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# Loss of Allosteric Control but Retention of the Bifunctional Catalytic Competence of a Fusion Protein Formed by Excision of 260 Base Pairs from the 3′ Terminus of *pheA* from *Erwinia herbicola*†

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**A bifunctional protein denoted as the P protein and encoded by *pheA* is widely present in purple gram-negative bacteria. This P protein carries catalytic domains that specify chorismate mutase (CM-P) and prephenate dehydratase. The instability of a recombinant plasmid carrying a *pheA* insert cloned from *Erwinia herbicola* resulted in a loss of 260 bp plus the TAA stop codon from the 3′ terminus of *pheA*. The plasmid carrying the truncated *pheA* gene (denoted *pheA**) was able to complement an *Escherichia coli pheA* auxotroph. *pheA** was shown to be a chimera composed of the residual 5′ part of *pheA* (901 bp) and a 5-bp fragment from the pUC18 vector. The new fusion protein (PheA*) retained both chorismate mutase and prephenate dehydratase activities. PheA* had a calculated subunit molecular weight of 33,574, in comparison to the 43,182-molecular-weight subunit size of PheA. The deletion did not affect the ability of PheA* to assume the native dimeric configuration of PheA. Both the CM-P and prephenate dehydratase components of PheA* were insensitive to L-phenylalanine inhibition, in contrast to the corresponding components of PheA. L-Phenylalanine protected both catalytic activities of PheA from thermal inactivation, and this protective effect of L-phenylalanine upon the PheA* activities was lost. PheA* was more stable than PheA to thermal inactivation; this was more pronounced for prephenate dehydratase than for CM-P. In the presence of dithiothreitol, the differential resistance of PheA* prephenate dehydratase to thermal inactivation was particularly striking. We conclude that the deletion defines a discrete regulatory domain for allosteric control of *pheA* and that this domain is located on the carboxy terminus of PheA and is separable from the two catalytic domains. Excision of the regulatory domain not only abolished allosteric control but secondarily influenced catalytic and physical properties.**

The biochemical pathways that specify the biosynthesis of L-tyrosine and L-phenylalanine in enteric bacteria utilize two bifunctional proteins that compete for chorismate at a metabolic branch point. The P protein encoded by *pheA* possesses catalytic domains for chorismate mutase (CM-P) and for prephenate dehydratase (PDT). The T protein encoded by *tyrA* possesses catalytic domains for a chorismate mutase (CM-T) and for prephenate dehydrogenase. This was originally shown in the landmark paper by Cotton and Gibson (8), who studied *Escherichia coli* and *Klebsiella pneumoniae* (originally named *Aerobacter aerogenes*). The rare occurrence in nature of the T protein (which is limited to the enteric bacteria *Alteromonas* and *Aeromonas* spp.) is in striking contrast to the widespread presence of the P protein throughout two of the three main subdivisions of gram-negative bacteria (1). The T-protein dehydrogenase has proven to be a cyclohexadienyl dehydrogenase (able to use prephenate and L-arogenate as alternative substrates), in contrast to the highly specific P-protein dehydratase, which cannot function as a cyclohexadienyl dehydratase (CDT) (2). Many enteric bacteria, but not *E. coli*, possess a third isozyme of chorismate mutase that is monofunctional (i.e., has one catalytic domain) and has been denoted as CM-F (4, 29).

The *pheA* and *tyrA* genes are contiguous in *E. coli* (18) and *Erwinia herbicola* (31). Homologous chorismate mutase domains are indicated by the high similarity of the N-terminal sequences of the P protein and the T protein. Since mutants lacking only CM-P activity or only PDT activity have been isolated (5, 27), it has been clear for some time that the two catalytic functions of the P protein are spatially distinct. The separability of the two T-protein functions remained in doubt (24) until molecular genetic approaches established the genetic separability of functional CM-T and dehydrogenase components in *E. coli* (20). More recently, the CM-T component of *E. herbicola* T protein was removed by deletion of the 5′ terminus of the *tyrA* gene in vitro, and altered enzymological features of the new monofunctional prephenate dehydrogenase were reported (30). However, defined deletions that selectively abolish allosteric control of either the P protein (by L-phenylalanine) or the T protein (by L-tyrosine) have not been described.

The pathway of phenylalanine biosynthesis in *E. herbicola* is more typical of enteric bacteria than is that of *E. coli* (4). In addition to the P protein, *E. herbicola* possesses monofunctional enzymes (CM-F and CDT) that can participate in a second pathway of phenylalanine biosynthesis (3, 28). In the process of selection for a CDT gene clone, an unexpected truncated *pheA* construct was obtained after transformation of an *E. coli pheA* auxotroph with a recombinant plasmid carrying the entire *phe* and *tyr* operons from *E. herbicola*. The expression product of the truncated *pheA* retained both CM-P and PDT domains with loss of allosteric

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TABLE 1. Bacterial strains and plasmids

| Strain or plasmid | Genotype or description | Source or reference |
|---|---|---|
| *E. herbicola* ATCC 33243 | Prototroph | ATCC |
| *E. coli* | | |
| JM83 | F⁻ ϕ80d*lacZ*ΔM15 Δ(*lac-proAB*) *ara rpsL* | BRL |
| KA197 | *thi-1 pheA97 relA1 spoT1* | CGSC 5243 |
| JP2255 | *aroF363 pheA361 pheO352 tyrA382 thi strR712 lacY1 xyl-5* | 5 |
| Plasmids | | |
| pUC18 | Ap$^r$ *lacZ* | BRL |
| pJX81 | Phe$^+$ and Tyr$^+$ clone from pUC18; *Hin*dIII-*Pst*I gene library of *E. herbicola* ATCC 33243 | This study |
| pJX82 | Phe$^+$ clone derived from spontaneous deletion of pJX81 | This study |
| pJX83 | Phe$^+$ clone derived by subcloning the 1.4-kb *Ssp*I-*Eco*RI fragment from pJX82 into pUC18 (at *Sma*I-*Eco*RI sites) | This study |
| pJX84 | Derivative of pJX82 lacking the *Pst*I fragment of the *Erwinia* DNA insert | This study |
| pJX183 | 1.87-kb *Eco*RI-*Eco*RV Phe$^+$ fragment ligated into pUC18 at *Eco*RI-*Sma*I sites | 31 |

control and with alterations of certain enzymological characteristics that are described here.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, and growth conditions.** The bacterial strains and plasmids utilized or constructed in this investigation are listed in Table 1. *E. herbicola* was cultured in LB medium (10) at 30°C, and the cells were harvested by centrifugation during the exponential phase of growth. *E. coli* strains were grown in M9 minimal medium (21) or LB medium at 37°C. L-Tyrosine (50 μg/ml), thiamine (17 μg/ml), and ampicillin (50 μg/ml) were supplemented when required. 5-Bromo-4-chloro-3-indoxyl-β-D-galactopyranoside (X-Gal) was added at 0.6 to 1.0 mg per plate when recombinants were screened in *E. coli* JM83 transformed with pUC18.

**Isolation of *E. herbicola* chromosomal DNA and construction of a gene library.** Chromosomal DNA, isolated by the method of Silhavy et al. (26), was partially digested with *Hin*dIII and *Pst*I. Fragments of 4 to 8 kb were isolated from agarose gel after electrophoresis and ligated into the *Hin*dIII-*Pst*I sites of pUC18. The ligation mixture was used to transform *E. coli* JM83. The transformants appearing on LB plates containing ampicillin and X-Gal were collected. Recombinant plasmids were purified from the transformants by the method of Davis et al. (11).

**DNA manipulations.** T4 DNA ligase and restriction enzymes were obtained from Bethesda Research Laboratories and Promega and were used according to the instructions of the manufacturers. Southern blot hybridization with biotinylated probes was conducted as recommended by Promega. *E. coli* strains were transformed by the $CaCl_2$ method (9).

Analysis of restriction sites, subcloning, and other standard molecular biology procedures were performed as described by Sambrook et al. (25).

**DNA sequencing and data analysis.** After the *Pst*I fragment was removed from pJX82 (Fig. 1), the remaining DNA was circularized by means of T4 DNA ligase to generate pJX84. pJX83 and pJX84 were purified by CsCl buoyant density gradient centrifugation (19). The first 300 bp of pJX83 and pJX84 were sequenced from the M13/pUC forward sequencing primer and the T7/T3α sequencing primer, respectively. At the DNA Core Facility of the University of Florida, fluorescent chain-terminating dideoxy nucleotides (22) were used to determine DNA sequences. The nucleotide sequence of DNA and the deduced peptide sequence were analyzed by using the University of Wisconsin Genetics Computer Group package (13).

**Crude extract preparation and enzyme assay.** *E. coli* JP2255 transformed by recombinant plasmids was grown at 37°C in 400 ml of LB medium plus ampicillin and harvested by centrifugation during the exponential phase of growth. Cells were suspended in 50 mM potassium phosphate buffer containing 1 mM dithiothreitol (pH 7.2) (buffer A) and disrupted by sonication. Cell debris was removed by 150,000 × *g* ultracentrifugation at 4°C for 40 min, the supernatant fluid was passed through a PD-10 Sephadex column equilibrated with buffer A to remove small molecules, and the eluate was saved. Unless otherwise specified, dithiothreitol was present to prevent inactivation.

Chorismate mutase and PDT were assayed as described by Cotton and Gibson (8). Prephenate dehydrogenase was assayed as previously described (7). One unit of enzyme activity is defined as the amount required to form 1 μmol of product per min at 37°C.

Protein was quantitated by the procedure of Bradford (6).

**DE52 column chromatography.** Approximately 100 mg of crude extract protein was applied to a DEAE-cellulose (DE52) column (1.5 by 20 cm) equilibrated with buffer A. The column was washed with 100 ml of buffer A, and then (for isolation of the wild-type P protein) the bound proteins were eluted with 500 ml of a linear gradient of KCl (0.0 to 0.4 M) in buffer A. Fractions of 3.0 ml were collected and assayed for $A_{280}$ and for chorismate mutase and PDT activities.

**Molecular mass estimation by gel filtration.** The molecular masses of both the partially purified PheA and PheA* were estimated by gel filtration on a Sephadex G-200 column (2.5 by 96 cm) in the presence or absence of L-phenylalanine or L-tyrosine. The elution buffer was buffer A plus 0.2 mM phenylalanine or tyrosine when the enzyme was run in the presence of the effector. Carbonic anhydrase (30 kDa), bovine serum albumin (67 kDa), alcohol dehydrogenase (150 kDa), and β-amylase (200 kDa) were used as molecular mass standards.

**Determination of L-phenylalanine overproduction.** Cultures (25 ml) of *E. coli* KA197(pJX83) and *E. coli* KA197(pJX183) were grown in M9 minimal medium at 37°C in 125-ml flasks

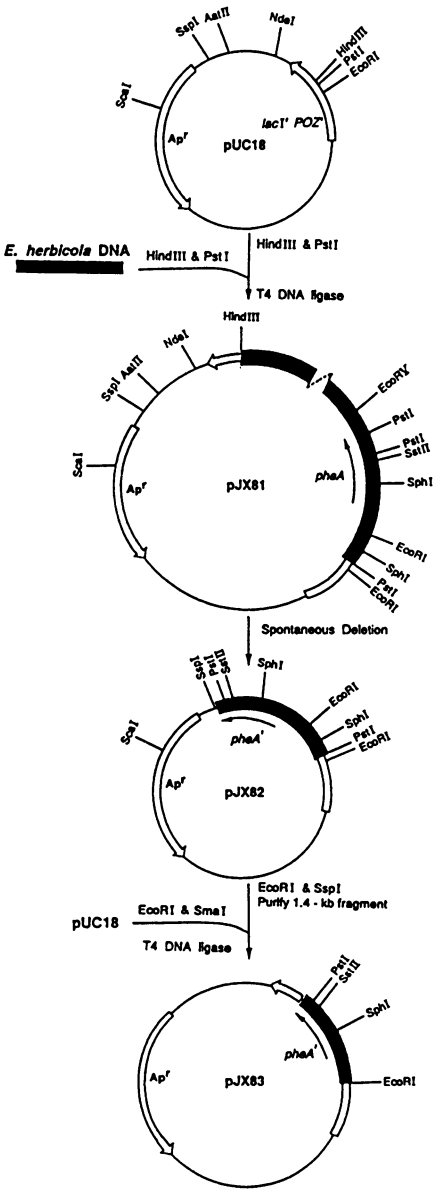


FIG. 1. Physical maps of plasmid pUC18 and the derivative recombinant plasmids pJX81, pJX82, and pJX83. The 5′ and 3′ boundaries of *pheA* and *pheA** (*pheA′*) are indicated by arrows.

with shaking at 300 rpm. Samples were taken at different times of growth, and cells were removed by centrifugation. L-Phenylalanine accumulated in the supernatant fluid was estimated by high-performance liquid chromatography as described by Fischer and Jensen (15).

**Biochemicals and chemicals.** Unless indicated otherwise, all biochemicals and chemicals were obtained from Sigma. Prephenate was prepared from culture supernatant fluids of a tyrosine auxotroph of *Salmonella typhimurium* (12). Chorismate was isolated from a triple auxotroph (62-1) of *K. pneumoniae* as described previously (17).

**Nucleotide sequence accession number.** The nucleotide sequence of *E. herbicola pheA** has been submitted to GenBank under accession number M74134.

TABLE 2. Expression of *E. herbicola* genes in *E. coli* JP2255

| Plasmid in strain JP2255 | Chorismate mutase (U/mg) | Sp act (U/mg) of: | |
|---|---|---|---|
| | | PDT | PDH[a] |
| pUC18 | 0 | 0 | 0 |
| pJX81 | 1.52 | 0.21 | 1.18 |
| pJX82 | 0.12 | 0.09 | 0 |
| pJX83 | 0.13 | 0.11 | 0 |

[a] PDH, prephenate dehydrogenase.

## RESULTS

**Isolation and expression of a truncated *pheA* gene (*pheA**).** Transformation with the ligation mixture of pUC18 and *Hin*dIII-*Pst*I fragments of *E. herbicola* chromosomal DNA into *E. coli* JM83 generated approximately 5,000 recombinants. Plasmids purified from a pool of colonies derived from these recombinants were used to transform *E. coli* KA197, a phenylalanine auxotroph lacking *pheA*. One prototrophic clone obtained on solid M9 medium containing ampicillin was designated pJX81. pJX81 is similar to the previously reported recombinant plasmid of pJX187 containing *pheA*, *tyrA*, and *aroF* genes from *E. herbicola* (31). The insert in pJX81 was ~7.0 kb in size, and transformation of pJX81 into *E. coli* JP2255 abolished auxotrophy for both phenylalanine and tyrosine. Chorismate mutase, PDT, and prephenate dehydrogenase activities, which are absent in JP2255, were found in the crude extract prepared from JP2255 harboring pJX81 (Table 2). These specific activities were much greater than those found in wild-type *E. coli* strains.

When pJX81 was retransformed into KA197, the plasmid isolated from one clone was only about 3.9 kb in total size. This plasmid, designated pJX82, conferred prototrophy to KA197 but not to JP2255. An enzyme assay showed that crude extract prepared from JP2255(pJX82) possessed the activities of chorismate mutase and PDT but not those of prephenate dehydrogenase (Table 2). Restriction analysis indicated that pJX82 was a deletion derivative of pJX81, with part of the pUC18 vector and most of the insert being lost. pJX82 DNA was invulnerable to cleavage by *Aat*II, *Nde*I, *Hin*dIII, and *Eco*RV (Fig. 1). Previous work had shown that the *Pst*I site adjacent to the downstream *Eco*RV site is located in the *pheA* structural gene, 81 bp upstream of the *pheA* stop codon (31). This *Pst*I site was not found in pJX82 (Fig. 1). Therefore, pJX82 carried a truncated *pheA* gene, denoted *pheA**, in which more than 81 bp had been deleted from the 3′ terminus of *pheA*.

The *Ssp*I-*Eco*RI fragment of pJX82 was placed in pUC18 to generate a recombinant plasmid named pJX83. KA197 transformed by pJX83 grew well on minimal medium, as did JP2255(pJX83) when M9 medium was supplemented with tyrosine. The same activity levels of chorismate mutase and PDT were found in crude extracts prepared from JP2255 (pJX83) or from JP2255(pJX82) (Table 2).

**Sequence comparison of *pheA* and *pheA*.*** When the nucleotide sequence of *pheA* (31) was compared with that of *pheA**, it was found that 260 bp plus the stop codon TAA had been deleted from the 3′ portion of *pheA*. Figure 2 shows a comparison of the 3′ regions of the 1,161-bp *pheA* and the 903-bp *pheA**.

The 3′ portion of *pheA** was identified as the location of a gene fusion, whereby the truncated *pheA* sequence was joined to bp 2509 of pUC18, just 3 bp downstream from the *Ssp*I restriction site (32). The sequence of *pheA** upstream

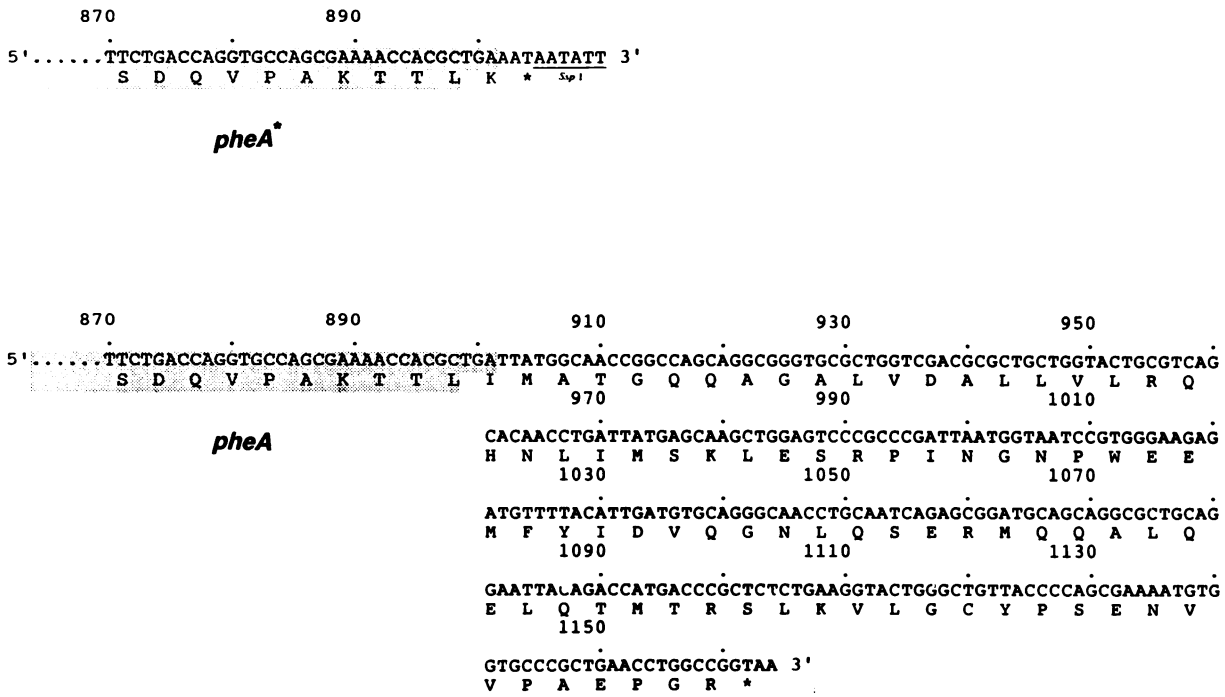


FIG. 2. Comparison of nucleotide and deduced amino acid sequences of the 3′ regions of *pheA* and *pheA**. The regions 3′ of bp 870 which are common to *pheA* and *pheA** are shaded.

from bp 901 matched exactly with the sequence of *pheA* (Fig. 2). A recombinant codon AAA was created by joining bp 901 of *pheA* (31) with bp 2509 and 2508 of pUC18 (32). The new TAA stop codon of *pheA** originated from bp 2507, 2506, and 2505 of pUC18. The two amino acid residues of the *pheA** stop codon are 2 of the 6 bp of a restriction site recognized by *Ssp*I (32).

**Characterization of the new P protein (PheA*).** Crude extracts of *E. coli* JP2255(pJX82) and *E. coli* JP2255(pJX81) were subjected to DE52 anion-exchange chromatography. The profile of the JP2255(pJX82) crude extract showed coincident chorismate mutase and PDT peaks in the wash fractions (Fig. 3A), indicating that PheA* did not bind to the anion-exchange resin at pH 7.2. The use of crude extract of JP2255(pJX81), on the other hand, resulted in the elution of a P-protein peak at 0.12 M KCl (Fig. 3B) and a T-protein peak at 0.22 M (data not shown) after chromatography. The profiles of PheA* and PheA are consistent with the data given by the Wisconsin Genetics Computer Group analysis of the predicted amino acid sequences, whereby the isoelectric points of PheA* and PheA were 7.17 and 6.75, respectively (31).

PheA* had 301 deduced amino acid residues with a calculated molecular mass of 33,574, in comparison to a molecular mass of 43,182 calculated from the 387 predicted residues of PheA (31). Sephadex G-200 gel filtration indicated that the native molecular mass of PheA* was ~68 kDa, whereas that of PheA was ~90 kDa. Thus, both PheA* and PheA are dimers. The presence of L-phenylalanine or L-tyrosine during gel filtration did not affect the molecular mass values obtained for PheA* or PheA.

PheA CM-P and PDT exhibited moderate sensitivity and high sensitivity to feedback inhibition by L-phenylalanine, respectively. Less than 25% inhibition of CM-P activity from PheA was obtained within a range of 2.0 to 5.0 mM phenylalanine under standard assay conditions. In view of results reported previously (29), assays were carried out at several pH values to detect any possible trend of variable feedback sensitivity as a function of pH; none was found. Greater than 60% inhibition of PheA PDT occurred with 1.0 mM phenylalanine, whereas 80% inhibition was obtained with 2.0 mM phenylalanine (Fig. 4). In contrast, phenylalanine had no inhibitory effect on either the CM-P (data not shown) or PDT (Fig. 4) activities of PheA*. Prephenate, commonly a product inhibitor of chorismate mutase (28), was not inhibitory to either the PheA or PheA* CM-P activities.

Kinetic analysis (data not shown) revealed that the substrate saturation curves were hyperbolic for both PDT activities of PheA and PheA* but sigmoidal for both CM-P activities of PheA and PheA*. The $S_{0.5}$ of PheA CM-P for chorismate was 0.47 mM and the $K_m$ of PheA PDT for prephenate was 0.25 mM. L-Phenylalanine was a competitive inhibitor for both activities. Moderately increased $S_{0.5}$ and $K_m$ values of 0.81 and 0.33 mM were obtained for the PheA* CM-P and PDT activities, respectively.

The pH optima of PheA CM-P and PDT were compared with those of the PheA* CM-P and PDT. The pH optimum of CM-P for both PheA and PheA* was 6.8 when assayed in 50 mM potassium phosphate buffer containing 1.0 mM dithiothreitol. Under the same conditions a pH optimum of 6.5 was determined for the PDT activities of both PheA and PheA*.

The intrinsic thermal stability of PheA* differed substantially from that of PheA. Although the CM-P component of PheA* was only marginally more resistant than that of PheA to thermal inactivation at 45°C, the PDT component of PheA* was strikingly stable under conditions of thermal inactivation compared with the PDT component of PheA (Fig. 5). The half-life of PheA* PDT was about 8 min, compared with about 2 min for the PheA PDT. In the presence of dithiothreitol the half-lives of PheA CM-P,

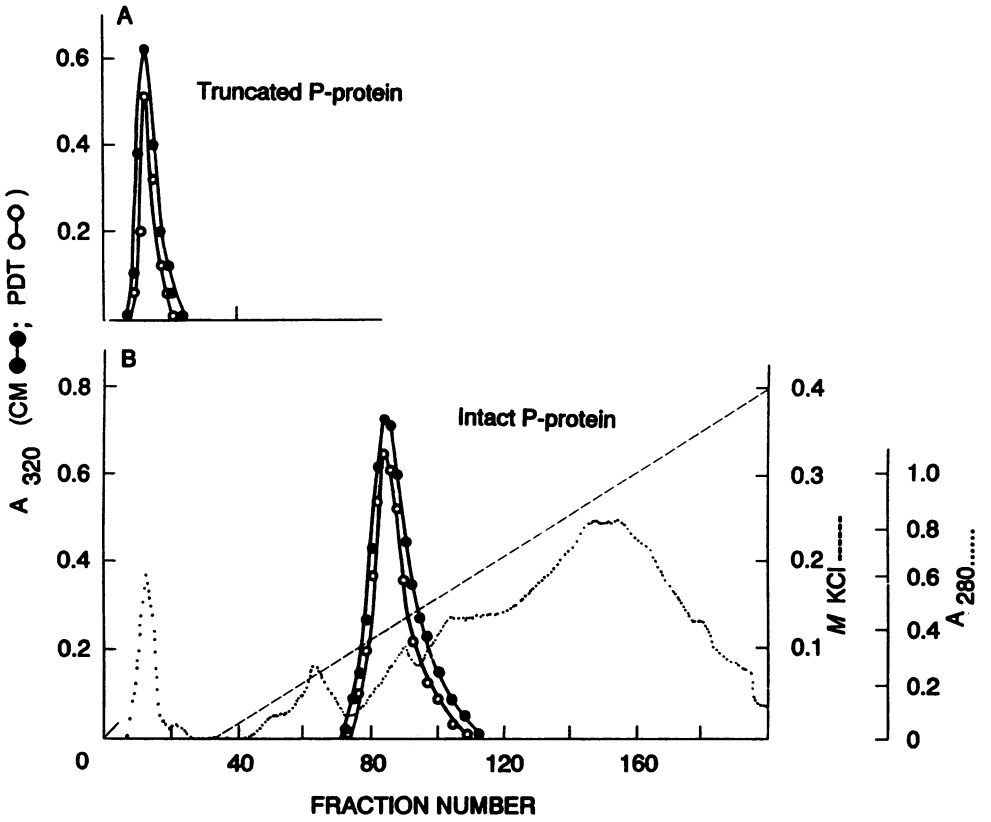


FIG. 3. Comparison of elution profiles of PheA* (A) and PheA (B) after DE52 column chromatography of crude extracts prepared from *E. coli* JP2255 host strains carrying pJX82 and pJX81, respectively. Enzyme activities indicated for chorismate mutase (●) and for PDT (○) were obtained at 37°C from 15 μl of eluate.

PheA* CM-P, and PheA PDT were extended about twofold or less. In contrast, the PheA* PDT was exceptionally stable, retaining about 70% of the starting activity after 30 min at 45°C. A comparable loss of activity for PheA PDT required only about 8 min.

As commonly observed for allosteric effectors, L-phenylalanine was a very effective protectant against thermal inactivation. About 75 to 80% of the starting CM-P and PDT activities of PheA were retained after 30 min at 45°C in the presence of L-phenylalanine (Fig. 5). Such protective effects of L-phenylalanine were not obtained for PheA* activities.

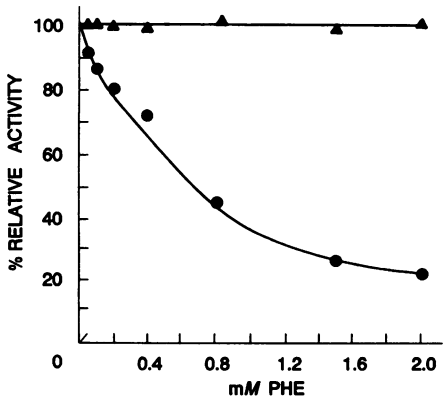


FIG. 4. Inhibition curves comparing the sensitivities of PDT activities of PheA (●) and PheA* (▲) to feedback inhibition by L-phenylalanine. Activity determinations were carried out as described under Materials and Methods. A relative activity of 100% corresponds to 0.047 U of activity.

As expected, *E. coli* KA197(pJX83) harboring *pheA** overproduced L-phenylalanine and excreted it into the growth medium (M9 minimal salts), resulting in 0.43 mM L-phenylalanine after 24 h of growth under the conditions described in Materials and Methods. *E. coli* KA197(pJX83) harboring *pheA* yielded a detectable level of L-phenylalanine (0.10 mM) in the growth medium under the same conditions and duration of culture. Although the PDT was fully sensitive to feedback inhibition in the latter strain, the low level of excretion was undoubtedly due to amplification of enzyme levels because of the plasmid copy number. The qualitatively superior ability of KA197(pJX83) to cross-feed KA197 (pUC18), compared with the ability of KA197(pJX83) to do so, was very obvious by visual inspection of cross-feeding competence on solid medium.

## DISCUSSION

The recent description of a truncated *Taq* DNA polymerase lacking the 5′-to-3′ exonuclease activity and its significance for certain polymerase chain reaction applications exemplifies the emerging value of defined deletions for achievement of objectives in protein engineering (14). The 260 bp deleted from the 3′ portion of *E. herbicola pheA* makes up more than 22% of the *pheA* gene. The chimeric

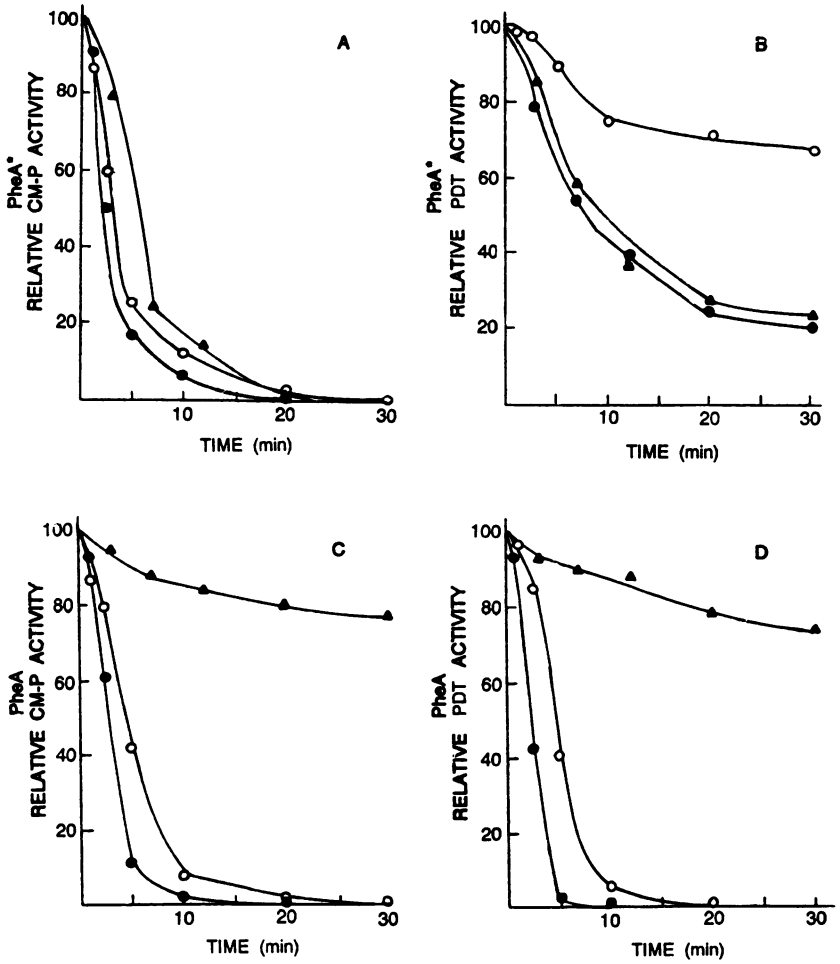


FIG. 5. Comparison of the sensitivities of the chorismate mutase (CM-P) and PDT activities of PheA (C and D) or PheA* (A and B) to thermal inactivation at 45°C in the absence (●) or presence of 1.0 mM dithiothreitol (○) or 1.0 mM L-phenylalanine (▲). Relative activities of 100 correspond to ~0.02 U of activity for CM-P or PDT components of PheA or PheA* in control reactions carried out under standard assay conditions described in Materials and Methods. Activities obtained under conditions of partial feedback inhibition in the presence of L-phenylalanine were normalized to relative activity values, where 100 equals the activity obtained in the absence of L-phenylalanine.

*pheA** was fully competent to form a gene product that can dimerize and carry out the chorismate mutase and PDT activities associated with the bifunctional P protein. In fact, because of the loss of sensitivity to feedback inhibition, strains carrying pJX83 excrete substantial amounts of phenylalanine. The discrete spatial location of the allosteric control region at the carboxy terminus is likely to apply to other P proteins, since alignment of sequenced P proteins (16, 18, 31) reveals high identity throughout the protein.

Baldwin and Davidson (5) found three amino acid residues to be important for the PDT activity of the P protein in *E. coli*: cysteine-216, threonine-278, and cysteine-374. Although these residues have all been conserved in PheA from the closely related *E. herbicola*, the results of this study show that cysteine-374 is in fact not essential for catalysis. In this connection, it is noteworthy that in the alignment of the *Pseudomonas stutzeri* P protein with the *E. coli* P protein, cysteine-216 and threonine-278 have been conserved in the *P. stutzeri* protein but cysteine-374 has undergone a change to serine. Thus, this latter residue must be nonessential for catalysis but capable of influencing the catalytic site at a distance. The ability of missense mutations at this site to disrupt catalytic competence is consistent with properties expected for an allosteric site.

One evolutionary scenario (1) is that an ancestor of *pheA* may have been a duplicate of the gene encoding CDT, which then fused with a duplicate of the gene encoding a monofunctional chorismate mutase (CM-F). If this is correct, then the spatial location of the domain for feedback inhibition by phenylalanine on the carboxy terminus well away from the catalytic site is consistent with acquisition of the phenylalanine-binding domain by a gene fusion event. This seems more likely than evolutionary changes that might have occurred in a nonessential 3′ section of the ancestral gene. Thus, *pheA* may have in its evolutionary history two gene fusion events that created a tripartite gene product with two catalytic domains and one regulatory domain. Additional changes would have been narrowed substrate specificity of PheA and acquisition of attenuation control of *pheA*.

It is intriguing in the latter connection that both CM-F and CDT enzymes from various bacteria inevitably fail to bind the anionic DE52 resin at pH 7.0 (3, 4), indicating that they have high (>7.0) isoelectric points. In contrast, all of the large number of P proteins studied have sufficient negative

charge to bind to DE52 at pH 7.0. In *E. herbicola*, the 87 amino acid residues missing from PheA* resulted in an increase of isoelectric point from pH 6.65 to pH 7.17. Thus, PheA*, unlike PheA, has the charge properties of CM-F and CDT.

Some enzymes have been shown to have sites that are essential for catalysis and feedback inhibition dispersed throughout the protein, e.g., the tryptophan-sensitive 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase of *E. coli* (23). On the other hand, proteins with discrete domains may have certain advantages for biotechnological manipulations. Stable deletions abolishing regulatory function can be acquired without disruption of catalytic competence. In the case of PheA*, the PDT component of the truncated protein exhibited a fortuitous increase in intrinsic stability under conditions of thermal denaturation. This increased stability was further accentuated in the presence of dithiothreitol. Changes that are induced in the physical and catalytic properties of the protein by excision of the regulatory region are not necessarily detrimental. The spatially large nonessential region provides a great deal of room for systematic probing of changes that might indirectly improve catalytic and physical properties.

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