Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 2339–2342
Bioorganic & Medicinal Chemistry Letters

Stereospecific deuteration of 2-deoxyerythrose 4-phosphate using 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase

Rachel M. Williamson, Amy L. Pietersma, Geoffrey B. Jameson and Emily J. Parker*

Institute of Fundamental Sciences, Massey University, Palmerston North 5301, New Zealand

Received 21 January 2005; revised 24 February 2005; accepted 28 February 2005
Available online 25 March 2005

Abstract—Racemic 2-deoxyerythrose 4-phosphate was synthesized and one enantiomer of this compound was found to be a substrate for *Escherichia coli* 3-deoxy-D-*arabino*-heptulosonate 7-phosphate synthase, the first enzyme of the shikimate pathway. When the reaction was carried out in deuterium oxide, an enzyme-catalyzed regio- and stereoselective incorporation of deuterium into the product was observed.

The enzyme 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAH7P) synthase catalyzes the first committed step of the shikimate pathway. This biosynthetic pathway occurs in plants and microorganisms, but not in mammals, and is responsible for the formation of aromatic compounds including the aromatic amino acids.[1] As the biosynthesis of aromatic compounds is required as a part of primary metabolism in many pathogenic organisms, the enzymes of the shikimate pathway are potential targets for new antimicrobial compounds.[2–5]

DAH7P synthase catalyzes a stereospecific aldol-like condensation between phosphoenolpyruvate (PEP) **1** and erythrose 4-phosphate (E4P) **2** to give DAH7P **3** and inorganic phosphate (Fig. 1). Many of the features of this enzymic reaction have been revealed through labelling studies and through structural analyses of the DAH7P synthases from *Escherichia coli* and *Saccharomyces cerevisiae*.[6–11] These studies have shown that the reaction involves an unusual cleavage of the C–O bond in PEP, and is likely to proceed through a linear bisphosphate intermediate formed by a *si*-face attack of the C3 of PEP on the C1 *re*-face of E4P.[12,13] However, several aspects regarding the interaction of the enzyme with its substrates and their subsequent activation for catalysis remain unresolved. DAH7P synthases belong to a family of enzymes that also includes the KDO8P synthases.[14,15] These enzymes catalyze an analogous reaction between PEP and a five-carbon phosphorylated monosaccharide, arabinose 5-phosphate.

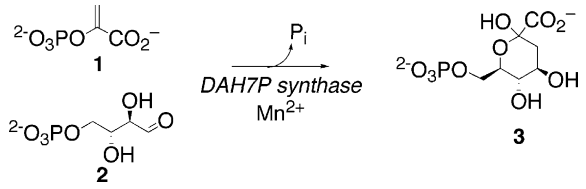


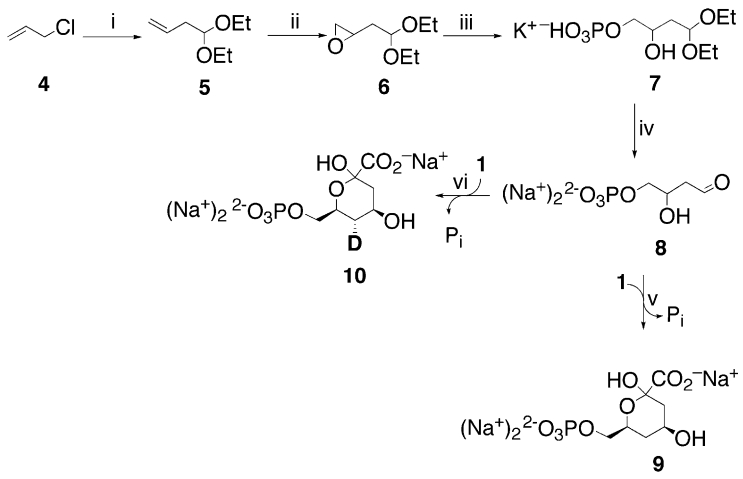
Figure 1. Reaction catalyzed by DAH7P synthase.

While there have been previous reports detailing the interaction of five-carbon phosphorylated sugars with DAH7P synthase,[16,17] there has been no investigation of the role(s) that the erythrose 4-phosphate hydroxyl groups play in binding and catalysis. In contrast, 2-, 3-, and 4-deoxyarabinose 5-phosphate derivatives have been tested on KDO8P synthases, and it has been shown that the 2- and 3-hydroxyl groups are important in this enzyme-catalyzed reaction.[18,19] We report here the synthesis of racemic 2-deoxyerythrose 4-phosphate (2-deoxyE4P), its interaction with the phenylalanine-sensitive DAH7P synthase from *E. coli*, and the isolation of 3,5-dideoxy-D-*arabino*-heptulosonate 7-phosphate. This is the first report of the interaction of an alternative four-carbon aldose phosphate substrate with this key biosynthetic enzyme.

2-DeoxyE4P was prepared in a four-step synthesis commencing with allyl chloride **4**, following a procedure

Keywords: Shikimate; Erythrose 4-phosphate; 3-Deoxy-D-*arabino*-heptulosonate 7-phosphate; Deoxyerythrose 4-phosphate.
* Corresponding author. Tel.: +64 356 9099x3566; fax: +64 6 350 5682; e-mail: e.j.parker@massey.ac.nz

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.02.080



Scheme 1. Chemical synthesis of racemic 2-deoxyE4P **8** followed by enzymic preparation of 3,5-dideoxy-D-*arabino*-heptulosonate 7-phosphate **9**. (i) Mg, $CH(OEt)_3$, 37%. (ii) *m*CPBA, CH_2Cl_2, 94%. (iii) KH_2PO_4, reflux, 68%. (iv) (1) Dowex-H^+, (2) Dowex-Na^+, 79% (v) DAH7P synthase, Mn^{2+}, H_2O, pH 6.8, 50%. (vi) DAH7P synthase, Mn^{2+}, D_2O, pD 6.8, 50%.

similar to that outlined by André et al.[20] and Guerard et al.[21] (Scheme 1). Allyl chloride was converted into 4,4-diethoxy-1-butene **5** by formation of the Grignard derivative and treatment with triethyl orthoformate.[22] Epoxidation with *meta*-chloroperbenzoic acid in dichloromethane gave 4,4-diethoxy-1,2-epoxybutane **6**. The epoxide ring was then opened using inorganic phosphate to give 4,4-diethoxy-2-hydroxybutyl phosphate, which was then isolated as its disodium salt **7**. The aldehyde moiety was released using Dowex-H^+ cation exchange resin. Treatment with Dowex-Na^+ and subsequent lyophilization of the aqueous solution gave 2-deoxyE4P **8** as its disodium salt.

The reaction catalyzed by DAH7P synthase is routinely monitored by the loss of PEP at 232 nm in the presence of E4P.[23] 2-DeoxyE4P was substituted for E4P in the enzyme assay and a time-dependent decline in absorbance was recorded, at a rate proportional to the DAH7P synthase concentration. The total change in absorbance (with PEP in excess) was proportional to the amount of 2-deoxyE4P added, but corresponded to the consumption of only 50% of the racemic 2-deoxyE4P present in the solution, suggesting that only a single enantiomer was acting as a substrate. The steady state kinetic parameters for this reaction were determined giving a K_M value of 650 μM for 2-deoxyE4P and a k_{cat} of 14 s^{-1}. While this corresponds to a specificity constant more than two orders of magnitude lower than that determined for the natural substrate ($K_{M(E4P)}$ = 21 μM, $k_{cat(E4P)}$ = 71 s^{-1}), 2-deoxyE4P is the best alternative substrate for *E. coli* DAH7P synthase so far reported.

To confirm this specificity, and to identify the product of the enzymic reaction, the product was prepared on a larger scale. 2-DeoxyE4P (4.5 mg) and PEP (4.5 mg) were dissolved in water (0.5 mL), manganese sulfate was added (to a final concentration of 60 μM), and the pH was adjusted to 6.8 using 10% sodium hydroxide solution. The enzymic reaction was then initiated by the addition of *E. coli* DAH7P synthase (20 μg). This

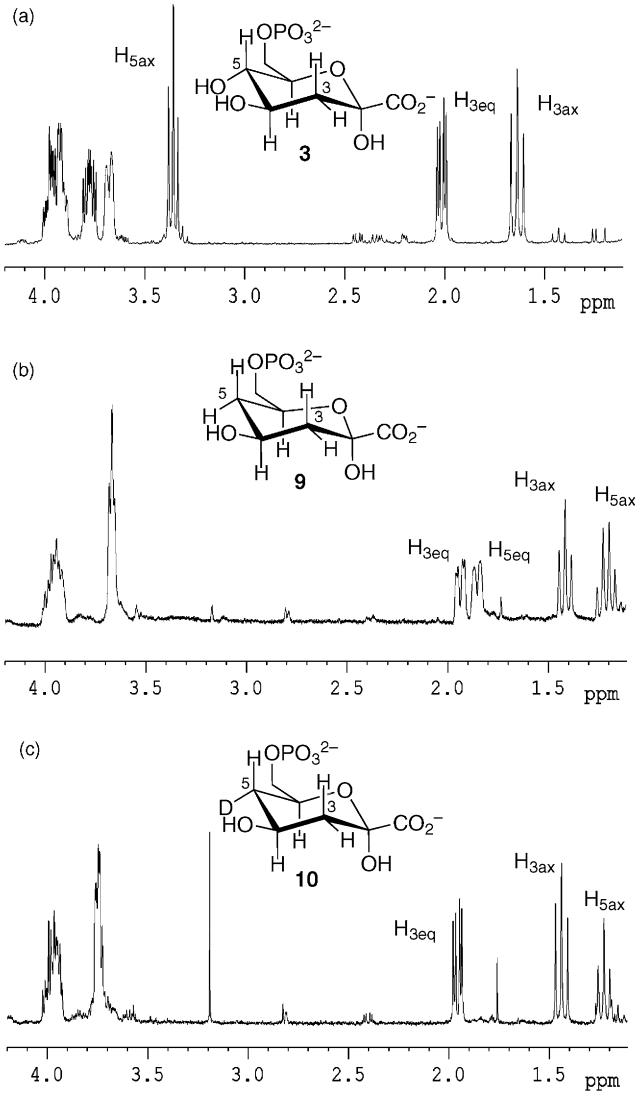


Figure 2. ^1H NMR spectra (400 MHz) of (a) DAH7P (b) 5-deoxy-DAH7P (c) (5*S*)-[5-^2H]-3,5-dideoxy-D-*arabino*-heptulosonate 7-phosphate. Minor peaks are attributed to the presence of small concentrations of the β-anomer.

reaction was monitored by the loss of PEP absorbance at 260 nm.[24] When the loss of PEP had ceased, the enzyme was removed by ultrafiltration and the solution was applied to an anion exchange column.[25] Fractions containing product were pooled, lyophilized, and analyzed by ^{1}H NMR spectroscopy (Fig. 2b). Comparison of this spectrum with that obtained when DAH7P **3** was prepared and purified using the same method (Fig. 2a) indicated that 3,5-dideoxy-D-*arabino*-heptulosonate 7-phosphate (5-deoxyDAH7P) **9** had been isolated. This product arises from the reaction of (3*S*)-2-deoxyE4P with PEP catalyzed by DAH7P synthase.[26] This enzyme therefore shows preferential binding and catalysis of the 2-deoxyE4P enantiomer with the same C3 configuration as the natural substrate E4P. As this product is isolated with the *arabino* configuration, this enzyme-catalyzed reaction proceeds with the same facial selectivity with respect to its monosaccharide substrate as is observed with E4P. No product with the alternative stereochemistry at C6 arising from reaction between PEP and the other enantiomer, (3*R*)-2-deoxyE4P, was observed.

In order to confirm the use of a single enantiomer of 2-deoxyE4P, the enzyme-catalyzed reaction was monitored by ^{1}H NMR in deuterium oxide. In this experiment, formation of a single product was observed, but signals corresponding to three methylene protons only were observed in the product spectrum (Fig. 2c). This product was isolated by anion exchange chromatography, and identified as (5*S*)-[5-^{2}H]-3,5-dideoxy-D-*arabino*-heptulosonate 7-phosphate **10**, using mass spectral and 2D NMR analysis. Interestingly, the deuterium is observed only in the 5*S* position. Under identical conditions without DAH7P synthase present, no exchange of deuterium at C2 of 2-deoxyE4P was observed. No incorporation of deuterium into DAH7P is found when the enzymic reaction is carried out with natural substrates, or when the nondeuterated 5-deoxyDAH7P is incubated with enzyme and phosphate in D_2O. These observations, combined with the stereospecificity of the deuterium incorporation and the predicted relative acidity of the C2 protons in 2-deoxyE4P, indicate that the deuteration occurs when (3*S*)-2-deoxyE4P is bound to the enzyme prior to reaction with PEP. As the deuterium is observed in the 5*S* position only (the position corresponding to the C2 hydroxyl group in E4P), it is tempting to speculate that the exchange is catalyzed by an enzymic residue normally responsible for forming a hydrogen bond contact to the C2 hydroxyl group of the natural substrate.

While to date there is no structure that shows E4P bound in the active site of DAH7P synthase in a manner competent for reaction, E4P has been modelled into the enzyme active site based on the binding of the nonreactive E4P analogue glycerol 3-phosphate (Fig. 3).[10] This model suggests that the C2 hydroxyl group of E4P is orthogonal to the carbonyl bond and interacts with Arg165 (proton donor) and the main chain carbonyl of Pro98 (proton acceptor), and that activation of the E4P carbonyl by metal coordination initiates the enzymic reaction. Coordination of (3*S*)-2-deoxyE4P in a similar manner would be expected to significantly increase the acidity of the pro*R* proton on C2 allowing for the stereospecific deuteration observed in these studies.

In summary, we report a short synthesis of racemic 2-deoxyE4P and the reaction of a single enantiomer of this compound with DAH7P synthase. Enzymic reaction led to the isolation of 5-deoxyDAH7P **9**, a previously unreported compound. When the reaction is carried out in deuterium oxide a novel stereospecific enzyme-catalyzed deuteration of the product at C5 is observed. Detailed kinetic analyses of the interaction of these compounds

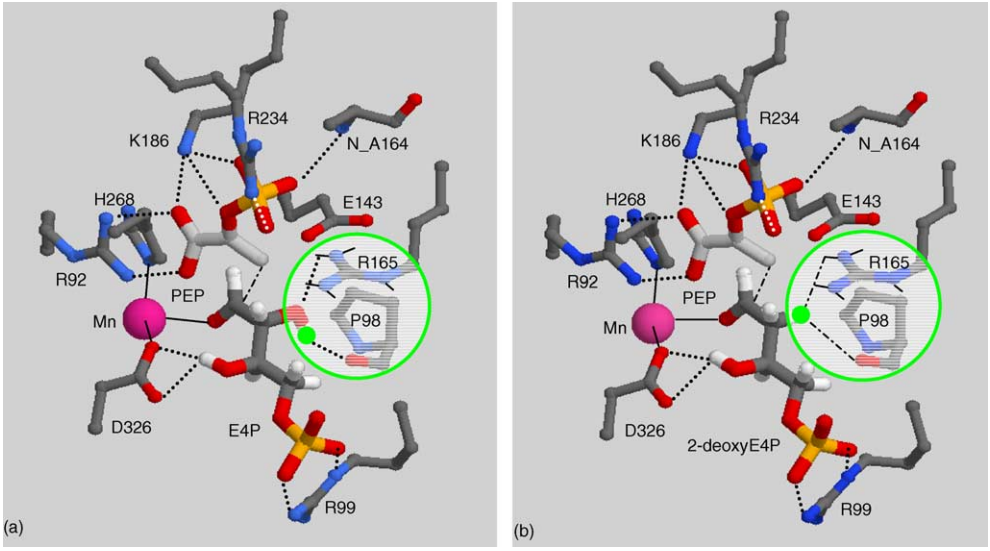


Figure 3. Model of E4P and (3*S*)-2-deoxyE4P in the active site of *E. coli* DAH7P synthase. The region of interest for the stereo- and regiospecific H/D exchange is highlighted. Model based on PEP and Mn^{2+} containing enzyme[8] (1n8f) and the *S. cerevisiae* structure[10] (1of8) containing Co^{2+}, PEP and glycerol 3-phosphate. Hydrogen bonds are shown as dotted lines and key interactions as dashed lines. Residues K97 and T100 are omitted, and only selected hydrogens in E4P, 2-deoxyE4P and R165 are shown for clarity.

with DAH7P synthases from a number of sources are currently underway.

These studies were funded by the Royal Society of New Zealand Marsden Fund (MAU008).

References and notes

1. Bentley, R. *Crit. Rev. Biochem. Mol. Biol.* **1990**, *25*, 307.
2. Campbell, S. A.; Richards, T. A.; Mui, E. J.; Samuel, B. U.; Coggins, J. R.; McLeod, R.; Roberts, C. W. *Int. J. Parasitol.* **2004**, *34*, 5.
3. Bulloch, E. M. M.; Jones, M. A.; Parker, E. J.; Osborne, A. P.; Stephens, E.; Davies, G. M.; Coggins, J. R.; Abell, C. *J. Am. Chem. Soc.* **2004**, *126*, 9912.
4. Coggins, J. R.; Abell, C.; Evans, L. B.; Frederickson, M.; Robinson, D. A.; Roszak, A. W.; Lapthorn, A. P. *Biochem. Soc. Trans.* **2003**, *31*, 548.
5. Frederickson, M.; Parker, E. J.; Hawkins, A. R.; Coggins, J. R.; Abell, C. *J. Org. Chem.* **1999**, *64*, 2612.
6. Shumilin, I. A.; Zhao, C.; Bauerle, R.; Kretsinger, R. H. *J. Mol. Biol.* **2002**, *320*, 1147.
7. Shumilin, I. A.; Kretsinger, R. H.; Bauerle, R. H. *Structure (London)* **1999**, *7*, 865.
8. Shumilin, I. A.; Bauerle, R.; Kretsinger, R. H. *Biochemistry* **2003**, *42*, 3766.
9. Stephens, C. M.; Bauerle, R. *J. Biol. Chem.* **1991**, *266*, 20810.
10. Hartmann, M.; Schneider, T. R.; Pfeil, A.; Heinrich, G.; Lipscomb, W. N.; Braus, G. H. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 862.
11. Konig, V.; Pfeil, A.; Braus, G. H.; Schneider, T. R. *J. Mol. Biol.* **2004**, *337*, 675.
12. Onderka, D. K.; Floss, H. G. *J. Am. Chem. Soc.* **1969**, *91*, 5894.
13. DeLeo, A. B.; Sprinson, D. B. *Biochem. Biophys. Res. Commun.* **1968**, *32*, 873.
14. Jensen, R. A.; Xie, G.; Calhoun, D. H.; Bonner, C. A. *J. Mol. Evol.* **2002**, *54*, 416.
15. Raetz, C. R. H.; Whitfield, C. *Annu. Rev. Biochem.* **2002**, *71*, 635.
16. Sheflyan, G. Y.; Howe, D. L.; Wilson, T. L.; Woodard, R. W. *J. Am. Chem. Soc.* **1998**, *120*, 11027.
17. Sundaram, A. K.; Woodard, R. W. *J. Org. Chem.* **2000**, *65*, 5891.
18. Ray, P. H.; Kelsey, J. E.; Bigham, E. C.; Benedict, C. D.; Miller, T. A. *ACS Symp. Ser.* **1983**, *231*, 141.
19. Kohen, A.; Jakob, A.; Baasov, T. *Eur. J. Biochem.* **1992**, *208*, 443.
20. André, C.; Demuynck, C.; Gefflaut, T.; Guerard, C.; Hecquet, L.; Lemaire, M.; Bolte, J. *J. Mol. Catal. B: Enzym.* **1998**, *5*, 113.
21. Guerard, C.; Alphand, V.; Archelas, A.; Demuynck, C.; Hecquet, L.; Furstoss, R.; Bolte, J. *Eur. J. Org. Chem.* **1999**, *1999*, 3399.
22. Cloux, R.; Schlosser, M. *Helv. Chim. Acta* **1984**, *67*, 1470.
23. Schoner, R.; Herrmann, K. M. *J. Biol. Chem.* **1976**, *251*, 5440.
24. A higher wavelength than the λ_{max} for PEP was used to keep absorbances within the linear range of the spectrophotometer.
25. Amersham SourceQ® (8 mL bed volume) eluted with a linear gradient of 0–500 mM ammonium bicarbonate.
26. Synthesis and enzyme testing of the enantiopure (3*S*)-2-deoxyerythrose 4-phosphate have discounted the unlikely possibility that this product arises from reaction of PEP on the *si*-face of (3*R*)-2-deoxyE4P.