JOURNAL OF BACTERIOLOGY, Jan. 1998, p. 119–127 Vol. 180, No. 1
0021-9193/98/$04.00+0

Substrate Ambiguity of 3-Deoxy-D-*manno*-Octulosonate 8-Phosphate Synthase from *Neisseria gonorrhoeae* in the Context of Its Membership in a Protein Family Containing a Subset of 3-Deoxy-D-*arabino*-Heptulosonate 7-Phosphate Synthases†

PREM S. SUBRAMANIAM, GANG XIE, TIANHUI XIA, AND ROY A. JENSEN*

Department of Microbiology and Cell Science, University of Florida, Gainesville, Florida 32611

Received 6 August 1997/Accepted 23 October 1997

3-Deoxy-D-*manno*-octulosonate 8-phosphate (KDOP) synthase and 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthase catalyze similar phosphoenolpyruvate-utilizing reactions. The genome of *Neisseria gonorrhoeae* contains one gene encoding KDOP synthase and one gene encoding DAHP synthase. Of the two nonhomologous DAHP synthase families known, the *N. gonorrhoeae* protein belongs to the family I assemblage. KDOP synthase exhibited an ability to replace arabinose-5-P with either erythrose-4-P or ribose-5-P as alternative substrates. The results of periodate oxidation studies suggested that the product formed by KDOP synthase with erythrose-4-P as the substrate was 3-deoxy-D-*ribo*-heptulosonate 7-P, an isomer of DAHP. As expected, this product was not utilized as a substrate by dehydroquinate synthase. The significance of the ability of KDOP synthase to substitute erythrose-4-P for arabinose-5-P is (i) recognition of the possibility that the KDOP synthase might otherwise be mistaken for a species of DAHP synthase and (ii) the possibility that the broad-specificity type of KDOP synthase might be a relatively vulnerable target for antimicrobial agents which mimic the normal substrates. An analysis of sequences in the database indicates that the family I group of DAHP synthase has a previously unrecognized membership which includes the KDOP synthases. The KDOP synthases fall into a subfamily grouping which includes a small group of DAHP synthases. Thus, family I DAHP synthases separate into two subfamilies, one of which includes the KDOP synthases. The two subfamilies appear to have diverged prior to the acquisition of allosteric-control mechanisms for DAHP synthases. These allosteric control specificities are highly diverse and correlate with the presence of N-terminal extensions which lack homology with one another.

3-Deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) and 3-deoxy-D-*manno*-octulosonate 8-phosphate (KDOP) are analogous seven- and eight-carbon 2-keto-3-deoxy sugars that are synthesized by enzymes which belong to functionally unrelated pathways. DAHP synthase forms DAHP as the acyclic precursor of the aromatic amino acids in bacteria, lower eukaryotes, and plants (3); KDOP synthase is best known for its role in the formation of KDOP as a critical component of the lipopolysaccharide of gram-negative bacteria (37), but its distribution in nature has recently been recognized to be broader (13). Both enzymes catalyze an overall condensation of phosphoenolpyruvate (PEP) with an aldose, i.e., erythrose-4-phosphate (E4P) in the case of DAHP synthase and arabinose-5-phosphate (A5P) in the case of KDOP synthase. The reactions are irreversible and are not aldol-type condensations, which unfortunately has been implied by the Enzyme Commission naming that has been recommended for DAHP synthase.

As might be expected from the close structural relationship of A5P and E4P, the reactions are strikingly similar. This similarity is reflected at the level of mechanistic detail (see reference 16 and references therein). DAHP synthase and KDOP synthase, along with enolpyruvoylshikimate 3-phosphate synthase and UDP-*N*-acetylglucosamine enolpyruvoyl transferase, comprise a small class of PEP-utilizing enzymes that catalyze C—O bond cleavage with respect to the release of P_i from PEP (1, 27). This contrasts with the more familiar nucleophilic attack at the phosphorous atom of PEP that results in P—O bond cleavage by the action of enzymes such as pyruvate kinase (25), PEP carboxylase (34), and PEP carboxykinase (8).

In classical studies with *Escherichia coli*, DAHP synthase (44, 45) and KDOP synthase (41) are specific for E4P and A5P, respectively. In contrast, we found that the KDOP synthase of *Neisseria gonorrhoeae* possessed the ability to utilize E4P in place of A5P. We addressed the question of whether KDOP synthase of *N. gonorrhoeae* in the presence of E4P and PEP was able to form DAHP, in which case it would also have the potential to function as a DAHP synthase. The time-dependent cleavage of the product was investigated by the periodate-oxidation-thiobarbituric acid (TBA) assay, and these results allow some speculation on the stereospecific course of the reaction in comparison with the reaction of DAHP synthase.

MATERIALS AND METHODS

Terminology. It is becoming increasingly awkward, especially when multiple molecular-genetic comparisons are under way, to use acronyms whose meanings are different for different organisms. Therefore, we employ the following definitions. *aroA* encodes monofunctional DAHP synthase, appropriately designated because this catalyzes the first reaction of aromatic biosynthesis. Unique feed-

* Corresponding author. Mailing address: Department of Microbiology and Cell Science, University of Florida, Gainesville, FL 32611. Phone: (352) 392-9677. Fax: (352) 392-5922. E-mail: rjensen@micro.ifas.ufl.edu.

† This paper is Florida Agricultural Station Series no. R-05902.

back inhibition specificities are designated by subscripts corresponding to the appropriate amino acid symbols. Thus, the three *E. coli* paralogs currently known as AroF, AroG, and AroH are denoted $AroA_Y$, $AroA_F$, and $AroA_W$, respectively. The species inhibited by both L-phenylalanine and L-tyrosine is denoted $AroA_{FY}$. •*aroA* encodes the DAHP synthase domain of the bifunctional chorismate mutase: DAHP synthase (AroG•AroA). The convention of using a bullet to separate the potentially independent domains of a fusion protein (or their coding regions in the gene) is according to the precedent of Crawford with tryptophan pathway fusions (10).

Data analysis. An updated version of the sequence analysis software offered by the Genetics Computer Group (GCG) (21) was used. The multiple alignment presented in Fig. 7 required some manual alignment. The GCG PILEUP program was used to align members of subfamily Iα and class $I\beta_D$ (see Fig. 6). Class $I\beta_K$ members were then manually added to the alignment, which was guided by use of a multiple alignment generated for subfamily Iβ alone by the PILEUP program.

Chemicals and biochemicals. E4P was prepared according to procedure B as described by Ballou (2), except that the monosodium salt of glucose-6-phosphate was used and the sulfuric acid treatment of the salt was omitted. The concentration of E4P was estimated by using a partially purified preparation of DAHP synthase from *N. gonorrhoeae* ATCC 27630. Dehydroquinate was synthesized according to the procedure of Haslam et al. (26). The product was obtained as an oil that could be dried to an extremely hygroscopic solid but could not be crystallized. The free acid was dissolved in water (to a concentration of 175 mM), the pH was adjusted to 7.0, and the solution was stored at −80°C. It was found to contain ~8% of dehydroshikimic acid by its A_{234}.

DAHP was isolated from the culture supernatant of an overproducing mutant of *E. coli* according to the procedure of Mehdi et al. (36). The mutant strain *E. coli* JB5 was a gift from Jeremy Knowles (Harvard University).

The trisodium salt of PEP was purchased from Sigma Chemical Co. (St. Louis, Mo.). Dithiothreitol (DTT) was purchased from Research Organics, Inc. (Cleveland, Ohio). Hydroxylapatite (Bio-gel HTP) was purchased from Bio-Rad (Rockville Centre, N.Y.). Bio-gel A (0.5 m, 100 to 200 mesh; exclusion limit, 500,000 Da) was purchased from Bio-Rad as a fully hydrated suspension in 10 mM Tris-EDTA buffer. This buffer was replaced with appropriate buffers after packing of the column.

Bacterial strains, media, and growth conditions. *N. gonorrhoeae* 2013 (ATCC 27630) is a clinical isolate which is auxotrophic for proline and resistant to growth inhibition by L-phenylalanine (4). Strain 2011 (ATCC 27628) is a clinical isolate which is prototrophic and sensitive to growth inhibition by L-phenylalanine (4). These strains were grown in the minimal medium formulated by Hendry (28). Growth at 525 nm was monitored by measuring the optical density of a 5-ml sample of the culture at regular intervals. Supplementation of media to appropriate concentrations with required components was done through a sterilizing membrane after the complete medium was made. Cells in the late-exponential phase of growth were harvested by centrifugation at 4°C, washed once with buffer A (20 mM K-phosphate at pH 7.0 containing 1.0 mM DTT), and stored at −89°C until used.

The source and cultivation of *Erwinia herbicola* (53) and *Pseudomonas acidovorans* (48) were as referenced.

Preparation of crude extracts. Cells were resuspended by thawing the frozen cells in buffer B (100 mM K-phosphate at pH 7.0 containing 1.0 mM DTT) at room temperature. A buffer-to-cell ratio of 2:1 (vol/wt) was used. The cells were broken in a French pressure cell at 16,000 lb/in^2. The suspension was centrifuged at 150,000 × *g* for 35 min, and the supernatant was collected as the crude extract. Extracts were desalted by passage through an Econo Pac 10DG (Bio-Rad) desalting column. All operations were conducted at 4°C.

Hydroxylapatite chromatography. A 75-mg amount of the desalted crude extract in buffer A was loaded on a column (1.5 by 20 cm) of hydroxylapatite equilibrated in buffer A. After the unbound protein was washed with 2 bed volumes of buffer A, adsorbed protein was eluted with a linear gradient between buffer A and 300 mM K-phosphate at pH 7.0 (containing 1.0 mM DTT) in a total volume of 340 ml. Fractions of 2.2 ml were collected. For KDOP synthase, the band within the two peak tubes exhibited a specific activity of 174.5 nmol/min/mg, compared to a specific activity of 3.1 nmol/min/mg in crude extract. This represents a purification factor of 563.

Enzyme assays. DAHP synthase was assayed as described by Jensen and Nester (30). Standard reaction mixtures contained 1.5 mM PEP, 1.0 mM of E4P, and 0.5 mM $MnSO_4$, and appropriate aliquots of enzyme, which were incubated at 37°C for 20 min. Activity was expressed as A_{549}, where a value of 1.0 corresponds to 0.3 mM DAHP formed. An ε value of $5.0 \times 10^4\ M^{-1}\ cm^{-1}$ was used (36). KDOP synthase was assayed and activities were quantitated as specified by Ray (41). In some experiments, we found it convenient to assay KDOP synthase in crude extracts. In such preparations, DAHP synthase activity could be reduced to less than 3% by omission of $MnSO_4$ and inclusion of 5 mM L-phenylalanine.

Dehydroquinate synthase was assayed by monitoring the disappearance of substrate DAHP by the TBA assay method for DAHP synthase. The reaction mixtures contained 0.3 mM DAHP, 50 μM NAD^+, and 0.5 mM Co^{2+} and were incubated at 37°C for 20 min. In some experiments, partially purified DAHP synthase from *N. gonorrhoeae* was used to generate DAHP. A DAHP synthase reaction mixture containing 0.8 mM PEP, 1.0 mM E4P, 0.5 mM $MnSO_4$, and an aliquot of DAHP synthase was prepared. Catalysis was conducted at 37°C until a DAHP yield equivalent to an A_{549} value of 1.0 to 1.5 was obtained. The DAHP synthase was destroyed by heating to 60°C. The formation of the product, dehydroquinate, was verified with a highly purified preparation of a bifunctional dehydroquinate dehydratase•shikimate dehydrogenase from *Nicotiana silvestris* (6).

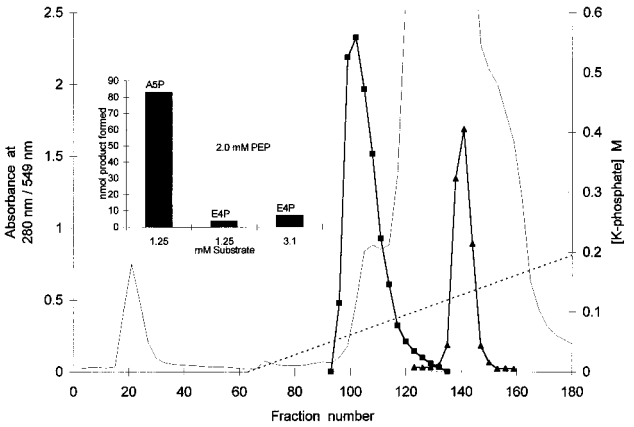


FIG. 1. Separation of DAHP synthase and KDOP synthase by chromatography of crude extracts of *N. gonorrhoeae* VHC 3102 on hydroxylapatite. A 100-mg amount of crude extract prepared as described in Materials and Methods was loaded on a column (1.5 by 23 cm) equilibrated with 20 mM K-phosphate (pH 7.0) containing 1.0 mM DTT (buffer A). After washing with 2 bed volumes of buffer A, absorbed proteins were eluted by application of a linear gradient between buffer A and 300 mM K-phosphate (pH 7.0, 1.0 mM DTT). DAHP synthase and KDOP synthase were assayed as described in Materials and Methods. The reaction mixtures contained 1.0 mM E4P/A5P, 1.0 mM PEP, and 0.5 mM Mn^{2+}. Enzyme activities are reported at A_{549}. Protein was monitored as A_{280}. Dashed line, progress of the gradient. The relative abilities of fraction 104 to use E4P and A5P at 2 mM PEP are shown in the inset. The amounts of product plotted on the ordinate scale were calculated by using an ε value of $1.03 \times 10^4\ M^{-1}\ cm^{-1}$ for the KDOP-derived chromophore and assuming an ε value equal to that for DAHP ($5.0 \times 10^4\ M^{-1}\ cm^{-1}$) for the E4P-derived product.

Cloning of $aroA_W$ from *E. herbicola*. The gene $aroA_W$ from *E. herbicola* was cloned by selection for the ability of amplified $AroA_W$ to suppress the nutritional requirement of a leaky *pheA* phenylalanine auxotroph of *E. coli* by the general methodology described by Xia et al. (53).

Nucleotide sequence accession number. The sequence for *E. herbicola* $aroA_W$ has been assigned GenBank accession no. U93355.

RESULTS

Relaxed substrate specificity of the KDOP synthase reaction. Figure 1 shows the activity profiles for DAHP synthase and KDOP synthase after fractionation of crude extracts of *N. gonorrhoeae* VHC 3102 on hydroxyapatite. KDOP synthase eluted ahead of the major protein eluate, thus yielding an excellent partial purification. When fractions under the KDOP synthase peak were assayed under conditions used to detect the DAHP synthase band (1 mM E4P), an activity just marginally above the background was detected. The substrate ambiguity of KDOP synthase was verified by more detailed work with the peak fractions. Accuracy was maximized by use of a longer assay duration (30 min) and use of saturating concentrations of E4P (3.1 mM). The inset of Fig. 1 shows a comparison of the activities of the peak fraction when assayed with 1.25 mM A5P, 1.25 mM E4P, and 3.1 mM E4P. The amount of the product formed with E4P was maximal at 3.1 mM E4P and equaled as much as 8% of the product formed with saturating concentrations of A5P (at a fixed concentration of 2 mM PEP). A standard periodate oxidation time of 30 min was used. No activity was seen with D- or L-glyceraldehyde-3-phosphate (1.5 mM). However, D-ribose-5-phosphate (3 mM), an isomer of A5P, was utilized to the same extent as E4P. Additional confirmation of substrate ambiguity was obtained by demonstrat-

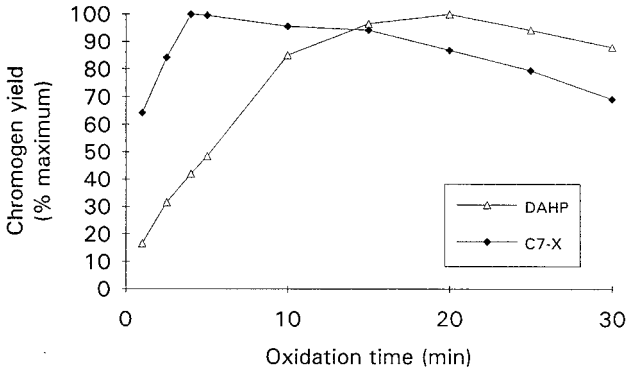


FIG. 2. Kinetics of periodate oxidation of the enzymatic products of KDOP synthase and DAHP synthase reactions with E4P and PEP. Products were accumulated as described in the text, and a 100-μl sample was used in the periodate-oxidation-TBA assay procedure. The oxidation was terminated at the specified time intervals. Maximal A_{549} values in each case were assigned a relative value of 100% and corresponded to 0.721 for DAHP and 1.482 for the E4P/KDOP synthase-derived product (C_7-X).

ing that E4P inhibited the KDOP synthase reaction with A5P in a competitive fashion.

The stereochemistry of the resultant C_7 product. Periodate oxidation provides a simple method to examine the relative configuration of the hydroxyl groups at C-4 and C-5 in 2-keto, 3-deoxy sugars such as DAHP and KDOP. The rate of chromophore formation measured by the TBA assay is directly proportionate to the ease of oxidative cleavage at C-4–C-5 (23); upon C-4–C-5 cleavage, 2-keto-3-deoxy-sugars yield the TBA-reactive product β-formylpyruvate. KDOP is rapidly oxidized by periodate as a consequence of the *cis* orientation of the C-4 and C-5 hydroxyl groups (23), in contrast to the much slower oxidation encountered when the *trans* configuration of these hydroxyls occurs, as seen in DAHP. The orientation of the C-4 hydroxyl in KDOP and DAHP is dictated by the stereochemistry of the addition across the face of the carbonyl group in the substrate sugar. In the case of both KDOP synthase and DAHP synthase, the orientation of the hydroxyl generated by the addition of PEP is such that C-4 has an (R) configuration in the products KDOP and DAHP.

In order to accumulate sufficient amounts of the C_7 product derived from the use of E4P by KDOP synthase (denoted C_7-X), the reaction was scaled up as follows. A 1-ml reaction mixture containing 3.6 mM E4P, 2 mM PEP, 1 mM DTT, 0.5 mM Mn^{2+}, and 375 μg of partially purified KDOP synthase in 75 mM potassium phosphate buffer (pH 7.0) was incubated at 37°C for 5 h. The reaction mixture was then transferred to 4°C and stored overnight. A 40-μl amount of 100% (wt/vol) trichloroacetic acid was added, mixed, and retained on ice for 5 min. The precipitated protein was removed by centrifugation, and the supernatant was diluted twofold with buffer and used for oxidation studies.

DAHP was accumulated in a similar 1-ml reaction mixture, except that it contained 1 mM E4P, 1 mM PEP, and 940 μg of crude extract from *Pseudomonas testosteroni* (ATCC 17409) incubated at 37°C for 20 min; the supernatant was diluted twofold with buffer before use. A 100-μl sample of each supernatant was used. A concentration corresponding to 0.35 mM DAHP was formed.

Figure 2 shows a comparison of the kinetics of periodate oxidation of the C_7-X product and authentic DAHP. It is evident that the C_7-X product is rapidly oxidized in comparison to DAHP, with the oxidation being complete within 4 min. Almost 65% of chromophore from C_7-X was released within 1 min of the oxidation (cf. 17% for DAHP). These results exactly parallel those of Doong et al. (13), who compared the rates of periodate oxidation of KDOP and DAHP under identical conditions. The C_7-X product behaves like authentic KDOP or KDO, i.e., maximal oxidization occurs within 5 min of incubation. Thus, the relative configuration of the hydroxyls at C-4–C-5 in the predominant C_7-X product must be *cis*. It follows that C_7-X is the 4(*S*)-isomer, unlike 4(*R*)-KDOP and 4(*R*)-DAHP. The product is accordingly concluded to be the D-*ribo*-analog of DAHP, i.e., 3-deoxy-D-*ribo*-heptulosonate 7-phosphate. Since DAHP is not formed, these results eliminate the possibility that the E4P-utilizing activity might have been due to the presence of a minor DAHP synthase species having a coincident elution profile with KDOP synthase.

The C_7-X product was tested as a substrate for dehydroquinate synthase, the enzyme that uses DAHP as a substrate to form dehydroquinate. Figure 3 shows the elution profile of *N. gonorrhoeae* dehydroquinate synthase from hydroxylapatite. The enzyme was assayed by monitoring the disappearance of DAHP by the TBA assay. The C_7-X product accumulated as described above was incubated with a partially purified preparation of dehydroquinate synthase for 20 min in a reaction mixture containing 0.5 mM Co^{2+} and 50 μM NAD^+. The C_7 product was not utilized as substrate (Fig. 3, insert). As a positive control, the enzyme was incubated with authentic DAHP (Fig. 3, insert). Under the same conditions used for C_7-X, almost 90% of the DAHP was utilized. Thus, the C_7-X product is not a substrate for dehydroquinate synthase, consistent with its likely identity as 3-deoxy-D-*ribo*-heptulosonic acid 7-phosphate. Absolute proof of product identity requires nuclear magnetic resonance and mass spectral analysis.

DISCUSSION

Mechanism of 3-deoxy-D-*ribo*-heptulosonate 7-phosphate acid formation by KDOP synthase. It was surprising to identify the D-*ribo* stereoisomer as the probable product of E4P utilization by KDOP synthase in view of the similarity of its reaction mechanism to that of the well-studied DAHP synthase

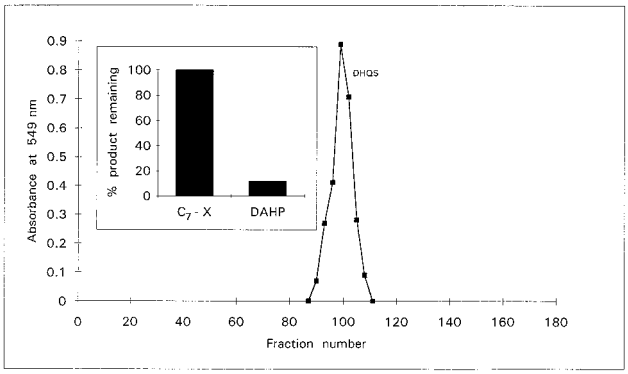


FIG. 3. The reaction of C_7-X with *N. gonorrhoeae* dehydroquinate synthase. Dehydroquinate synthase activities are profiled as eluted off the column described in the legend to Fig. 1. Enzyme activities are reported for simplicity as A_{549}. Note that the values actually represent ΔA_{549} where a value of 1.0 corresponds to 48.0 nmol of DAHP consumed. The insert shows the fraction of product remaining after incubation of C_7-X and DAHP with dehydroquinate synthase as described in the text. A value of 100% corresponds to 0.24 mM DAHP and 0.1 mM DAHP equivalents of C_7-X.

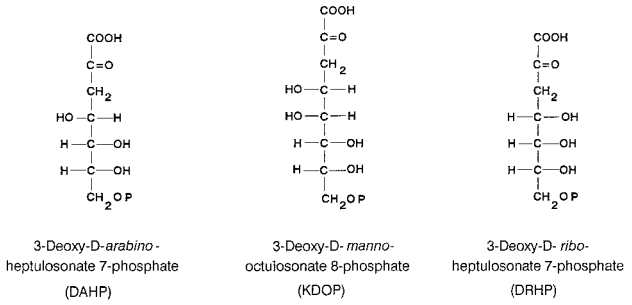


FIG. 4. Chemical structures of DAHP, KDOP, and DRHP.

(12, 19). The condensation of E4P and PEP by DAHP synthase proceeds by a stereospecific addition across the *si* face of C-3 of PEP and the *re* face of C-1 of E4P (18). The resulting DAHP has an (*R*) configuration at C-4 (Fig. 4 and 5), with the newly formed hydroxyl being in *trans* to the C-5 hydroxyl. In the case of the KDOP synthase reaction, based on the established structure of KDOP, the same stereochemistry, 4(*R*), is generated at C-4 and the reaction most likely proceeds with the same stereospecificity as that of the DAHP synthase reaction. The configuration of the substrate (A5P) disposes the C-5 in *cis* to the newly formed hydroxyl at C-4. To form the 4(*S*)-isomer, as occurs when 3-deoxy-D-*ribo*-heptulosonic acid is formed by *N. gonorrhoeae* KDOP synthase with E4P, the addition should proceed across the *si* face of C-3 of PEP and the *si* face of C-1 of E4P. E4P is presumably accommodated at the active site such that the C-1 carbonyl presents the face opposite to that across which PEP adds in E4P in the DAHP synthase reaction and across which PEP adds in A5P in the true KDOP synthase reaction. It is assumed that the *si* face of PEP is consistently presented in all of these reactions, since this appears to prevail in other reactions involving PEP, including those that do not involve C—O bond cleavage (42). The D-*ribo* product appears to be the predominant product of *N. gonorrhoeae* KDOP synthase when offered PEP and E4P. This KDOP synthase thus lacks the ability to function as a DAHP synthase. Whether the D-*ribo* product might be produced in vivo for some unknown function, as implied by the rather broad distribution of 2-keto, 3-deoxy sugars in nature (35), is an intriguing possibility for future discovery.

Substrate ambiguity and reversed modes of stereospecificity. A precedent exists in which another species of KDOP synthase exhibits broad substrate specificity. KDOP synthase from spinach (13) was able to use E4P as a substrate 24% as well as A5P. It also catalyzed the condensation of PEP and E4P to the D-*ribo* product, the reaction thus proceeding with the opposite stereochemistry performed in condensing PEP and A5P to form KDOP. The *N. gonorrhoeae* and higher-plant KDOP synthases are thus far the only reported broad-specificity KDOP synthases.

While most DAHP synthase enzymes are relatively specific (to the extent that these characterizations have been made), one species exhibits a remarkable relaxation of substrate specificity. A cytosolic enzyme (denoted DS-Co) present in most if not all higher plants (14, 15) is able to condense PEP with an array of aldoses that include the phospho and dephospho counterparts of glycolaldehyde, glyceraldehyde, erythrose, threose, arabinose, ribose, and glucose. The efficiency of catalysis, as defined by the V_{max}/K_m ratio, was highest with glycolaldehyde, E4P, or glyceraldehyde-3-P. Whereas E4P yielded a *trans* C-4–C-5 product, glyceraldehyde-3-P and A5P produced *cis* C-4–C-5 products. The higher-plant DAHP synthase (DS-Co) thus has a minor capability to function as a KDOP synthase. In contrast, the chloroplast-localized DAHP synthase (denoted DS-Mn) exhibits narrow substrate specificity (20).

2-Keto, 3-deoxy sugars are common in the cell wall, lipopolysaccharide, and extracellular polysaccharides of bacteria (35), but their biosynthetic routes are not known. It would be logical to envisage their synthesis by reactions analogous to those of DAHP synthase and KDOP synthase. From an evolutionary standpoint, given the existence of the broad catalytic ability to condense PEP and aldoses, selection for specific function could occur at two levels: one at the level of the stereospecificity of the reaction which has a fourfold freedom and the other at the level of specificity for the appropriate sugar substrates.

Gene families encoding AroA proteins. The current database contains two distinct classes of sequenced genes that specify DAHP synthase. Walker et al. (49) defined type I DAHP synthases as "*E. coli*-like" homologs having a subunit M_r of about 39,000, while type II DAHP synthases were defined as "plant-like" homologs having a subunit M_r of about 54,000. Our analysis shows that the type I DAHP synthases belong to a family which further subdivides into two subfamilies, as illustrated by the dendrogram presented in Fig. 6. Subfamily Iα consists entirely of DAHP synthase proteins, whereas the previously unrecognized subfamily Iβ contains one group of DAHP synthase proteins (class $I\beta_D$) and one group of KDOP synthase proteins (class $I\beta_K$). Proteins within subfamily Iβ are 28.5 to 30.8 kDa, which is smaller than their subfamily Iα counterparts. The levels of overall identities between individual members of Iα and $I\beta_D$ (e.g., 18% for *E. coli* $AroA_F$ and *Synechocystis* sp. $AroA_F$) or between individual members of Iα and $I\beta_K$ (e.g., 17% for *E. coli* $AroA_F$ and *E. coli* KdsA) are sufficiently low that homology is not apparent. However, the multiple alignment given in Fig. 7 indicates that all three groups comprise a family of homologs which share 17 invariant residues. Even though members of class $I\beta_D$ are functionally equivalent to members of subfamily Iα in that they catalyze the DAHP synthase reaction, they exhibit greater overall similarity to members of class $I\beta_K$, which catalyze the closely related KDOP synthase reaction. The class $I\beta_D$ DAHP synthases possess 17 residues that are conserved with subfamily Iα DAHP synthases, but not with class $I\beta_K$ KDOP synthases. These res-

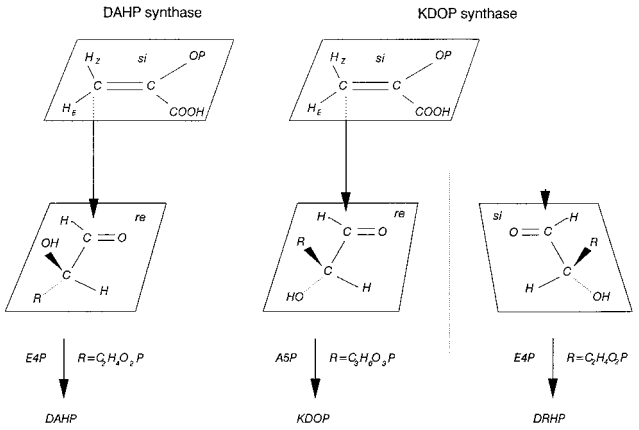


FIG. 5. Representation of the mechanism of formation of DAHP by DAHP synthase, KDOP by KDOP synthase and 3-deoxy-D-*ribo*-heptulosonate 7-phosphate (DRHP) by KDOP synthase. The mechanism for the formation of DRHP is hypothetical. In the case of the formation of DRHP, it is assumed that the addition is across the *si* face of PEP by analogy.

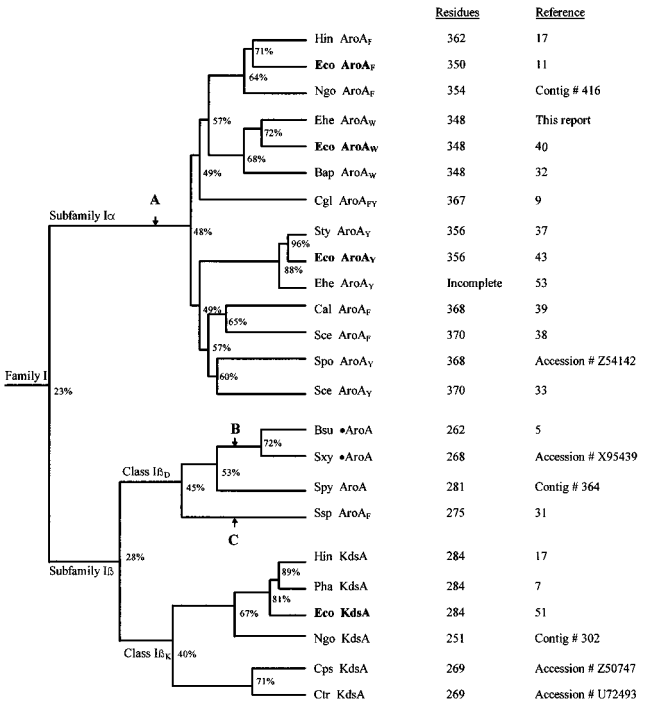


FIG. 6. Dendrogram (PILEUP) showing family I relationships. Averaged percent identities of deduced amino acid sequences are indicated at the node positions. The four *E. coli* paralogs are highlighted in boldface. Abbreviations: Hin, *Haemophilus influenzae*; Eco, *E. coli*; Ngo, *N. gonorrhoeae*; Ehe, *E. herbicola*; Bap, *B. aphidicola*; Cgl, *C. glutamicum*; Sty, *Salmonella typhimurium*; Cal, *Candida albicans*; Sce, *S. cerevisiae*; Spo, *Schizosaccharomyces pombe*; Bsu, *B. subtilis*; Sxy, *S. xylosus*; Spy, *Streptococcus pyogenes*; Ssp, *Synechocystis* sp.; Pha, *Pasteurella haemolytica*; Cps, *Chlamydia psittaci*; and Ctr, *Chlamydia trachomatis*. Hypothetical evolutionary events included acquisition of a binding pocket for aromatic amino acids (A), fusion of genes encoding an unregulated AroA (carboxy terminal) and chorismate mutase (N terminal) (B) and acquisition of an N-terminal domain specifying allosteric control by L-phenylalanine (C).

idues may, therefore, be important for specificity relationships which dictate E4P utilization. The class Iβ_D DAHP synthases possess an additional 19 residues which are conserved with the KDOP synthases of class Iβ_K, but not with the subfamily Iα DAHP synthases.

The 30-kDa size of the class Iβ_K proteins appears to define the basic catalytic domain, and homology to this span of residues is apparent throughout family I. The approximate 40-residue N-terminal extensions of the differentially regulated isoenzyme types within subfamily Iα show little identity with one another and probably function in allosteric control. N-terminal extensions in class Iβ_D specify catalytic domains for chorismate mutase which also serve as allosteric domains for DAHP synthase (30) in two cases. In the *Synechocystis* protein, the extension may specify phenylalanine-mediated allostery. The streptococcal protein (*Streptococcus* AroA) which lacks an N-terminal extension probably lacks allosteric control, a feature noted for a number of streptococcal DAHP synthases (unpublished data).

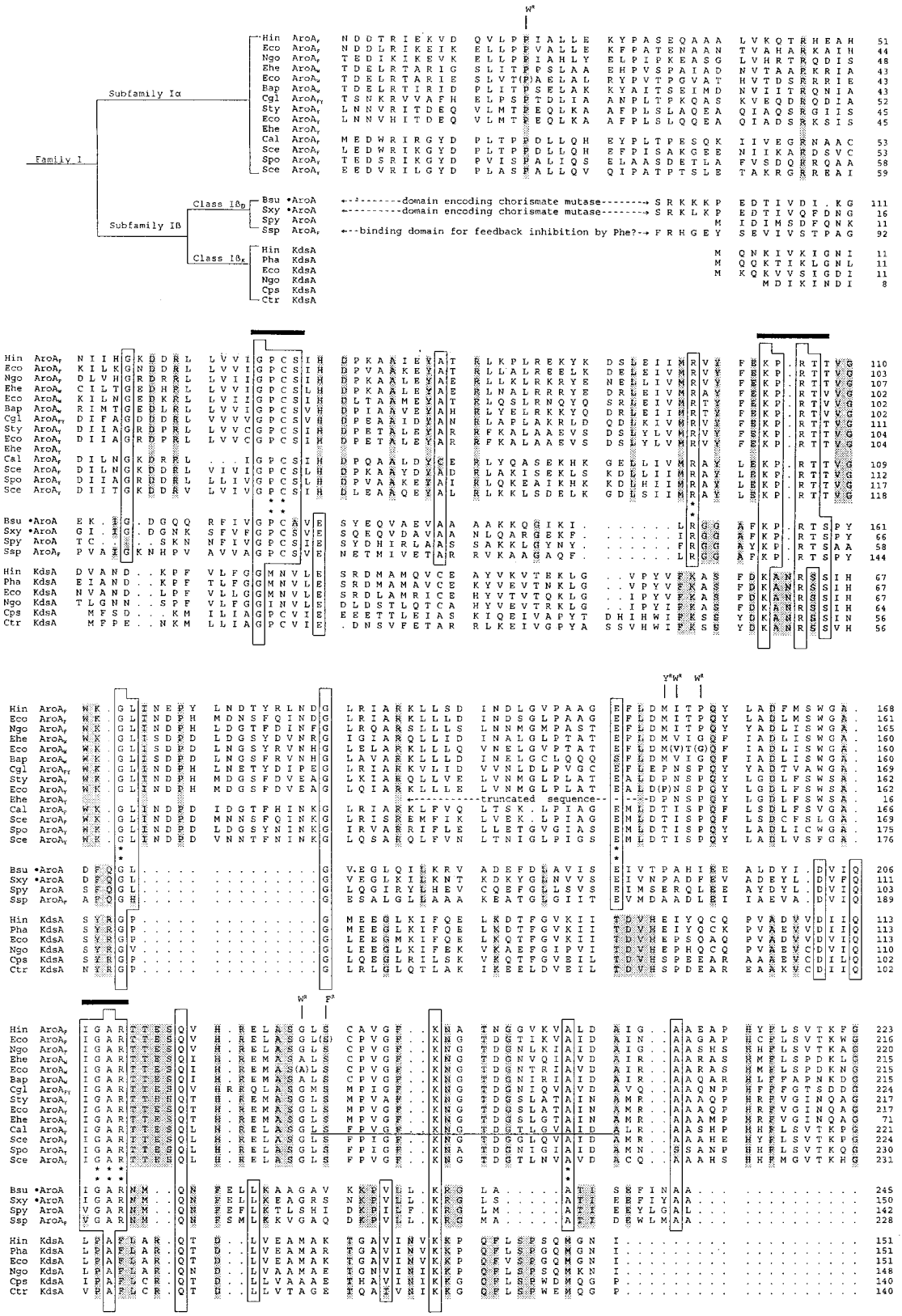
Active-site motifs. Several conspicuous motifs exist which may correspond to active-site residues of DAHP synthase, but not KDOP synthase. GPCS, KPRTS/T, and IGAR motifs are indicated in Fig. 7. Missense mutants within the GPCS and IGAR motifs of *E. coli* AroA$_W$ have been reported (40). (Interestingly, eight family II [plant-type] DAHP synthases in the database possess a KPRS motif in the same region of the primary sequence occupied by KPRT in the enzyme members of family I.) Of the remaining nine conserved residues which are potential active-site residues of DAHP synthase but not KDOP synthase, three have been shown to be essential for activity in *E. coli* AroA$_W$ (40), as indicated in Fig. 7.

Subfamily Iα DAHP synthases, as exemplified by studies with *E. coli* isoenzymes (46), are dependent on divalent metals for activity and are inactivated by EDTA treatment. The conserved C-61 residue of *E. coli* AroA$_F$ has been shown to be a catalytic residue, and it has been noted that the nearby conserved H-64 provides an appropriate motif for a metal coordination center (47). If so, this center would not be available for subfamily Iβ members, which lack the H-64 residue. Consistent with this, *B. subtilis* •AroA was unaffected by EDTA (30), and KDOP synthase does not require divalent metals for activity (41). *B. subtilis* •Aro may lack an active-site sulfhydryl, since sulfhydryl reagents failed to inhibit activity (30).

Independently evolved regulatory domains. *Bacillus subtilis* and *Staphylococcus xylosus* possess putative gene fusions yielding DAHP synthase and chorismate mutase as coexisting domains of a bifunctional protein (AroG•AroA). The chorismate-mutase catalytic domain is also the regulatory domain governing allosteric control by chorismate and prephenate (29). Since the AroG• domains belong to a different protein family, these have been excluded from the multiple alignment of Fig. 7. The *Synechocystis* AroA$_F$ protein is known to be sensitive to feedback inhibition by L-phenylalanine (24). It possesses a unique N-terminal extension of 72 residues that also is not shown in Fig. 7. We suggest that this might correspond to a regulatory domain which governs feedback inhibition by L-phenylalanine. A discrete domain governing sensitivity to feedback inhibition of *E. herbicola* PheA by L-phenylalanine which is similar in size has been reported (52), although obvious homology is not apparent. KdsA is not known to be sensitive to allosteric control. Thus, the portion of sequences that align with class Iβ_K proteins for the entire family I assemblage would appear to correspond to the basic KDOP-DAHP synthase catalytic domains.

All members of subfamily Iα are sensitive to feedback inhibition by one of the aromatic amino acids (two in the case of Cgl AroA$_{FY}$). A number of residues shown to be important for feedback inhibition in a given isoenzyme of *E. coli* are conserved in all other members of subfamily Iα, regardless of substrate specificity, e.g., P-18 and S-179 (numbered with reference to *E. coli* AroA$_W$). On the other hand, other residues important for feedback inhibition are uniquely conserved in correlation with the specificity for feedback inhibitor, e.g., V-160 for *E. herbicola* AroA$_W$, *E. coli* AroA$_W$, and *Buchnera aphidicola* AroA$_W$. Ray et al. (40) concluded that a common ancestor evolved an aromatic binding pocket which utilizes residues scattered throughout the primary sequence. If so, the DAHP synthase of *Corynebacterium glutamicum* may be the contemporary protein that is closest to the ancestral DAHP synthase. It further seems likely that the N-terminal residues within subfamily Iα (which have no counterparts in subfamily Iβ) interact with other residues interspersed throughout the primary sequence to accomplish feedback inhibition. Indeed, a conserved proline within the N-terminal extension (P-18 with reference to *E. coli* AroA$_W$) has been shown to be essential for sensitivity to feedback inhibition by L-tryptophan (40).

Evolutionary scenario. The foregoing information indicates that an ancient gene encoding a fundamental broad-specificity catalytic entity duplicated to produce the initial ancestors of subfamily Iα and subfamily Iβ. Members of class Iβ_K exemplify contemporary catalytic proteins which never acquired allosteric regulation. Class Iβ_D proteins acquired allosteric specificities by recruiting N-terminal allosteric domains through fusion


Family I
Subfamily Iα
Subfamily Iβ
Class Iβ$_D$
Class Iβ$_K$
Hin AroA$_F$
Eco AroA$_F$
Ngo AroA$_F$
Ehe AroA$_W$
Eco AroA$_W$
Bap AroA$_W$
Cgl AroA$_{FY}$
Sty AroA$_Y$
Eco AroA$_Y$
Ehe AroA$_Y$
Cal AroA$_F$
Sce AroA$_F$
Spo AroA$_F$
Sce AroA$_Y$
Bsu •AroA
Sxy •AroA
Spy AroA
Ssp AroA$_F$
←-binding domain for feedback inhibition by Phe?-→
Hin KdsA
Pha KdsA
Eco KdsA
Ngo KdsA
Cps KdsA
Ctr KdsA
←truncated sequence→

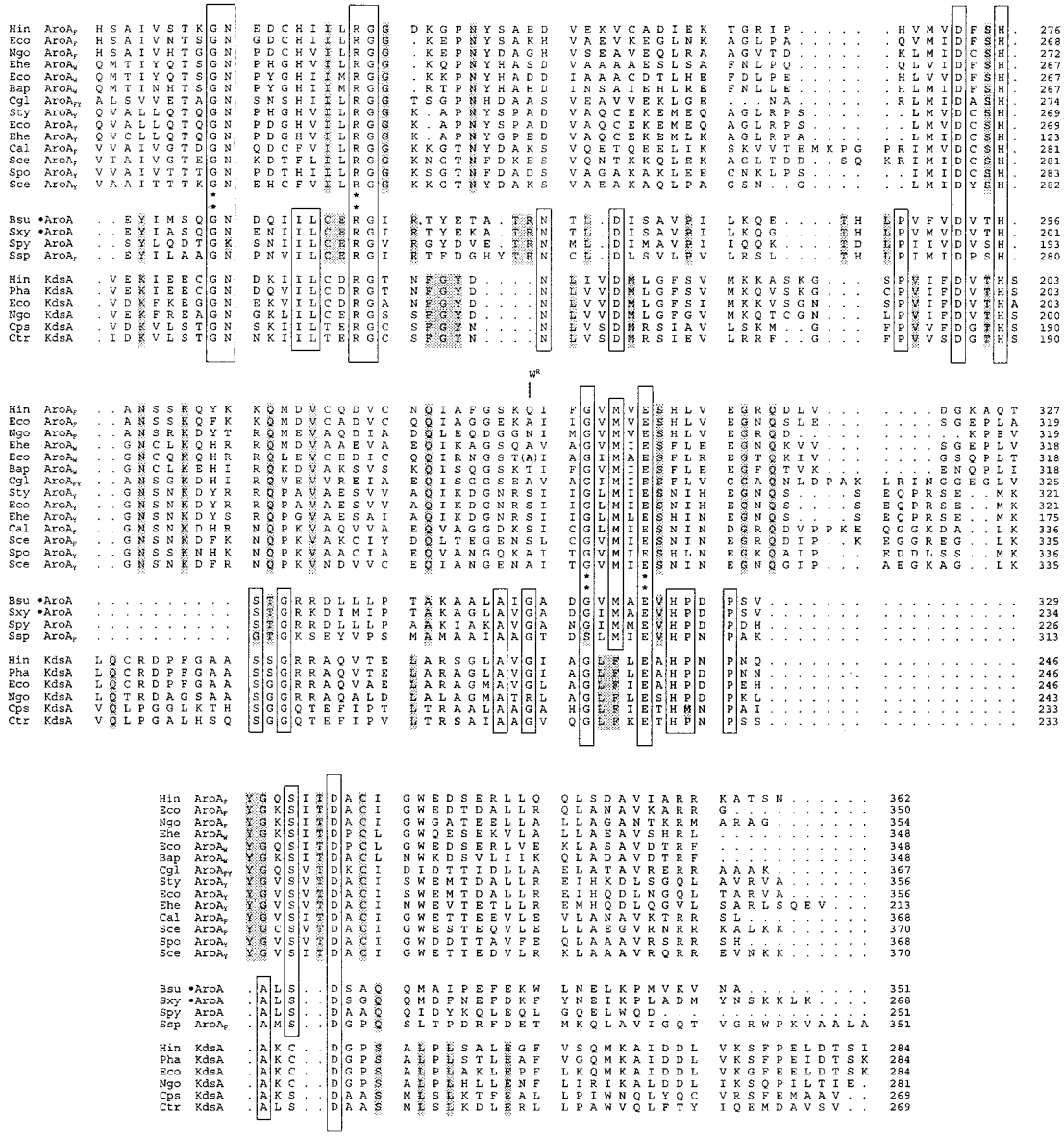


FIG. 7. Multiple alignment of family I DAHP and KDOP synthases. Amino acid residues which are conserved between class $I\beta_D$ and either or both of the other two groupings are boxed. In a few cases, a variant residue within a box is shaded. Invariant residues that are restricted to one of the three groups are shaded. Possible motif regions for DAHP synthase catalysis are indicated with heavy overbars. Residues shown (40) to be essential for catalytic activity of *E. coli* $AroA_W$ are indicated by double asterisks within a given box. Residues shown to be important for feedback inhibition by L-tryptophan (W^R), L-phenylalanine (F^R), or L-tyrosine (Y^R) are shown at the top, with the organism used for the study (references 40, 22, and 50, respectively) indicated in parentheses. A frameshift error between residues 184 to 204 of *C. albicans* $AroA_F$ (indicated by underlining) was corrected. Residue numbers are given at the far right. Gaps used to optimize the alignment are indicated by dots. Abbreviations are as indicated in the legend to Fig. 6.

mechanisms. In subfamily Iα, acquisition of an N-terminal extension may also have been crucial for an aromatic binding pocket needed for allostery. Following gene duplication, alterations of key residues narrowed specificity for a given aromatic amino acid, thus yielding, for example, the three *E. coli* isoenzyme paralogs $AroA_F$, $AroA_Y$, and $AroA_W$. Hence, members of family I are homologs with respect to catalytic domain, but not necessarily with respect to regulatory domains. Narrowed specificity for inhibition of DAHP synthase by L-phenylalanine occurred within subfamily Iα at least twice (e.g., leading to *E. coli* $AroA_F$ and *Saccharomyces cerevisiae* $AroA_F$), but these both were derived from the putative ancestral aromatic amino acid binding pocket. In contrast, the phenylalanine-binding region acquired by *Synechocystis* $AroA_F$ within subfamily Iβ was a completely independent event of domain acquisition.

The atomic relationships which dictate substrate specificity will be of future interest at several levels. (i) An established narrow-specificity DAHP synthase in class $I\beta_D$ (*B. subtilis* •AroA) exhibits greater overall similarity to a known narrow-specificity KDOP synthase (*E. coli* KdsA) than to a known narrow-specificity DAHP synthase homolog in subfamily Iα (*E. coli* Aro_F). (ii) Each of the three homolog groups considered in this report may prove to contain both broad-specificity and narrow-specificity members. Established broad-specificity DAHP synthases have not yet been sequenced, and most of the

proteins encoded by sequenced genes have not been characterized for specificity.

ACKNOWLEDGMENTS

We appreciate the input of Anne Hendry (Hamilton General Hospital), who initiated studies of *N. gonorrhoeae* in our laboratories. We thank Carol A. Bonner for the generous provision of the bifunctional dehydroquinase•shikimate dehydrogenase from *Nicotiana tabacum*. We thank the Gonococcal Genome Sequencing Project (University of Oklahoma) for availability of sequence data prior to publication.

REFERENCES

1. **Anderson, K. S., and K. A. Johnson.** 1990. Kinetic and structural analysis of enzyme intermediates: lessons from EPSP synthase. Chem. Rev. **90:**1131–1149.
2. **Ballou, C. E.** 1963. Preparation and properties of D-erythrose-4-phosphate. Methods Enzymol. **6:**479–484.
3. **Bentley, R.** 1990. The shikimate pathway: a metabolic tree with many branches. Crit. Rev. Biochem. Mol. Biol. **5:**307–384.
4. **Bhatnagar, R. K., A. Berry, A. T. Hendry, and R. A. Jensen.** 1989. The biochemical basis for growth inhibition by L-phenylalanine in *Neisseria gonorrhoeae*. Mol. Microbiol. **3:**429–435.
5. **Bolotin, A., V. Khazak, N. Stoynova, K. Ratmanova, Y. Yomantas, and Y. Kozlov.** 1995. Identical amino acid sequence of the *aroA*(G) gene products of *Bacillus subtilis* 168 and *B. subtilis* Marburg strain. Microbiology **141:**2219–2222.
6. **Bonner, C. A., and R. A. Jensen, R. A.** 1994. Cloning of cDNA encoding the bifunctional dehydroquinase•shikimate dehydrogenase of aromatic amino acid biosynthesis in *Nicotiana tabacum*. Biochem. J. **302:**11–14.
7. **Burrows, L. L., J. S. Lam, and R. Y. C. Lo.** 1996. The *kdsA* gene of *Pasteurella trehalosi* (*haemolytica*) serotype T3 is functionally and genetically homologous to that of *Escherichia coli*. J. Endotoxin Res. **3:**353–359.
8. **Chang, H.-C., H. Maruyama, R. S. Miller, and M. D. Lane.** 1966. The enzymatic carboxylation of phosphoenolpyruvate. J. Biol. Chem. **241:**2421–2430.
9. **Chen, C., C. Liao, and W. Hsu.** 1993. The cloning and nucleotide sequence of a *Corynebacterium glutamicum* 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase gene. FEMS Microbiol. Lett. **107:**223–230.
10. **Crawford, I. P.** 1989. Evolution of a biosynthetic pathway: the tryptophan paradigm. Annu. Rev. Microbiol. **43:**567–600.
11. **Davies, W. D., and B. E. Davidson.** 1982. The nucleotide sequence of *aroG*, the gene for 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetase (phe) in *Escherichia coli* K-12. Nucleic Acids Res. **10:**4045–4058.
12. **DeLeo, A. B., J. Dayan, and D. B. Sprinson.** 1973. Purification and kinetics of tyrosine-sensitive 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthetase from *Salmonella*. J. Biol. Chem. **248:**2344–2353.
13. **Doong, R. L., S. Ahmad, and R. A. Jensen.** 1991. Higher plants express 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase. Plant Cell Environ. **14:**113–120.
14. **Doong, R. L., J. E. Gander, R. J. Ganson, and R. A. Jensen.** 1992. The cytosolic isoenzyme of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in *Spinacia olearacea* and other higher plants: extreme substrate ambiguity and other properties. Physiol. Plant **84:**351–360.
15. **Doong, R. L., and R. A. Jensen.** 1992. Synonymy of the three apparent isoenzymes of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in *Pisum sativum* L. with 3-deoxy-D-*manno*-octulosonate 8-phosphate synthase and the DS/Co DS-Mn isoenzyme pair. New Phytol. **121:**165–171.
16. **Dotson, G. D., R. K. Dua, J. C. Clemens, E. W. Wooten, and R. W. Woodward.** 1995. Overproduction and one-step purification of *Escherichia coli* 3-deoxy-D-*manno*-octulosonic acid 8-phosphate synthase and oxygen transfer studies during catalysis using isotopic-shifted heteronuclear NMR. J. Biol. Chem. **270:**13698–13705.
17. **Fleischmann, R. D., M. D. Adams, O. White, R. A. Clayton, E. F. Kirkness, A. R. Keriavage, C. J. Bult, J.-F. Tomb, B. A. Dougherty, J. M. Merrick, K. McKenny, G. Sutton, W. FitzHug, C. Fields, J. D. Gocayne, J. Scott, R. Shirley, L.-L. Liu, A. Glodek, J. M. Kelley, J. F. Weidman, C. A. Phillips, T. Spriggs, E. Hedblom, M. D. Cotton, T. R. Utterback, M. C. Hanna, D. T. Nguyen, D. M. Saudek, R. C. Brandon, L. D. Fine, J. L. Fritchman, J. L. Fuhrmann, N. S. M. Geoghagen, C. L. Gnehm, L. A. McDonald, K. B. Small, C. M. Fraser, H. O. Smith, and J. C. Venter.** 1995. Whole-genome random sequencing and assembly of *Haemophilus influenzae* Rd. Science **269:**496–512.
18. **Floss, H. G., D. K. Onderka, and M. Carroll.** 1972. Stereochemistry of the 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetase reaction and the chorismate mutase reaction. J. Biol. Chem. **247:**736–744.
19. **Ganem, B.** 1978. From glucose to aromatics: recent developments in natural products of the shikimic acid pathway. Tetrahedron **34:**3353–3383.
20. **Ganson, R. J., T. A. d'Amato, and R. A. Jensen.** 1986. The two-isozyme system of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase in *Nicotiana silvestris* and other higher plants. Plant Physiol. **82:**203–210.
21. **Genetics Computer Group, Inc.** 1995. Wisconsin sequence analysis package, version 8.0. Genetics Computer Group, Inc. Madison, Wis.
22. **Ger, Y., S. Chen, H. Chiang, and D. Shiuan.** 1994. A single Ser-180 mutation desensitizes feedback inhibition of the phenylalanine-sensitive 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP) synthetase in *Escherichia coli*. J. Biochem. **116:**986–990.
23. **Ghalambor, M. A., E. M. Levine, and E. C. Heath.** 1966. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. J. Biol. Chem. **241:**3207–3215.
24. **Hall, G. C., M. B. Flick, R. L. Gherna, and R. A. Jensen.** 1982. Biochemical diversity for biosynthesis of aromatic amino acids among the cyanobacteria. J. Bacteriol. **149:**65–78.
25. **Harrison, W. H., P. D. Boyer, and A. B. Falcone.** 1955. The mechanism of enzymic phosphate transfer reactions. J. Biol. Chem. **215:**303–317.
26. **Haslam, E., R. D. Haworth, and P. F. Knowles.** 1963. The preparation and identification of 5-dehydroquinic and 5-dehydroshikimic acids. Methods Enzymol. **6:**498–501.
27. **Hedstrom, L., and R. Abeles.** 1988. 3-Deoxy-D-*manno*-octulosonate-8-phosphate synthase catalyzes the C—O bond cleavage of phosphoenolpyruvate. Biochem. Biophys. Res. Commun. **157:**816–820.
28. **Hendry, A. T.** 1983. Growth responses of *Neisseria gonorrhoeae* auxotypes to required amino acids and bases in liquid medium. Can. J. Microbiol. **29:**1309–1313.
29. **Jensen, R. A., and E. W. Nester.** 1965. The regulatory significance of intermediary metabolites: control of aromatic acid biosynthesis by feedback inhibition in *Bacillus subtilis*. J. Mol. Biol. **12:**468–481.
30. **Jensen, R. A., and E. W. Nester.** 1966. Regulatory enzymes of aromatic amino acid biosynthesis in *Bacillus subtilis*. I. Purification and properties of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthetase. J. Biol. Chem. **241:**3365–3372.
31. **Kaneko, T., S. Sato, H. Kotani, A. Tanaka, E. Asamizu, Y. Nakamura, N. Miyajima, M. Hirosawa, M. Sugiura, S. Sasamoto, T. Kimura, T. Hosouchi, A. Matsuno, A. Muraki, N. Nakazaki, K. Naruo, S. Okumura, S. Shimpo, C. Takeuchi, T. Wada, A. Watanabe, M. Yamada, M. Yasuda, and S. Tabata.** 1996. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC 6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. **3:**109–136.
32. **Kolibachuk, D., D. Rouhbakhsh, and P. Baumann.** 1995. Aromatic amino acid biosynthesis in *Buchnera aphidicola* (endosymbiont of aphids): cloning and sequencing of a DNA fragment containing *aroH-thrS-infC-rpmT-rplT*. Curr. Microbiol. **30:**313–316.
33. **Künzler, M., G. Paravicini, C. M. Egli, S. Irniger, and G. H. Braus.** 1992. Cloning, primary structure and regulation of the *ARO*4 gene, encoding the tyrosine-inhibited 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase from *Saccharomyces cerevisiae*. Gene **113:**67–74.
34. **Maruyama, H., R. L. Easterday, H.-C. Chang, and M. D. Lane.** 1966. The enzymatic carboxylation of phosphoenolpyruvate. J. Biol. Chem. **241:**2405–2412.
35. **Mayer, H., U. R. Bhat, H. Masoud, J. Radziejewska-Lebrecht, C. Widemann, and J. H. Krauss.** 1989. Bacterial lipopolysaccharides. Pure Appl. Chem. **61:**1271–1282.
36. **Mehdi, S., J. W. Frost, and J. R. Knowles.** 1987. Dehydroquinate synthase from *Escherichia coli* and its substrate 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate. Methods Enzymol. **142:**306–314.
37. **Muday, G. K., and K. M. Herrmann.** 1990. Regulation of the *Salmonella typhimurium aroF* gene in *Escherichia coli*. J. Bacteriol. **172:**2259–2266.
38. **Paravicini, G., H.-U. Mosch, T. Schmidheini, and G. Braus.** 1989. The general control activator protein GCN4 is essential for basal ARO3 gene expression in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **9:**144–151.
39. **Pereira, S. A., and G. P. Livi.** 1993. Cloning and expression of the *ARO*3 gene encoding DAHP synthase from *Candida albicans*. Gene **132:**159–165.
40. **Ray, J. M., C. Yanofsky, and R. Bauerle.** 1988. Mutational analysis of the catalytic and feedback sites of the tryptophan-sensitive 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase of *Escherichia coli*. J. Bacteriol. **170:**5500–5506.
41. **Ray, P. H.** 1980. Purification and characterization of 3-deoxy-D-*manno*-octulosonate 8-phosphate from *Escherichia coli*. J. Bacteriol. **141:**635–644.
42. **Rose, I. A., E. L. O'Connel, P. Noce, M. F. Utter, H. G. Wood, J. M. Willard, T. G. Cooper, and M. Benziman.** 1969. Stereochemistry of the enzymatic carboxylation of phosphoenolpyruvate. J. Biol. Chem. **244:**6120–6133.
43. **Shultz, J., M. A. Hermodson, C. Garner, and K. M. Herrman.** 1984. The nucleotide sequence of the *aroF* gene of *Escherichia coli* and the amino acid sequence of the encoded protein, the tyrosine-sensitive 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase. J. Biol. Chem. **259:**9655–9661.
44. **Smith, L. C., J. M. Ravel, S. R. Lax, and W. Shive.** 1962. The control of 3-deoxy-D-*arabino*-heptulosonic acid 7-phosphate synthesis by phenylalanine and tyrosine. J. Biol. Chem. **237:**3566–3570.
45. **Srinivasan, P. R., and D. B. Sprinson.** 1959. 2-Keto-3-deoxy-D-*arabo*-heptonic acid 7-phosphate synthetase. J. Biol. Chem. **234:**716–722.
46. **Stephens, C. M., and R. H. Bauerle.** 1991. Analysis of the metal requirement of 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase from *Escherichia*

coli. J. Biol. Chem. **266:**20810–20817.
47. **Stephens, C. M., and R. Bauerle.** 1992. Essential cysteines in 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase from *Escherichia coli*. J. Biol. Chem. **267:**5762–5767.
48. **Subramaniam P., R. Bhatnagar, A. Hooper, and R. A. Jensen.** 1994. The dyamic progression of evolved character states for aromatic amino acid biosynthesis in gram-negative bacteria. Microbiology **140:**3431–3440.
49. **Walker, G. E., B. Dunbar, L. S. Hunter, H. G. Nimmo, and J. R. Coggins.** 1996. Evidence for a novel class of microbial 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase in *Streptomyces coelicolor* A3(2), *Streptomyces rimosus* and *Neurospora crassa*. Microbiology **142:**1973–1982.
50. **Weaver, L. M., and K. M. Herrmann.** 1990. Cloning of an *aroF* allele encoding a tyrosine-insensitive 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase. J. Bacteriol. **172:**6581–6584.
51. **Woisetschläger, M., and G. Högenauer.** 1987. The *kdsA* gene coding for 3-deoxy-D-*manno*-octulosonic acid 8-phosphate synthetase is part of an operon in *Escherichia coli*. Mol. Gen. Genet. **207:**369–373.
52. **Xia, T., G. Zhao, and R. A. Jensen.** 1992. 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*. Appl. Environ. Microbiol. **58:**2792–2798.
53. **Xia, T., G. Zhao, and R. A. Jensen.** 1993. The *pheA*/*tyrA*/*aroF* region from *Erwinia herbicola*: an emerging comparative basis for analysis of gene organization and regulation in enteric bacteria. J. Mol. Evol. **36:**107–120.