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EVIDENCE FOR OPPOSITE STEREOCHEMICAL COURSES FOR THE REACTIONS CATALYSED BY TYPE I AND TYPE II DEHYDROQUINASES

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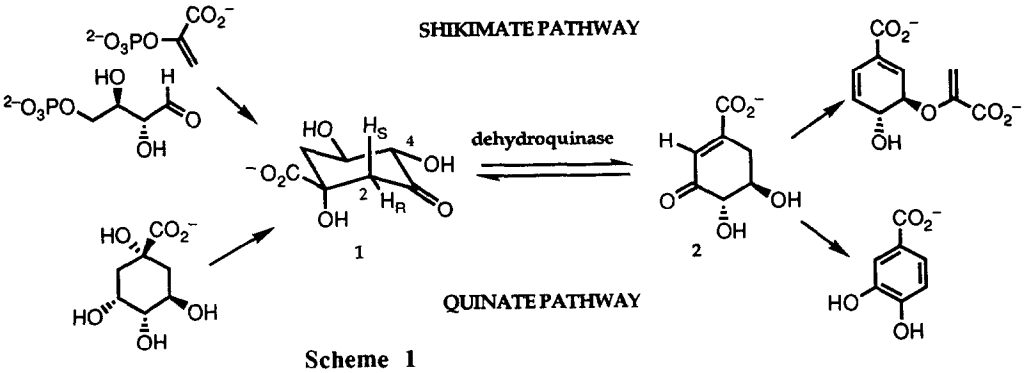
Abstract There are two distinct types of dehydroquinase which catalyse the dehydration of dehydroquinate **1** to dehydroshikimate **2**. Stereochemical studies using deuterated dehydroquinate are described which show that the reaction catalysed by the type II enzyme proceeds with *anti* stereochemistry, in contrast to the *syn* elimination catalysed by the type I enzyme.

Dehydroquinase (3-dehydroquinate dehydratase, EC 4.2.1.10) catalyses the dehydration of 3-dehydroquinate **1** to 3-dehydroshikimate **2**. This reaction is the third reaction on the shikimate pathway.[1] The same reaction is also part of the quinate pathway which enables fungi to utilise quinate as a carbon source via the β-ketoadipate pathway[2] (Scheme 1).



Scheme 1

It is now recognised that there are two distinct classes of dehydroquinase which appear to be quite different in physical and biochemical properties.[3] The type I enzyme is typified by the *Escherichia coli* dehydroquinase, and the type II enzyme by the quinate pathway enzyme from *Aspergillus nidulans*. Of mechanistic significance is the observation that there is a conserved lysine in type I dehydroquinases[4] but not in the type II enzyme.[5] Correspondingly, treatment of the enzyme with an equilibrium mixture of substrate and product followed by borohydride trapping results in irreversible inactivation of only the type I enzyme.[3,4,6]

The mechanism of the type I enzyme has been shown to involve Schiff base formation between the substrate and an active site lysine.[4,5] The lysine has been identifed as Lys-170,[5] and an imine intermediate on the enzyme has been observed directly by electrospray mass spectrometry.[7] The stereochemical course of the type I catalysed reaction involves loss of the *pro-R* hydrogen from C-2, corresponding to an overall *syn* elimination.[8,9] Although there are examples of enzymic *syn* dehydrations,[10] this result is surprising because the axial *pro-S* hydrogen is more acidic when the substrate is in its preferred chair conformation.[11] Indeed, the non-enzymic conversion of **1** to **2** involves loss of the *pro-S* hydrogen and so proceeds with overall *anti* stereochemistry.[11] In an attempt to rationalise the stereochemical course of the type I enzyme it has been suggested that there must be some distortion of the imine intermediate to bring the *pro-R* C-2 hydrogen coplanar with the π-acceptor orbital of the imine, thereby increasing its acidity.[11,12]

Several factors indicate that the type II dehydroquinase does not use a Schiff base mechanism to catalyse the conversion of **1** to **2**. The most striking is the lack of a conserved lysine.[4] This is coupled with the failure to trap an imine intermediate in borohydride trapping experiments,[3] or to detect any covalent adducts on the enzyme by electrospray mass spectrometry.[13] If a different mechanism is followed, then there is no reason why the reaction must follow the same stereochemical course as the type I enzyme, particularly as the non-enzymic elimination proceeds with *anti* stereochemistry.

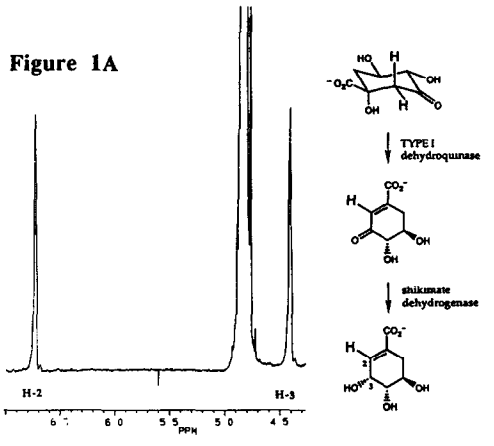
We therefore decided to determine the stereochemical course of the type II dehydroquinase reaction. This could be most easily achieved using [2-^{2}H]dehydroquinate differentially labelled in the C-2 *pro-R* and *pro-S* positions as a substrate for both the type I and type II enzymes. This was obtained by treatment of dehydroquinate with triethylamine in D_2O.[14] The extent of deuteration was measured by the reduction in the intensity of the corresponding signal in the ^{1}H NMR spectrum as approximately 20% in the C-2 *pro-R* position, 80% in the *pro-S* position, with 30% exchange at C-4. This difference in extent of deuteration of the two hydrogens at C-2 (approx 1:4) was sufficient to allow the unambiguous determination of the stereochemical course of the enzyme-catalysed elimination.

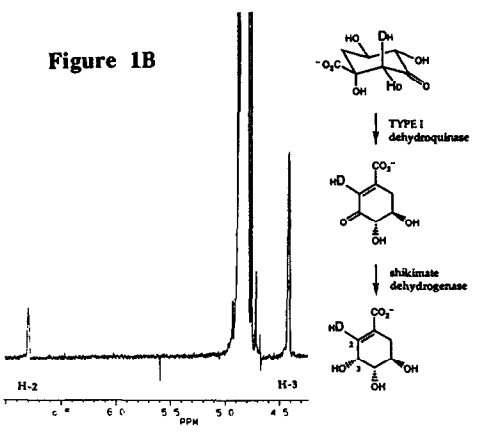
Because of the relative instability of dehydroshikimate formed in the dehydroquinase reaction, it was decided to reduce it *in situ* with shikimate dehydrogenase[15] and isolate shikimate as the reaction product. The shikimate was then purified by ion exchange chromatography and the extent of deuteration at C-2 measured by ^{1}H NMR spectroscopy.[16]

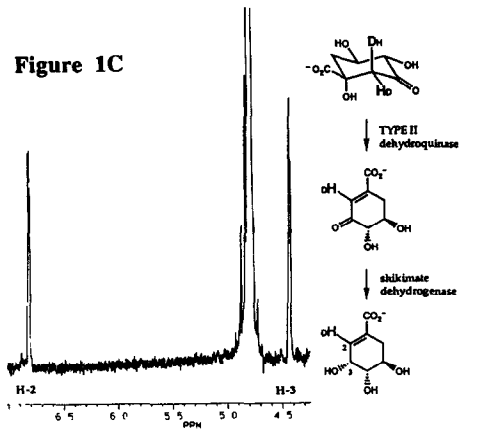
The first experiment was a control using unlabelled dehydroquinate and type I dehydroquinase from *E. coli*.[17] Figure 1A shows the signals for H-2 (vinylic) and H-3 in the ^{1}H NMR spectrum of the shikimate product. H-3 is derived from the NADPH cofactor and its signal acts as a standard against which the integral for the H-2 signal can be measured.

In the second experiment, the differentially deuterated dehydroquinate was treated with type I dehydroquinase from *E. coli*. The NMR spectrum (Figure 1B) of the shikimate product shows a very much reduced signal for the H-2. This derives from the *pro-S* hydrogen in the dehydroquinate which had been extensively exhanged for deuterium. This confirms the loss of the *pro-R* hydrogen in a *syn* elimination.

The final experiment was performed using type 2 dehydroquinase from *A. nidulans*.[18] In this case, the NMR spectrum of the derived shikimate (Figure 1C) shows a slightly reduced signal for H-2. This derives from the *pro-R* hydrogen in the substrate, indicating that it is the *pro-S* hydrogen which is eliminated.[19] The results from second and third experiments are complementary and show clearly that the reaction catalysed by type I and type II dehydroquinases follow opposite stereochemical courses.







The observation that the reactions catalysed by the type I and type II enzymes proceed with opposite stereochemistries is the clearest evidence so far that the different enzymes use different mechanisms. Different mechanisms for the same *type* of reaction are well precedented, for instance enzyme-catalysed decarboxylation reactions can go via a Schiff base mechanism (with retention), or be metal-catalysed (generally with inversion).[20] However, this is a comparison of reactions on different substrates by different enzymes. What is remarkable about the result reported in this paper is that it is the same reaction, the conversion of dehydroquinate to dehydroshikimate, that is being catalysed by two different enzymes, using different mechanisms that proceed with opposite stereochemistries. The catalytic mechanism of the type II dehydroquinase remains to be determined. It will be of particular interest to see if the enzyme has any metal dependence.

From a comparison of the amino acid sequences it has been suggested that the type I and type II dehydroquinases arose by convergent evolution.[21] It is now clear that they found different solutions to the same chemical problem. This has very exciting implications and opens up the way for a detailed comparison of the enzyme structures and catalytic mechanisms.

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References and Notes

1. Bentley, R. *Critical Reviews in Biochemistry and Molecular Biology* **1990**, *25*, 307-384.

2. Giles, N. H.; Case, M. E.; Baum, J. A.; Geever, R. F.; Huiet, L.; Patel, V. B.; Tyler, B. M. *Microbial. Rev.* **1985**, *49*, 338-358.

3. Kleanthous, C.; Deka, R.; Davis, K.; Kelly, S. M.; Cooper, A., Harding, S. E.; Price, N. C.; Hawkins, A. R.; Coggins, J. R. *Biochem. J.* **1992**, *282*, 687-695.

4. Chauduri, S.; Duncan, K.; Graham, L. D.; Coggins, J. R. *Biochem. J.* **1990**, *275*, 1-6.

5. Garbe, T.; Servos, S.; Hawkins, A. R.; Young, D.; Dougan, G.; Charles, I. G. *Mol. Gen. Genet.* **1991**, *228*, 385-392.

6. Butler, J. R.; Alworth, W. L.; Nugent, M. J. *J. Am. Chem. Soc.* **1974**, *96*, 1617-1618.

7. Shneier, A.; Kleanthous, C.; Deka, R.; Coggins, J. R.; Abell, C. *J. Am. Chem. Soc.* **1991**, *113*, 9416-9418.

8. Hanson, K. R.; Rose, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *50*, 981-988.

9. Smith, B. W.; Turner, M. J.; Haslam, E. *J. Chem. Soc., Chem. Commun.* **1970**, 842-843. Haslam, E.; Turner, M. J.; Sargent, D.; Thompson, R. S. *J. Chem. Soc.* (C) **1971**, 1489-1495.

10. Schwab, J. M.; Klassen, J. B.; Habib, A. *J. Chem. Soc., Chem. Commun.* **1986**, 357-358.

11. Smith, B. W.; Turner, M. J.; Haslam, E. *J. Chem. Soc., Perkin Trans.* **1975**, *1*, 52-55.

12. Vaz, A. D. N.; Butler, J. R.; Nugent, M. J. *J. Am. Chem. Soc.*1**975**, *97*, 5914-5915.

13. Shneier, A; Abell, C. unpublished results

14. Grewe, R.; Haworth, H. *Biochem. Prep.***1968**, 11, 21-26.

15. *E. coli* shikimate dehydrogenase was purified as described by Chaudari, S.; Anton A. I.; Coggins, J. R. *Methods Enzymol.* **1987**, *142*, 315-320.

16. Ammonium 3-dehydroquinate (5 mg) was dissolved in water (50 µl) and NADPH (1.2 equivalents) in buffer (450 µl, 50 mM, pH 7.0 [potassium phosphate buffer for type I, Tris acetate buffer for type II]) was added. Dehydroquinase (0.5 units) and shikimate dehydrogenase (1.7 units) were added and the mixture left at 25 °C. The reaction was monitored by removing aliquots and following the decrease in A_{340} due to the conversion of NADPH to $NADP^+$. After transformation, the reaction mixture was loaded onto a DEAE Sephadex column (bicarbonate form), which was eluted with a linear gradient of 0.05-0.5 M TEAB. The fractions containing shikimate were dried, and further purified on a BioRad Organic Acids column. Elution with 0.05 M formic acid gave shikimic acid (4 mg).

17. *E. coli* dehydroquinase was purified as described by Chaudhuri, S.; Lambert , J. M.; McColl, L. A.; Coggins, J. R. *Biochem. J.* **1986**, *239*, 699-704.

18. *A. nidulans* type 2 dehydroquinase was purified as described by Beri, R. K.; Grant, S.; Roberts, C. F.; Smith, M.; Hawkins, A. R. *Biochem. J.* **1990**, *265*, 343-349.

19. Because the reaction catalysed by the type II enzyme and the non-enzymic reaction both proceed with *anti* stereochemistry, it was important to show that the formation of dehydroquinase in the third experiment was enzyme catalysed. Therefore a control experiment was carried out under identical conditions but without the type II dehydroquinase. In this experiment there was no detectable formation of dehydroshikimate.

20. Rozell, J. D.; Benner, S. A. *J. Am. Chem. Soc.* **1984**, *106*, 4937-4941.

21. Hawkins, A. R. *Curr. Genet.* **1987**, *11*, 491-498.