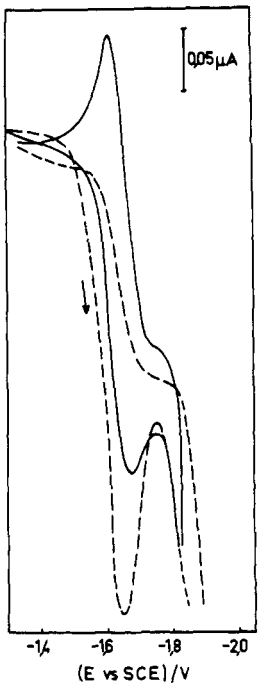

Figure 3. Cyclic voltammograms of 4^+ (—) and $Cu(7)_2^+$ (------) in DMF; 0.1 M $N(C_4H_9)_4^+ \cdot ClO_4^-$; room temperature; mercury cathode; scan rate = 10 mV/s.

preparation of a formally copper(0) complex of exceptional stability.

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Formation of Ferryltetraphenylporphyrin by Laser Irradiation

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The crucial step in the reaction cycle of cytochrome P-450 is the formation of the ferryl group via the cleavage of dioxygen bonded to iron protoporphyrin. The main purpose of this communication is to report the first observation of the resonance Raman (RR) spectra of ferryltetraphenylporphyrin, OFe(TPP), which was formed unexpectedly during our measurement of the RR spectra of $Fe(TPP)O_2$ in pure O_2 matrices at ~15 K.

A stable, "base-bound" complex, $Fe(TPP)(pip)_2$ (pip = piperidine), was placed in a miniature oven under the cold tip of our matrix isolation Raman apparatus.[2] The oven was heated at ~180 °C under vacuum until the vacuum gauge indicated complete dissociation of the base from the complex. The "base-free" Fe(TPP) thus obtained was vaporized by heating the oven at ~225 °C by laser beam and cocondensed with pure oxygen on the inclined surface of the cold tip which was cooled to ~15 K by a CTI Model 21 closed-cycle helium refrigerator. As our previous matrix-isolation IR studies[3] indicate, such cocondensation reactions produce five-coordinate, "base-free" $Fe(TPP)O_2$. Resonance Raman spectra of the cocondensation

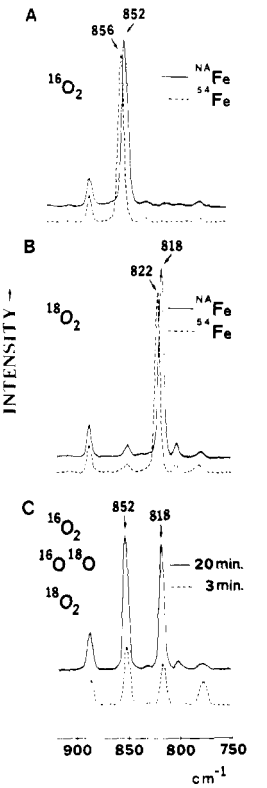

Figure 1. Resonance Raman spectra of Fe(TPP) cocondensed with dioxygen at ~15 K (406.7-nm excitation, 1-2 mW). (A) $^{NA}Fe(TPP)$ with $^{16}O_2$ (solid line) and $^{54}Fe(TPP)$ with $^{16}O_2$ (broken line). (B) $^{NA}Fe(TPP)$ with $^{18}O_2$ (solid line) and $^{54}Fe(TPP)$ with $^{18}O_2$ (broken line). (c) $^{NA}Fe(TPP)$ with isotopically mixed dioxygen ($^{16}O_2/^{16}O^{18}O/^{18}O_2$ = 1/2/1). The solid and broken lines indicate that spectra after 20 and 3 min of laser irradiation, respectively.

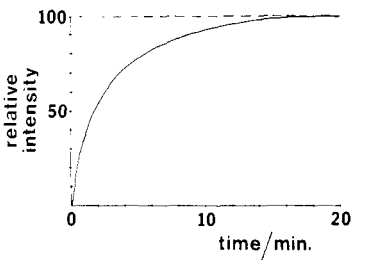

Figure 2. A plot of relative intensity of the 852-cm^{-1} band of $^{16}O^{NA}Fe(TPP)$ vs. time of laser irradiation.

products were measured using a Spex Model 1401 double monochromator in conjunction with a Spectra-Physics Model 164-01 Kr ion laser. Throughout this investigation, the samples were excited with 1-2 mW of the 406.7-nm radiation (Soret excitation).

The solid line of Figure 1A shows the RR spectrum of $^{NA}Fe(TPP)$(^{NA}Fe: Fe in natural abundance, 92% pure ^{56}Fe) cocondensed with $^{16}O_2$. It shows a strong signal at 852 cm^{-1} in addition to the normal Fe(TPP) bands. The intensity of this band is time dependent, reaching the maximum after about 20 min. This intensity remains constant as long as the matrix temperature is kept at ~15 K. The intensities of all other bands remain almost unchanged during this period. A plot of the relative intensity of the 852-cm^{-1} band vs. the time of laser irradiation is shown in Figure 2. It is clear that the above photolysis follows the first-order kinetics.

The 852-cm^{-1} band was shifted to 818 cm^{-1} in an $^{18}O_2$ matrix (Figure 1, trace B). Similar experiments with an isotopically scrambled oxygen ($^{16}O_2/^{16}O^{18}O/^{18}O_2$ = 1/2/1) produced two bands at 852 and 818 cm^{-1} as shown in trace C. (The broken line in trace C indicates the spectrum obtained after 3 min of laser irradiation). These results indicate quite clearly that the 852- and 818-cm^{-1} bands are due to the $\nu(^{16}O\text{-Fe})$ and $\nu(^{18}O\text{-Fe})$,

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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.

Acknowledgment. This work was supported by a National Science Foundation Grant (PCM-8114676).

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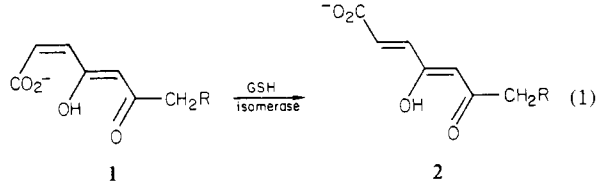
Maleylacetone cis–trans isomerase, which catalyzes the reactions shown in eq 1,[1] requires glutathione (GSH) as a coenzyme.[2]

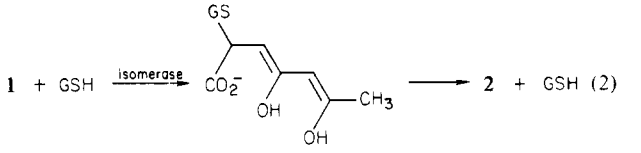


(1)

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]

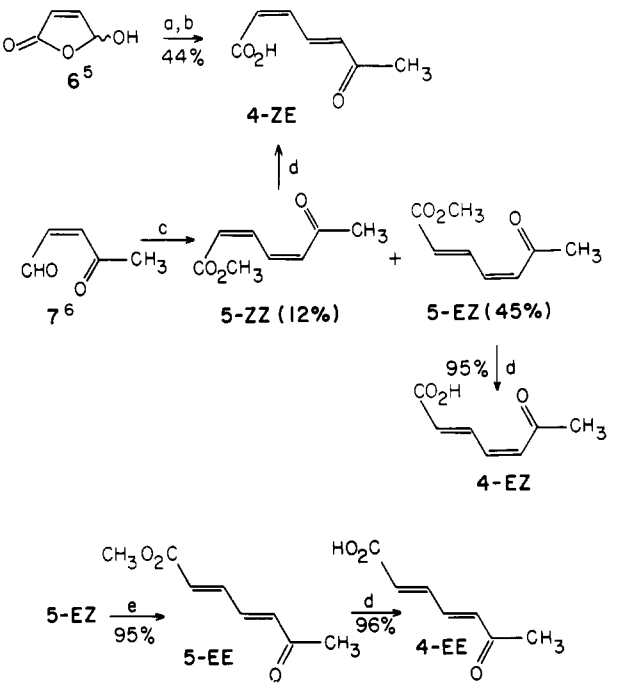


3

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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