respectively, of ferryltetraphenylporphyrin, OFe(TPP), which is formed by the cleavage of dioxygen of $Fe(TPP)O_2$ via laser photolysis. The observed isotopic shift (852 - 818 = 34 cm^{-1}) is in good agreement with that expected for a perturbed FeO molecule (38 cm^{-1}). These bands cannot be attributed to the μ-oxo dimer, $(Fe(TPP))_2O$, since no strong Raman bands are seen near 360 cm^{-1}.[4]

Further support for our conclusion is provided by ^{NA}Fe-^{54}Fe isotope substitution. As are shown by the broken lines of Figure 1, A and B, the bands at 852 and 818 cm^{-1} of $^{16}O^{NA}Fe(TPP)$ and $^{18}O^{NA}Fe(TPP)$, respectively, are shifted by 4.0 cm^{-1} to higher frequencies by the substitution, and these values are again in good agreement with those expected for a perturbed FeO molecule (3.5 cm^{-1}).

A simple diatomic approximation gives a force constant of 5.32 mdyn/Å for the above ferryl group. This is much larger than that of the Fe-O bonds in $(Fe(TPP))_2O$ (3.8 mdyn/Å)[4] and in oxyhemoglobin (3.09 mdyn/Å).[5,6] In this respect, the formulas such as $PFe^{IV}{=}O^{2-}$ or $PFe^{V}{=}O^{2-}$ describe the ferryl group better than $PFe^{III}{-}O^{-}$ or $PFe^{IV}{-}O^{-}$ (P: porphyrin).

According to recent ab initio MO calculations,[7] the negative charge and polarization of the dioxygen greatly increase upon coordination to an iron porphyrin (i.e., $Fe{-}O_1(-0.46e){-}O_2(-0.19e)$). This trend will be accelerated by the donation of the second electron from NADH to the iron center and the presence of a cysteinyl sulfur (S^-) at the trans position to the dioxygen in cytochrome P-450. It is then not surprising that the O-O bond cleavage occurs quite easily under biological conditions. We are now conducting experiments to answer the question of whether we can mimic the hydroxylation reaction of cytochrome P-450 in a matrix environment.

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Unique, One-Step, Double Isomerization (2*E*,4*Z* ⇌ 2*Z*,4*E*) of 6-Oxo-2,4-heptadienoic Acid Catalyzed by Maleylacetone Cis-Trans Isomerase

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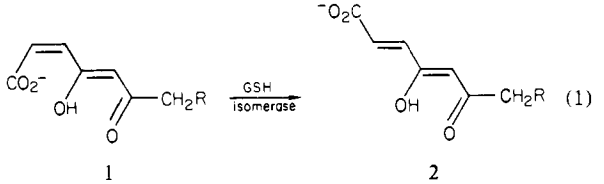
Maleylacetone cis-trans isomerase, which catalyzes the reactions shown in eq 1,[1] requires glutathione (GSH) as a coenzyme.[2]

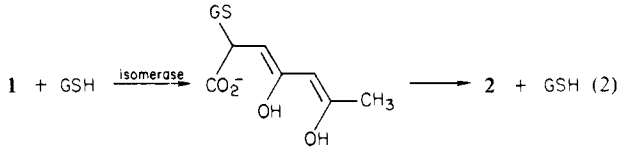


(1)

1 2

a, R = H; b, R = CO_2^-

Previous studies indicate that GSH binds to the enzyme along the backbone of the tripeptide, pointing its SH group away from the enzyme's surface.[3] In the enzymatic and nonenzymatic reaction, reversible nucleophilic addition of GSH to C2 of **1** forming a dienediol intermediate (**3**) thereby allows internal rotation about the C2-C3 bond; ketonization with expulsion of GSH provides **2** (eq 2).[4]

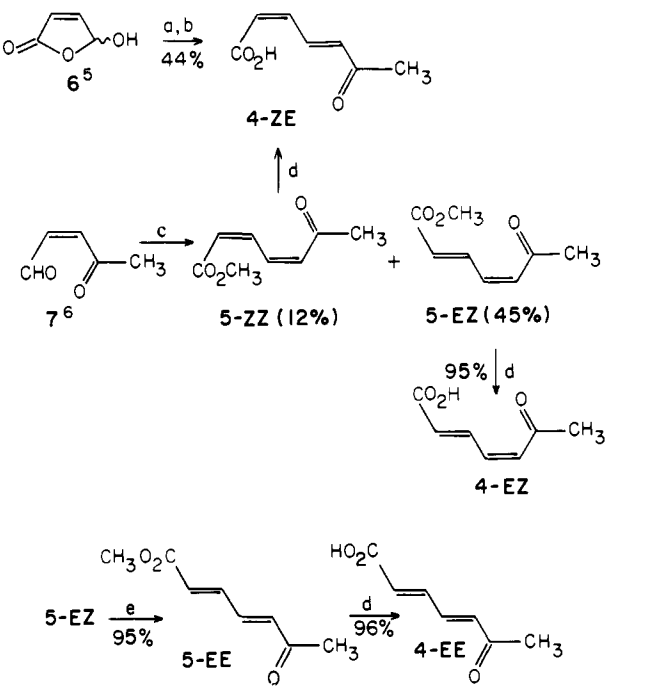


3

To examine the structural requirements of the enzyme, 6-oxo-2,4-heptadienoic acids (**4**) and methyl esters **5** were synthesized (Scheme I) to provide the four possible cis-trans isomeric skeletons, only one of which was reported previously. Base-catalyzed hydrolysis of the esters **5**-*EZ* and **5**-*EE* yielded the corresponding acids. Repeated attempts to hydrolyze methyl 6-oxo-2(*Z*),4(*Z*)-heptadienoate (**5**-*ZZ*), however, led only to the **4**-*ZE* acid. The routes of synthesis (Scheme I) and the NMR spectra[7] establish the structures of these new compounds.

As expected, **4**-*ZE* acid is isomerized to **4**-*EE* by the enzyme and obligatory GSH.[8] The K_M for **4**-*ZE* is 3.2×10^{-3} M vs. 8.4×10^{-4} M for maleylacetone;[3,9] k_{cat} is ~0.36 times that for maleylacetone.

Scheme I[a]



[a] (a) NaH/THF; (b) $(C_6H_5)_3P{=}CHCOCH_3$; (c) $(C_6H_5)_3P{=}CHCO_2CH_3$/THF; (d) t-C_4H_9OH/1 N NaOH (1:10), 0 °C, 4 min; (e) I_2/THF, reflux.

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(7) See supplementary material.
(8) A highly purified preparation of the vibrio 01 maleylacetone isomerase (specific activity 23 units/mg) was obtained by affinity chromatography on GS-agarose.[3]
(9) The kinetics of isomerization were followed by HPLC analysis on a C-18 column (1% acetic acid, 5% acetonitrile, 94% water or 2.5 mM tetra-*n*-butyl ammonium phosphate in 93.3% 0.01 M phosphate buffer, pH 7.4, 6.7% acetonitrile).

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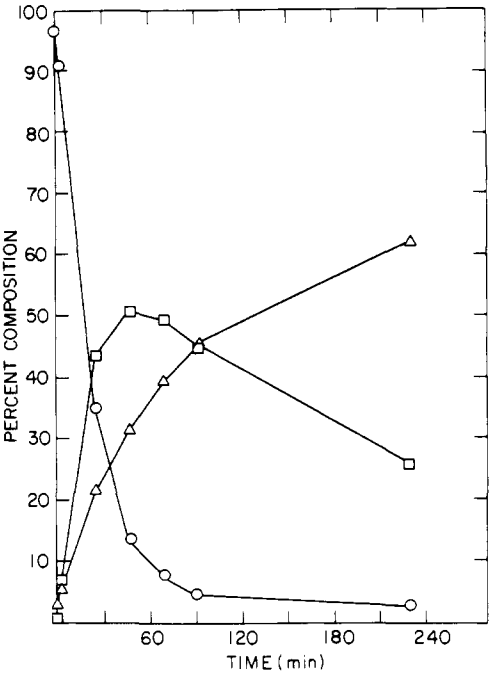
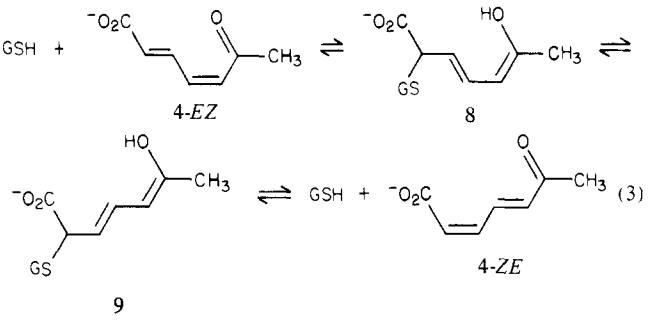


Figure 1. Typical kinetics of the maleylacetone cis–trans isomerase catalyzed isomerization of 6-oxo-2,4-heptadienoic acids at pH 7.4 in the presence of coenzyme, glutathione. Note the early, predominant formation of 4-*ZE* from 4-*EZ*. Circles, 4-*EZ*; squares, 4-*ZE*; triangles, 4-*EE*.

Surprisingly, the isomerase also processes 4-*EZ*. Moreover, the product generated is remarkable because *the enzyme converts 4-EZ directly to 4-ZE; it catalyzes a double cis–trans isomerization.* The first product formed in the enzyme-catalyzed isomerization of 4-*EZ* is 4-*ZE*; 4-*EE* forms on a slower time scale (Figure 1). Similarly, 4-*EZ* is formed from 4-*ZE* when GSH and the isomerase are present.

The K_M for 4-*EZ* is 1.9×10^{-3} M. The enzyme processes 4-*EZ* ~1.6 times faster than maleylacetone. When 4-*EZ* is the initial enzyme substrate, 4-*EE* and 4-*ZE* form in a branching ratio of 1:3. 4-*EE* and 4-*EZ* form, however, in a branching ratio of approximately 2.7:1 when 4-*ZE* is the initial substrate. A calculated equilibrium constant,[10] [4-*ZE*]/[4-*EZ*] ≃ 21, compares reasonably with a measured value of 16 ± 7 for the enzyme equilibration.

Since the enzyme-catalyzed double isomerization requires GSH and substrate maleylacetone inhibits enzymatic processing of either 4-*ZE* or 4-*EZ*, the same active site is probably used by all three substrates. The mechanism for this extraordinary transformation remains to be elucidated, but we note here that it could be accommodated by a mechanism parallel to that for isomerization of maleylacetone (eq 2). As with maleylacetone, enzyme-bound GSH could add to C2 of enzyme-bound 4-*EZ* or 4-*ZE* to form a dienol intermediate (eq 3). If the carboxyl, the GS backbone, and the enolic oxygen are utilized for binding to the enzyme then the termini of the intermediate would be relatively immobile. Internal concerted rotation about the C2–C3 and the C4–C5 single bonds could still occur, however, provided that the enzyme topology does not interfere.[12,13] Rotation in this way would cause the two



dienol intermediates, **8** and **9**, to equilibrate and subsequent ketonization with expulsion of GSH would provide 4-*EZ* and 4-*ZE*, respectively. Formation of 4-*EE* from either analogue could occur from the same dienol intermediate by suggesting that the enolic oxygen binding is weak. If the intermediate were held only at the GS/carboxyl terminus, the relaxation to the 4-*EE* geometry would appear to have a strong driving force. Consistent with this is the observation that 4-*EE* is the sole product of nucleophilic catalysis of isomerization of 4-*EZ* or of 4-*ZE* by thiocyanate ion at the same pH.

The present results provide support for proposals which suggest that the enzyme binds maleylacetone in the region of the 6-oxo function as well as by its carboxyl group.[1]

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Supplementary Material Available: A table of vinyl ^{1}H NMR chemical shifts and coupling constants for 4-*EZ*, 4-*ZE*, 5-*ZZ*, and 5-*EE* (1 page). Ordering information is given on any current masthead page.

(10) If 4-*EZ* equilibrates with 4-*ZE* through several intermediates it can be shown[11] that the equilibrium constant, [4-*ZE*]/[4-*EZ*], equals $(V_{max,4\text{-}EZ}/V_{max,4\text{-}ZE})(K_{M,4\text{-}ZE}/K_{M,4\text{-}EZ})C$, where C is a correction factor, (3/4)/(1/3.7), to take account of the branching ratio for each substrate.

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(12) During rotation (**8** ⇌ **9**) some small lateral movement of one terminus with respect to the other is necessary.

(13) Similar molecular dynamics have been postulated in a model for the photoconversion of 11-*cis*-retinal in rhodopsin to its all-trans isomer by a concerted rotation ("bicycle pedal motion") in which the cis double bond migrates toward the Schiff base terminus (Warshel, A. *Nature (London)* **1976**, *260*, 679).

Intramolecular Long-Distance Electron Transfer in Radical Anions. The Effects of Free Energy and Solvent on the Reaction Rates[1]

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As an extension of the work on long-distance *inter*molecular electron transfer (ET) in rigid matrices carried out by one of us[2-5] for some time, we have embarked on a study of *intra*molecular long-distance ET in fluid solution. In a recent communication we have reported not only that such reactions can occur over considerable distance (~10 Å) but also that, given the appropriate driving force (exothermicity), the observable rates can be very fast indeed.[6] In this communication we wish to demonstrate the dependence of the ET rates on the exothermicity of the reaction and report a remarkable solvent dependence of the observed rates. The rates show a strong deviation from the classical Brønsted

(1) Work was supported by US-DOE Contract Number W31 109 ENG-38 (Argonne) and NSF Grant CHE-8218164 (University of Chicago).

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