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Registry No. DBBQ, 3383-21-9; $Fe(DBSQ)_3$, 64020-89-9; $Fe_4(DBSQ)_4(DBCat)_4^-$, 119009-91-5; $Fe(CO)_5$, 13463-40-6.

Supplementary Material Available: Complete listings of anisotropic thermal parameters and hydrogen atom coordinates $Fe(DBSQ)_3$ and $Fe_4(DBSQ)_4(DBCat)_4$ (7 pages); observed and calculated structure factors (63 pages). Ordering information is given on any current masthead page.

Communications to the Editor

Dehydroquinate Synthase: A Sheep in Wolf's Clothing?

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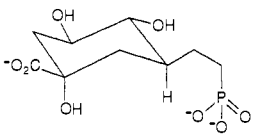
The shikimate pathway[2,3] is responsible for the biosynthesis of aromatic amino acids in plants and microorganisms. The second enzyme of this metabolic sequence, dehydroquinate synthase, catalyzes the conversion of the seven carbon keto acid 3-deoxy-D-*arabino*-heptulosonate 7-phosphate (DAHP, **1**) to dehydroquinate (DHQ, **2**), which is the first carbocyclic metabolite in the pathway. As is shown in Scheme I, the enzyme, which is a monomer of M_r 38 880, appears to catalyze an unusually complex concatenation of chemical changes.[4] The secondary alcohol at C-5 of DAHP is oxidized by enzyme-bound NAD^+, facilitating the β-elimination of inorganic phosphate across C-6 and C-7. After reduction of the C-5 ketone, the pyranose ring opens, and a subsequent intramolecular aldol reaction yields the product dehydroquinate. Following the recent suggestion that DHQ synthase may not, in fact, mediate the last two steps of Scheme I,[5] we now report experiments that suggest that the enzyme plays only a minor role in the second step of the sequence.

Recent work in our laboratory has shown that the enzyme-catalyzed elimination of inorganic phosphate occurs with syn stereochemistry from the cyclic α-pyranose form of the substrate.[6] This result is in accord with the stereochemical course of all enzymes that catalyze β-eliminations from ketones or from thioesters[7] and suggests that the elimination of P_i from DAHP is a stepwise (E1cB) process. If this is true, the *minimum* number of enzyme-bound species associated with the overall reaction is seven! While examining the behavior of substrate analogues that cannot complete the sequence shown in Scheme I, we have studied several carbocyclic DAHP analogues (e.g., **3**, **4**, **5**, and **6**)[8] that can be oxidized at C-5 and suffer abstraction of the C-6 proton, but that cannot complete the elimination reaction because the phosphate group has been converted into a phosphonate.

The homophosphonate analogue **3**, which is isosteric with DAHP, has a K_i value (1.7 μM) that is close to the K_m of DAHP



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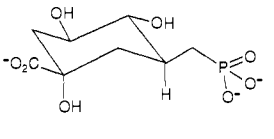

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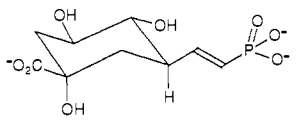

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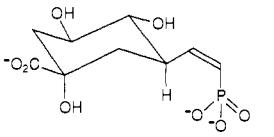

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($\sim$4 μM).[9] Incubation of **3** with the enzyme in D_2O leads to enzyme-catalyzed exchange of the C-6 proton with the solvent.[11] In contrast, the phosphonate analogue **4** binds much more tightly to DHQ synthase[10] (this analogue is the best inhibitor of the enzyme yet found: $K_i \sim 0.8$ nM), and despite the fact that **4** is readily oxidized at C-5 when bound,[12] *no* exchange of the C-6 proton with the solvent is observed. The positioning of the phosphonate group is evidently critical (albeit in different ways) both for binding and for proton exchange.

To define the position of the phosphate ester group of enzyme-bound DAHP, we have examined the behavior of the unsaturated analogues **5** and **6**. The *E*-vinyl homophosphonate **5** mimics an expected low-energy conformation of the substrate DAHP and of the homophosphonate **3**, with the side chain in an enforced extended conformation. The *E*-vinyl analogue **5** is weakly bound by DHQ synthase ($K_i \sim 25$ μM), no oxidation at C-5 can be observed even at saturating concentrations, and no proton exchange at C-6 is detectable. In contrast, the *Z*-vinyl homophosphonate **6** inhibits the enzyme even better than the saturated analogue **3** ($K_i \sim 0.16$ μM), is oxidized to the same extent, and undergoes C-6 proton exchange at the same rate as does **3**. The *Z*-vinyl homophosphonate **6** is thus the only analogue that is *both* tightly bound *and* exchanges its C-6 proton with the solvent.[13]

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(8) The carbocyclic analogue series was originally designed to complement the 2-deoxy substrate analogues,[6] neither of which can suffer ring opening after the elimination of P_i. Satisfactory spectroscopic data were obtained for all new compounds. Details of the synthetic work will be published elsewhere.

(9) At pH 7.5, 20 °C, in 50 mM potassium 3-(*N*-morpholino)propanesulfonate buffer containing $CoSO_4$ (50 μM), NAD^+ (10 μM), and substrate, using the *E. Coli* enzyme (Frost, J. W.; Bender, J. L.; Kadonaga, J. T.; Knowles, J. R. *Biochemistry* **1984**, *23*, 4470) coupled to dehydroquinase (1 unit) and monitored at 234 nm. Earlier reports (e.g. ref 10) of higher K_m values appear to derive from the use of a less sensitive assay method and the difficulty of obtaining true initial rates at the low substrate concentrations required.

(10) This behavior parallels that shown earlier for the pyranose derivatives: Le Maréchal, P.; Froussios, C.; Level, M.; Azerad, R. *Biochem. Biophys. Res. Commun.* **1980**, *92*, 1104.

(11) The rate of proton exchange is approximately 0.1% of k_{cat}.

(12) The formation of enzyme-bound NADH is observed at 340 nm. The proportion of the enzyme that contains NADH and oxidized substrate analogue seems to be related to the redox potential of the analogue and approaches 100% for **3**, **4**, and **6**.

(13) Although molecular mechanics calculations show that the charged groups of **4** and **6** are positioned similarly, the attack trajectory required for an intramolecular deprotonation in **4** and that required for intramolecular deprotonation in **6** are substantially different. The barrier for proton transfer via a five-membered ring may be more than 3 kcal/mol higher than that for a six-membered ring (Menger, F. M. *Tetrahedron* **1983**, *39*, 1025).

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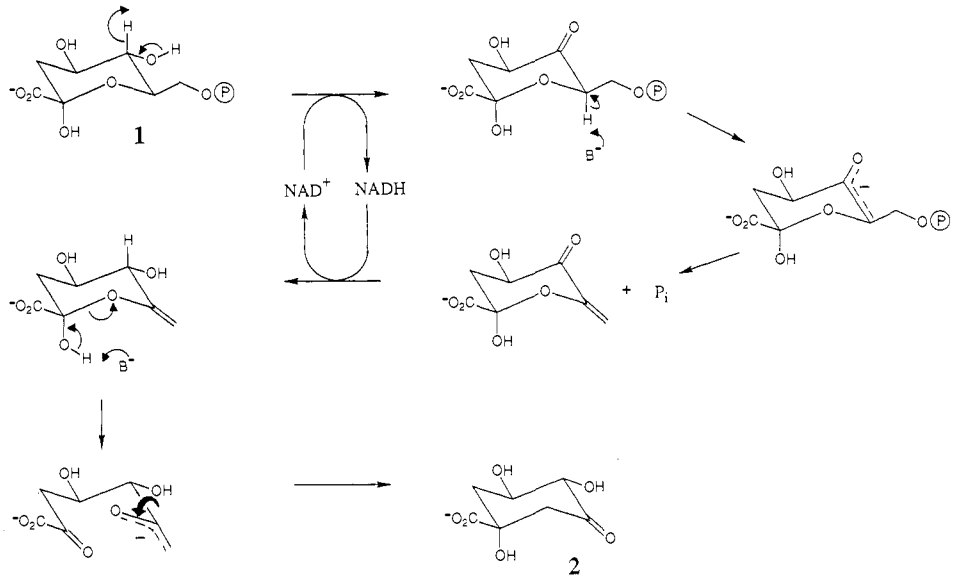
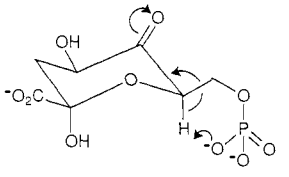
Scheme I. Suggested Mechanistic Pathway for Dehydroquinate Synthase



All these results are simply accommodated by postulating that the base responsible for removal of the proton at C-6 is a peripheral oxygen of the phosphate group of the substrate DAHP itself (or, for **3** and **6**, of the analogous phosphonate group). This suggestion (i) requires the enzyme to bind a conformer of DAHP that is mimicked by **6**, accounting for the relatively tight binding of this analogue; (ii) explains the fact that the analogue **4** does not undergo exchange even though it is tightly bound; and (iii) explains why the *E*-vinyl homophosphonate **5** is poorly bound and does not suffer either oxidation or exchange.

This proposal has several attractive features. First, the enzyme exploits one of the strongest bases available at physiological pH: the dianionic phosphate ester group. Second, the enzyme avoids the steric problem associated with deprotonating a tertiary center where the proton is 1,3 diaxial to a hydroxyl group (at C-2). Third, the difficulty of bringing an enzymic base close to the charged phosphate side chain is side-stepped. Finally, the act of substrate deprotonation produces a better leaving group for the (presumably subsequent) departure of P_i. Several of these features have gratifying precedent, for example in the facile intramolecularly-catalyzed elimination of P_i from 3-hydroxypropionaldehyde phosphate.[14]

The present proposal, coupled with the recent suggestion of Bartlett and his group[5] that the pyranose ring opening and the aldol reaction (the last two steps of Scheme I) may occur off the enzyme, helps to explain how a relatively small monomeric protein can catalyze such a complicated molecular transformation. Oxidation of DAHP (bound as in **7**) at C-5 results in the facile



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elimination of P_i to give the ene-one, reduction of which provides the enol pyranose that is then lost from the enzyme and rearranges rapidly and stereospecifically to DHQ. Although the evidence presented herein is only suggestive, the possibility that the phosphate group of DAHP promotes the β-elimination of P_i both accommodates all that we know about the mechanism of this unusual enzyme and reduces the number of required catalytic groups to what might reasonably exist at the active site of the enzyme. What appeared at first sight to be an impressively complex mechanism may, in fact, be ingeniously simple.

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Symmetric Addition of SO_2 to Linear Bi- and Trinuclear Gold(I) Compounds. Partial Oxidation To Form $[Au(\mu\text{-}(CH_2)_2PPh_2)]_2(SO_2)_2$ and $Au_2Pt(\mu\text{-}C,S\text{-}CH_2PPh_2S)_4(SO_2)_2$

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A gold-gold bond forms when $[Au(\mu\text{-}(CH_2)_2PPh_2)]_2$ (Au···Au = 2.977 (1) Å) is oxidized to $[Au(\mu\text{-}(CH_2)_2PPh_2)]_2XY$ (XY = Cl_2, CH_3Br, CF_3CH_2I, $(NO_2)_2$, etc.) (Au–Au = 2.55–2.7 Å).[1,2] Metal–metal bond formation may occur during the initial step of the oxidative addition[3] or subsequent to metal–ligand bond formation. To further explore possible metal–metal bond formation related to this initial step, we have crystallized and structurally studied the adduct of SO_2 with $[Au(\mu\text{-}(CH_2)_2PPh_2)]_2$. SO_2 coordinates axially as a Lewis acid by removing electron

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