, A., and S. Garen.** 1963. Complementation *in vivo* between structural mutants of alkaline phosphatase from *E. coli*. J. Mol. Biol. **7**:13–22.
6. **Garen, A., and C. Levinthal.** 1960. A fine structure genetic and chemical study of the enzyme alkaline phosphatase of *E. coli*. I. Purification and characterization of alkaline phosphatase. Biochim. Biophys. Acta **38**:470–483.
7. **Glover, S. W.** 1968. The induction, isolation and analysis of auxotrophic mutants, p. 17–21. *In* R. C. Clowes and H. Hayes (ed.), Experiments in microbial genetics. Blackwell Scientific Publications, Oxford.
8. **Kelley, P. M., P. A. Neumann, K. Shriefer, F. Cancedda, M. J. Schlesinger, and R. A. Bradshaw.** 1973. Amino acid sequence of *Escherichia coli* alkaline phosphatase. Amino- and carboxyl-terminal sequences and variations between two isozymes. Biochemistry **12**:3499–3503.
9. **Kulbe, K. D.** 1974. Micropolyamide thin-layer chromatography of phenylthiohydrantoin amino acid (PTH) at subnanomolar level. A rapid microtechinique for simultaneous multisample identification after automated Edman degradations. Anal. Biochem. **59**:564–573.
10. **Lazdunski, C., and M. Lazdunski.** 1967. Les isophosphatases alcalines d'*Escherichia coli*. Séparation, propriétés cinétiques et structurales. Biochim. Biophys. Acta **147**:280–288.
11. **Levinthal, C., E. Signer, and K. Fetherolf.** 1962. Reactivation and hybridization of reduced alkaline phosphatase. Proc. Natl. Acad. Sci. U.S.A. **48**:1230–1237.
12. **Malamy, M. H., and B. L. Horecker.** 1964. Purification and crystallization of the alkaline phosphatase in *E. coli*. Biochemistry **3**:1893–1897.
13. **Miller, J. H.** 1972. Selection of thy$^-$ strains with trimethoprim, p. 218–220. *In* Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
14. **Nakata, A., M. Amemura, M. Yamaguchi, and K. Izutani.** 1977. Factors affecting the formation of alkaline phosphatase isozymes in *Escherichia coli* K-12. Biken J. **20**:47–55.
15. **Nakata, A., G. R. Peterson, E. L. Brooks, and F. G. Rothman.** 1971. Location and orientation of the *phoA* locus on the *Escherichia coli* linkage map. J. Bacteriol. **107**:683–689.
16. **Natori, S., and A. Garen.** 1970. Molecular heterogeneity in the amino-terminal region of alkaline phosphatase. J. Mol. Biol. **49**:577–588.
17. **Ostrove, S., and J. V. Maizel, Jr.** 1971. Polyacrylamide gel electrophoresis of viral proteins. Methods Virol. **5**:179–246.
18. **Piggot, P. J., M. D. Sklar, and L. Gorini.** 1972. Ribosomal alterations controlling alkaline phosphatase isozymes in *Escherichia coli*. J. Bacteriol. **110**:291–299.
19. **Reid, T. W., and I. B. Wilson.** 1970. *E. coli* alkaline phosphatase, p. 373–415. *In* P. D. Boyer (ed.), The enzymes IV. Academic Press Inc., New York.
20. **Rothman, F., and R. Byrne.** 1963. Fingerprint analysis of alkaline phosphatase of *E. coli* K12. J. Mol. Biol. **6**:330–340.
21. **Schlesinger, M. J., and L. Andersen.** 1968. Multiple molecular forms of the alkaline phosphatase of *Escherichia coli*. Ann. N.Y. Acad. Sci. **151**:159–170.
22. **Schlesinger, M. J., W. Bloch, and P. M. Kelley.** 1975. Differences in the structure, function, and formation of two isozymes of *Escherichia coli* alkaline phosphatase, p. 333–342. *In* C. L. Markert (ed.), Isozymes, vol. 1. Molecular structure. Academic Press Inc., New York.
23. **Signer, E., A. Torriani, and C. Levinthal.** 1961. Gene expression in intergeneric merozygotes. Cold Spring Harbor Symp. Quant. Biol. **26**:31–34.
24. **Suzuki, T., and A. Garen.** 1969. Fragments of alkaline phosphatase from nonsense mutants. I. Isolation and characterization of fragments from amber and ochre mutants. J. Mol. Biol. **45**:549–566.
25. **Weiner, A. M., T. Platt, and K. Weber.** 1972. Amino-terminal sequence analysis of proteins purified on a nanomole scale by gel electrophoresis. J. Biol. Chem. **247**:3242–3251.