Different Mechanistic and Stereochemical Courses for the Reactions catalysed by Type I and Type II Dehydroquinases

Joanna Harris,[a] Colin Kleanthous,[b] John R. Coggins,[c] Alastair R. Hawkins[d] and Chris Abell*[a]

[a] *University Chemical Laboratory, Lensfield Road, Cambridge, UK CB2 1EW*
[b] *School of Biological Sciences, University of East Anglia, Norwich, UK NR4 7TJ*
[c] *Department of Biochemistry, University of Glasgow, Glasgow, UK G12 8QQ*
[d] *Department of Biochemistry and Genetics, The Medical School, The University, Newcastle upon Tyne, UK NE2 7HH*

The reaction catalysed by type II dehydroquinase is shown to be exclusively *anti* in contrast to the *syn* stereochemistry of the reaction catalysed by type I dehydroquinase.

Enzymes are generally considered to catalyse reactions by optimised mechanisms. It is unexpected, and therefore of great interest, when two mechanistically distinct enzymes are found which catalyse the same reaction.[1] Dehydroquinase catalyses the dehydration of 3-dehydroquinate **1** to 3-dehydroshikimate **2**.[2] Recent biochemical and genetic studies have shown that there are two different dehydroquinases (type I and type II), which appear to be a result of convergent evolution.[3] Both types of dehydroquinase are found in eukaryotes and procaryotes, and in some circumstances can be coproduced in the same organism.[3,4] Not only do type I and type II dehydroquinases exhibit different physical and biochemical properties, but preliminary studies suggest they catalyse reactions which have different mechanistic and stereochemical courses.[3,5] We now describe experiments which exploit the different stereochemical courses of the reactions catalysed by type I and type II dehydroquinases, and show that the reaction catalysed by the type II enzyme is exclusively *anti*.

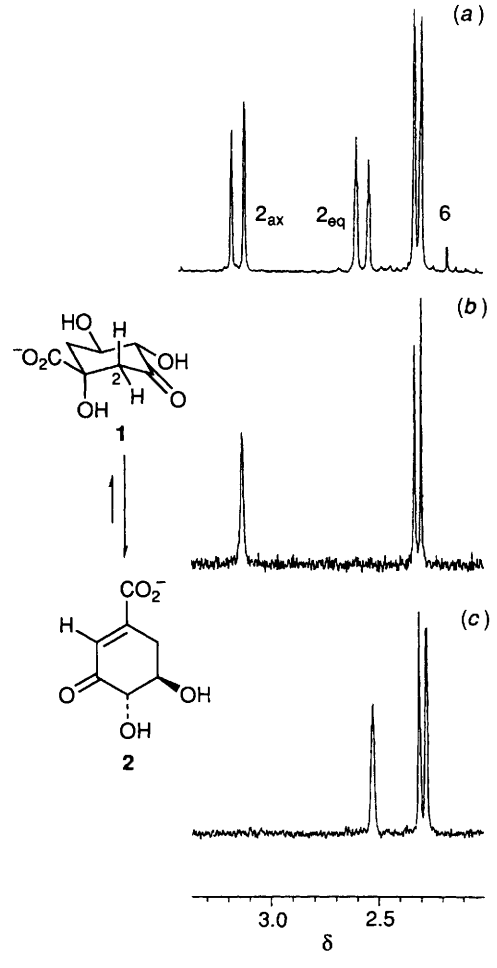
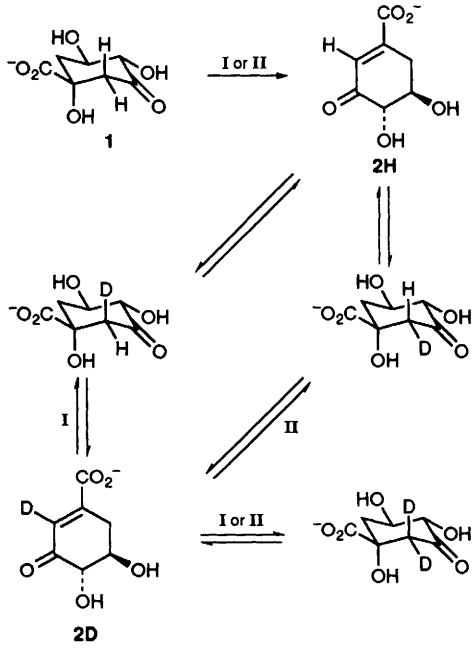


Fig. 1 Partial ^{1}H NMR spectra (D_2O, 250 MHz) of dehydroquinate **1**: (*a*) standard sample; (*b*) (2*R*)-[2-^{2}H]dehydroquinate formed from dehydroshikimate **2** by the type I dehydroquinase from *E. coli* in D_2O; (*c*) (2*S*)-[2-^{2}H]dehydroquinate formed from dehydroshikimate **2** by the type II dehydroquinase from *A. nidulans* in D_2O

Escherichia coli dehydroquinase has been extensively studied and its mechanism shown to involve Schiff base formation. A conserved lysine (Lys-170 in *E. coli*) in type I dehydroquinases has been shown to form a Schiff base with the substrate,[6] and an imine intermediate on the enzyme has been observed directly by electrospray mass spectrometry.[7] Treatment of the enzyme with an equilibrium mixture of substrate and product followed by borohydride trapping results in irreversible inactivation of the type I enzyme.[3,6] In contrast, there is no conserved lysine in type II dehydroquinases, and borohydride trapping experiments do not inactivate the enzyme.[3]

The reaction catalysed by *E. coli* dehydroquinase involves loss of the equatorial *pro-R* hydrogen from C-2, corresponding to an overall *syn* elimination.[8] This result is in contrast to the non-enzymic elimination which involves loss of the more acidic axial *pro-S* hydrogen,[9] but can be accommodated by a mechanism which involves some distortion of the imine intermediate to align the bond to the *pro-R* C-2 hydrogen with the π-orbital of the imine, thereby increasing its acidity.[9] The conjugate acid of the enzyme base (His-143)[10] is then ideally placed to act as a general acid catalyst for the departing hydroxy group. As the type II enzyme appears to be mechanistically distinct, it cannot be assumed it will have equivalent catalytic functionality, and so it is of interest to determine the stereochemical course of its reaction. To do this we have fully exploited the availability of the type I enzyme.



Scheme 1 Incorporation of deuterium at C-2 of dehydroshikimate only occurs if the substrate has undergone a reaction catalysed by both the type I enzyme **I** and the type II enzyme **II**

(2*R*)-[2-^{2}H]Dehydroquinate was prepared by incubating dehydroshikimate with the type I dehydroquinase from *E. coli* in D_2O.[11] The equilibrium for this reaction is 15 in favour of dehydroshikimate,[12] and so only small amounts of dehydroquinate were isolated. After purification by HPLC, spectroscopic analysis showed that the product was stereospecifically deuteriated. In a parallel experiment (2*S*)-[2-^{2}H]dehydroquinate was prepared by incubating dehydroshikimate with the type II dehydroquinase from *Aspergillus nidulans*[13] in D_2O (Fig. 1). This result shows that the reaction catalysed by type II dehydroquinase is exclusively *anti*.

These labelled dehydroquinates were then used to study the forward reaction. Incubation of (2*R*)-[2-^{2}H]dehydroquinate with type I dehydroquinase resulted in the formation of dehydroshikimate with complete loss of deuterium. In contrast incubation with the type II dehydroquinase produced [2-^{2}H]dehydroshikimate retaining all the deuterium at C-2. Control experiments without type II enzyme showed no formation of dehydroshikimate, confirming that the elimination was indeed enzyme catalysed. In the complementary experiment, treatment of (2*S*)-[2-^{2}H]dehydroquinate with type I enzyme resulted in complete retention of deuterium in the dehydroshikimate formed.

Finally, the different stereochemical courses of the type I and type II enzymes were demonstrated in a unique experiment. Dehydroquinate was incubated with a mixture of type I and type II enzymes in D_2O. Incorporation of deuterium at C-2 of dehydroshikimate was observed (conversion of **2H** to **2D**, Scheme 1). This can only occur if say, (2*R*)-[2-^{2}H]dehydroquinate formed by the hydration of dehydroshikimate by type I dehydroquinase, is converted back to dehydroshikimate by type II dehydroquinase which proceeds with the opposite sterochemical course. Incubation of dehydroquinate with only type I, or only type II dehydroquinase results in no deuterium incorporation into dehydroshikimate, consistent with the stereochemical integrity of these reactions.

The opposite sterochemical outcomes of the reactions catalysed by the type I and type II dehydroquinases provides conclusive evidence that the respective reactions proceed by different mechanisms. This is a very unusual finding, and offers an excellent experimental system for addressing questions of convergent evolution and catalytic efficiency. It is now a matter of great interest to establish the catalytic mechanism of the type II enzyme.

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