A COMPARISON OF THE AMINO-TERMINAL SEQUENCES OF 3-DEOXY-D-*ARABINO*-HEPTULOSONATE-7-PHOSPHATE SYNTHASE ISOENZYMES FROM *ESCHERICHIA COLI*

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1. Introduction

The first committed step in the biosynthesis of aromatic compounds in bacteria and plants is the condensation of phosphoenolpyruvate and erythrose-4-phosphate to form 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (DAHP) and P_i [1]. This reaction is catalyzed by DAHP synthase [7-phospho-2-keto-3-deoxy-D-*arabino*-heptonate D-erythrose-4-phosphate lyase (pyruvate phosphorylating) EC 4.1.2.15]. In *Escherichia coli* there are 3 DAHP synthase isoenzymes [2]: phenylalanine-sensitive DAHP synthase (PHE); tyrosine-sensitive DAHP synthase (TYR); tryptophan-sensitive DAHP synthase (TRP). All 3 isoenzymes have been isolated as apparently homogeneous preparations [3–5], and some of their molecular parameters have been described [3–8].

While one expects extensive structural similarities for isoenzymes, especially when they are isolated from a unicellular organism, a comparison of the *E. coli* DAHP synthases reveals some strikingly different molecular parameters. DAHP synthase (PHE) is a tetramer of 4 identical subunits of M_r 35 000 [3], the other 2 DAHP synthases are dimeric protein with subunit M_r 39 000 [4,5]. Precipitating rabbit antibodies raised against pure DAHP synthase (PHE) or (TYR) do not crossreact with the other 2 isoenzymes [7,9]. The lack of cross reactivity among the 3 isoenzymes points to large structural differences of these proteins. To show precisely the extent of these differences we have undertaken the primary structure analysis of DAHP synthase. We report here the amino-terminal sequence of the 3 isoenzymes.

2. Materials and methods

Phosphoenolpyruvate [10] and erythrose-4-phosphate [11] were synthesized and assayed as in [12]. All other chemicals were obtained commercially in the highest purity available and were used without further purification.

DAHP synthase (PHE) was isolated from extracts of *E. coli* K12 strain HE401 [3]. DAHP synthase (TYR) was isolated from extracts of strain HE102/pKB45 [4]. This strain was obtained through transformation of strain HE102 [4] with plasmid pKB45 [13]. DAHP synthase (TRP) was isolated [5] from extracts of strain 37-1/pJS01. This strain was obtained by transformation with plasmid pJS01 [5] of strain 37-1, a *trpR* deletion mutant [14]. Plasmid pJS01 is a pBR322 [15] derivative that carries a 6.8 kilobase *Eco*R1 fragment of plasmid pKB23 [16] containing *aroH*. Plasmids pKB45 and pKB23 were kindly provided by Dr K. D. Brown. The construction of all plasmid carrying strains were performed under the National Institutes of Health guidelines for recombinant DNA technology.

The enzymes were carboxymethylated with iodoacetic acid [17] and subjected to sequence analysis without fragmentation. Automated amino acid sequencing was performed on a Beckman Sequencer, model 890, as in [18]. Sequenator products were identified by high-pressure liquid chromatography. The molar amounts of products observed in the early cycles were >60% of the molar amounts of the protein degraded. No identifications were made with a peak/background ratio <2.

3. Results and discussion

Fig.1 shows the sequences of the first 40 amino acid residues for the 3 *E. coli* DAHP synthase isoenzymes. The boxes indicate common residues. Homology

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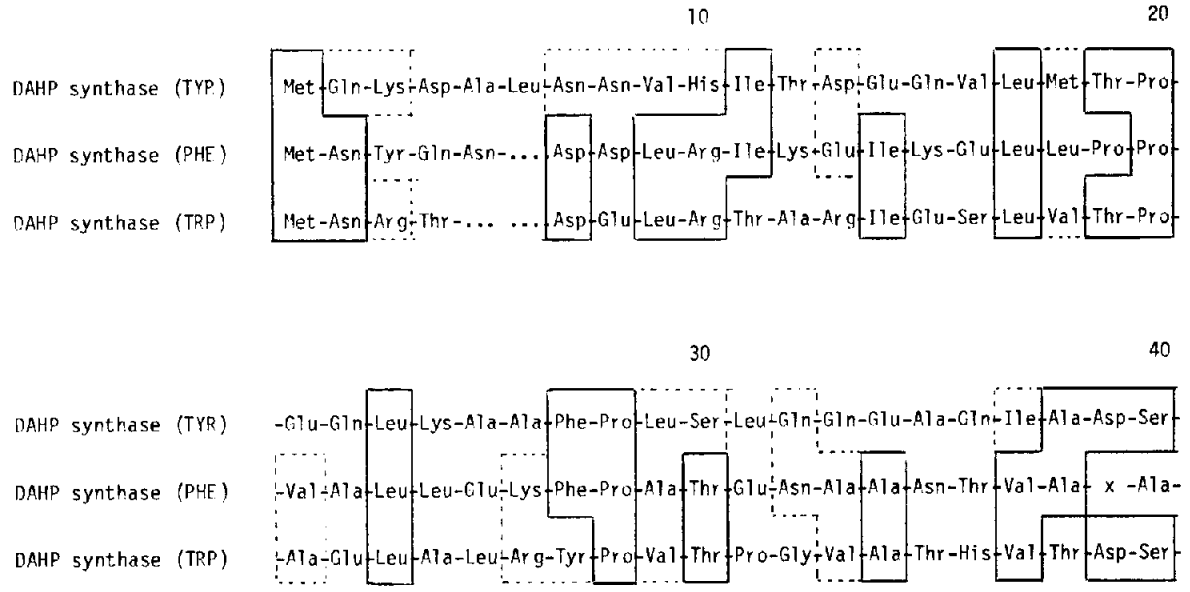

Fig.1. Amino-terminal sequences of DAHP synthase isoenzymes from *E. coli*. Invariant residues are enclosed by solid line boxes; dashed line boxes enclose residues of similar chemical character; (. . .) one residue deletions introduced to maximize homology; (X) an unknown residue.

is maximized through introduction of a one-residue deletion in position 6 for DAHP synthase (PHE) and a two-residue deletion for positions 5–6 of DAHP synthase (TRP). Even with these adjustments, only 5 of 40 residues are common to all 3 sequences: Met_1, Leu_{17}, Pro_{20}, Leu_{22}, Pro_{28}.

However, when one compares sequences of isoenzyme pairs the degree of similarity increases somewhat. DAHP synthase (TYR) has 8 residues in common with DAHP synthase (PHE): Ile_{11}, Phe_{27} and Ala_{38}, and the 5 residues common to all 3 sequences. DAHP synthase (TYR) also has 8 residues in common with DAHP synthase (TRP): Thr_{19}, Asp_{39} and Ser_{40}, and the 5 residues common to all 3 sequences. DAHP synthase (PHE) and DAHP synthase (TRP) have 13 of 40 common residues. Thus, the 3 pairs have 20%, 20% and 32.5% identical residues in the first 40 residues, respectively. This indicates that the proteins are only distantly related.

All 3 isoenzymes contain iron as an integral part of the polypeptide chain [6]. Through sequence comparison [8] with hemerythrin, the iron-containing oxygen carrier in the hemolymph of sea worms, His_{10} and Glu_{14} of DAHP synthase (TYR) have been suggested to be part of the iron-binding site. These two residues are not common to all 3 isoenzymes. The phenyl-

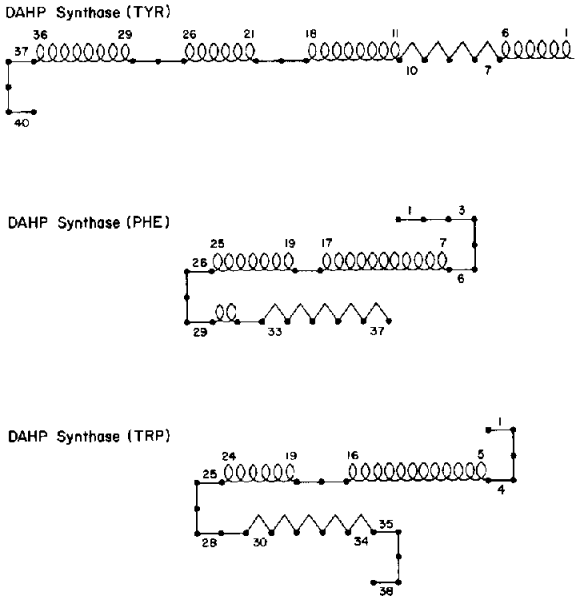

Fig.2. Secondary structures for the amino-termini of DAHP synthase isoenzymes from *E. coli*, predicted according to [19] as coil or turn (•—•), α-helix (ꕥ) or β-sheet (•⟋⟍•). Numbers mark boundary residues. Since no deletions are introduced for DAHP synthase (PHE) and (TRP), the numbers are not identical to all residue numbers given for these polypeptides in fig.1.

alanine-sensitive and the tryptophan-sensitive isoenzymes both have Arg in position 10 and Ile in position 14, indicating either different ligands for the iron binding sites or an altogether different distribution of the iron ligands through the polypeptides. From our primary structural analysis of the amino terminal portion of the *E. coli* DAHP synthase isoenzymes we conclude that the phenylalanine-sensitive and the tryptophan-sensitive isoenzymes are more closely related to each other than either one is to the tyrosine-sensitive isoenzyme.

Secondary structure predictions [19] suggest a fair resemblance of DAHP synthase (PHE) and DAHP synthase (TRP), with less similarity to the third isoenzyme (fig.2). This confirms that the first two isoenzymes are more closely related than either is to the third one. However, common secondary structures are also predicted for the 3 isoenzymes; most convincing is the α-helix starting at residue 21 or 19 with $\langle P_\alpha \rangle$ propensities of 1.31, 1.16, 1.29 and $\langle P_\beta \rangle$ propensities of 0.86, 0.97, 0.93 for the DAHP synthase (TYR), (PHE) and (TRP), respectively. Given the weak amino acid sequence homology and the many instances of similar amino acid character where identities do not occur (fig.1), and given the substantial similarity between the secondary structural predictions for DAHP synthase (PHE) and (TRP) and weaker similarity for the tyrosine-sensitive isoenzyme, we suggest that the enzymes arose by divergence of a common ancestral gene specifying a DAHP synthase. Further support of this hypothesis will come from a more detailed comparison of the 3 isoenzymes, which will have to await completion of total sequence analysis that is under way in our laboratory.

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