enantiomeric enrichment involve the destruction of the sp^3-hybridized asymmetric carbon atom, thereby creating the sp^2-hybridized carbon followed by regeneration of the original sp^3-hybridized carbon atom under the asymmetric environment. For instance, proton abstraction from the asymmetric carbon atom followed by enantioselective protonation has been reported for α-amino acid derivatives[12] and α-substituted carbonyl compounds.[13] Enamine formation followed by hydrolysis with chiral acids has been used for the enantiomeric enrichment of α-substituted carbonyl compounds.[14,15] All of those reported methods involve the enantioface differentiation by protonation. The present method provides the first example of the enantiomeric enrichment in which the sp^3-hybridized carbon is not destroyed throughout the overall process.

The method for the asymmetric lactonization described here can be applied to other hydroxy dicarboxylates and dihydroxy monocarboxylates possessing σ-symmetry. Further studies along these lines are currently under way.

Supplementary Material Available: Tables of crystal data, bond lengths, bond angles, atomic coordinates, and thermal parameters for the (*S*)-(−)-α-methylbenzylamine salt of (*S*)-(−)-**2** and (1*S*)-(+)-10-camphorsulfonic acid monohydrate (5 pages). Ordering information is given on any current masthead page.

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Resonance Raman Scattering from Horseradish Peroxidase Compound I

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Horseradish peroxidase (HRP) catalyzes the oxidation of various substrates in the presence of hydrogen peroxide by forming sequential intermediates, compounds I and II (HRP-I and HRP-II).[1] The active site of resting HRP contains ferric protoheme, which is ligated in one axial position by histidine nitrogen. HRP-I is formed by the reaction of hydrogen peroxide with the resting enzyme and is two oxidation equivalents above the native ferric state. While one oxidation equivalent is thought to be located on the metal center as a low-spin ($S = 1$) oxyferryl structure ($Fe^{IV}O$),[2] the other equivalent most likely resides on the porphyrin ring as a π cation radical.[3] The second intermediate, HRP-II, is a single oxidation equivalent above the ferric state and retains the low-spin oxyferryl center.

Though resonance Raman (RR) techniques have been applied to the ferrous and ferric enzyme[4] and to HRP-II,[5-10] Raman data for HRP-I have been difficult to obtain owing to the reactivity and photolability of the first intermediate.[11] Cryogenic techniques may be insufficient to stabilize HRP-I to laser irradiation and past attempts to obtain RR measurements of HRP-I[9] resulted in photoreduction to a mixture of HRP-II, ferric, and ferrous species.[12] Thus a reliable RR spectrum of HRP-I has not yet

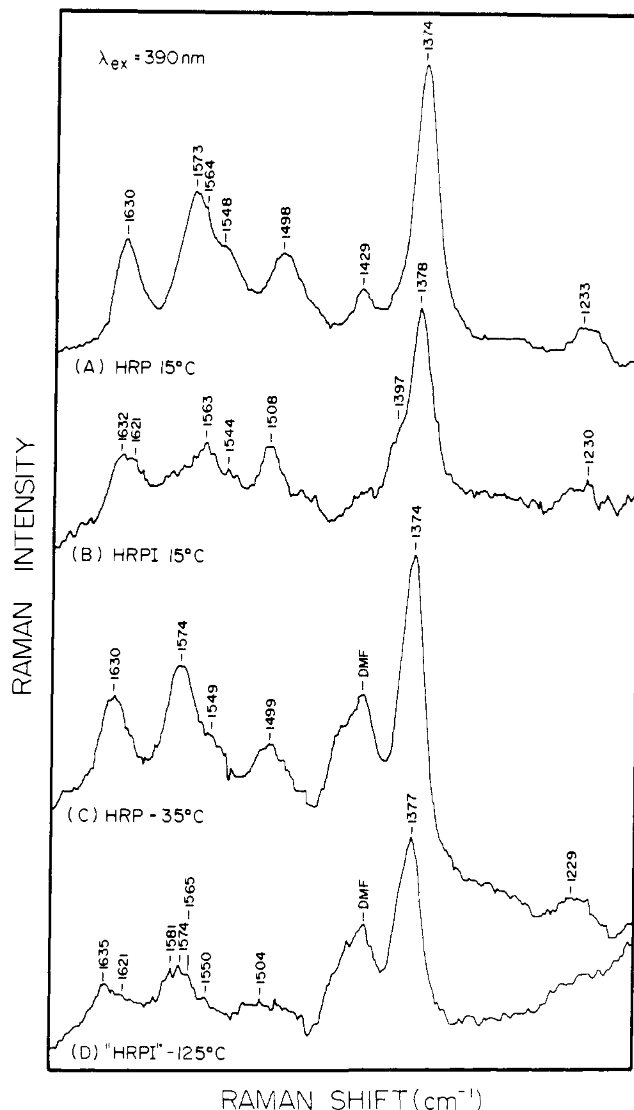

Figure 1. RR spectra of native HRP and HRPI, pH 7.2, excited at 390 nm (10-ns pulses, 10 Hz): (A) native HRP, 0.1 mM, 50 mM phosphate, 15 °C, 90° scattering, 10–15 mW, 25-min acquisition time; (B) HRPI, 0.05 mM, 50 mM phosphate, 15 °C, 90° scattering, 10–15 mW, sum of eight 5-min runs; (C) native HRP, 0.6 mM, 66% DMF, 34% 50 mM phosphate, −35 °C, spinning EPR tube, backscattering, 20 mW, 12 min; (D) HRPI, 0.6 mM, generated at −40 °C in 66% DMF, 34% 50 mM phosphate by the reaction of resting HRP with a slight excess of H_2O_2; spectra were recorded at −125 °C by backscattering from a spinning EPR tube with vertical translation of the sample in the laser beam; 15 mW, 6 min.

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Table I. RR Frequencies (cm^{-1}) of HRP Species

	native HRP[a,b]	HRP·CN[b]	HRPII[b]	HRPI[a]
ν_4	1374	1375	1382	1378
ν_3	1498	1506	1508	1508
ν_{10}	1630	1642	1641	1632

[a]This work. [b]From ref 5.

been reported. We have avoided the problems noted above by using pulsed, near-UV laser excitation of a flowing sample of HRP-I, which was generated by rapid mixing of native HRP with hydrogen peroxide. The flow rate ensured that each laser pulse used in accumulating the spectrum reported here was incident on a fresh aliquot of sample.

Oxidation of HRP (Sigma type VI)[13] to HRP-I was carried out by rapid mixing of equal volumes of cooled, 50 mM phosphate (pH 7.2) solutions of 0.1 mM HRP and 1 mM hydrogen peroxide. Peroxide solutions were prepared by dilution of 30% hydrogen peroxide (Mallinckrodt) and quantified by assuming an extinction coefficient at 240 nm of 43.6 M^{-1} cm^{-1}.[14] Solutions were driven through two eight-jet tangential mixers in series[15] at flow rates of 0.65–0.85 mL/min by using a SAGE 355 syringe pump. After mixing, the HRP-I sample passed through a 0.5-mm-i.d. quartz capillary. The time between mixing and laser excitation was 2.4–3.1 s.[16] Raman scattering was collected with a Spex 1459 Illuminator, dispersed in a Spex 1877 Triplemate, and detected and analyzed by using an EG&G PAR 1420 diode array detector and its associated OMA II electronics. Laser irradiation at 390 nm (10-ns pulses, 1.0–1.5 mJ per pulse, 10 Hz) was provided by pumping Exciton LD390 laser dye in a Quanta Ray Pulsed Dye Laser (PDL) with the third harmonic output (355 nm) from a Quanta Ray Nd:YAG DCR1A laser. Sample flow rate, spatial beam width, and capillary geometry were such that 2–3 complete scattering volumes passed through the capillary between pulses.

Figure 1, parts A and B, shows RR spectra of flowing samples of HRP and HRP-I, respectively, at 15 °C. In the HRP-I spectrum, the absence of the strong bands of the native enzyme at 1498 (ν_3) and 1573 cm^{-1} (ν_2) and the shifted position of ν_4 (1378 cm^{-1}) show that reaction with peroxide was complete. The absence of bands at 1587 (ν_2), 1382 (ν_4), and 1641 cm^{-1} (ν_{10}), which are characteristic of HRP-II,[5] as well as the predominantly HRP-I absorption spectra we recorded for the samples after each RR measurement,[17] shows that contributions from HRP-II were minimal. The experiment was repeated with pulsed laser excitation at 420 nm, the absorption maximum of HRP-II, and ν_4 remained at 1378 cm^{-1} (not shown). This provides confirmation that HRP-II was not present in significant amounts and, excluding the possibility of a pre-HRP-I species,[18] we can conclude that the spectrum presented here represents scattering from HRP-I.

We have also explored an alternative, low-temperature technique to stabilize HRP-I and record its Raman spectrum. The intermediate was generated in 66% dimethyl formamide as described by Douzou et al.[19] Figure 1D shows the RR spectrum of this species recorded at −125 °C in a spinning 3-mm tube. Though photoreduction to HRP-II and native HRP is evidenced by the features at 1581 and 1574 cm^{-1}, respectively, and the position of ν_{10} at 1635 cm^{-1} is high owing to contributions from HRP-II (ν_{10} = 1641 cm^{-1}), the 1377 cm^{-1} maximum of ν_4[20] and the feature at 1565 cm^{-1} represent significant scattering from HRP-I. This spectrum confirms the general features of the HRP-I spectrum in Figure 1A but indicates that photoreduction artifacts persist even to fairly low temperature.

In order to assess the effects of ring oxidation on the high-frequency porphyrin vibrational modes,[21] it is useful to compare frequencies observed for HRP-I with those of native HRP, HRP-II, and HRP–cyanide (Table I). The similar frequencies for the core size markers, ν_3 and ν_{10}, in HRP–cyanide and HRP-II indicate a low-spin iron for both species. For HRP-II, the high value of the oxidation state marker, ν_4, at 1382 cm^{-1}, reflects the influence of the $Fe^{IV}O$ unit on the π^* population of the ring. Thus, oxidation of the iron to the ferryl state simply causes an increase in ν_4 and little change in the high-frequency core-size marker bands, ν_3 and ν_{10}. Upon oxidation of the ring an electron is lost, presumably from the porphyrin a_{2u} π orbital.[22] Since this orbital has electron density centered on the nitrogens and methine carbons, bonds involving these atoms are expected to be weakened and normal modes to which they contribute should, in general, decrease in frequency.[9] Owing to a high percentage of $\nu(C_\alpha\text{–}N)$ character,[23] the frequency decrease in ν_4 between HRP-II (1382 cm^{-1}) and HRP-I (1378 cm^{-1}) is consistent with depopulation of the a_{2u} orbital HRP-I. Similarly, loss of electron density from the methine carbon may be reflected in the frequency decrease in the ν_{10} mode, which is predominantly $\nu(C_\alpha\text{-}C_m)$ in character. We have observed qualitatively similar frequency shifts in Soret-enhanced, high-frequency modes for the series $Co^{II}OEP$, $Co^{III}OEP{\cdot}ClO_4^-$, $Co^{III}OEP^{+}{\cdot}2ClO_4^-$, which models the $HRP{\cdot}CN^-$, HRP-II, HRP-I set.[24] Oxidation of the ring to the $^2A_{2u}$ state occurs upon going from the second to the third members of each series and in both the ν_{10} and possibly ν_4 frequencies decrease.

Though cryogenic RR measurements on HRP-I are complicated by photoreduction, rapid mixing techniques produce spectra of HRP-I free of these complications. Frequency shifts between RR modes of HRP-II and HRP-I are consistent with loss of an electron from the a_{2u} orbital. Changes in electron density in the a_{2u} orbital, along with possible structural differences between HRP-II and HRP-I,[18] influence the RR spectra of these species.

Acknowledgment. We thank Prof. M. Zerner for communicating his calculations on metalloporphyrin cation radicals in advance of publication. Thanks to Deak Watters for machining the mixers. This research was supported by NIH (GM25480). T.O. was a Yates Memorial Summer Fellow.

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