Low-Temperature Magnetic Circular Dichroism Studies of the Photoreaction of Horseradish Peroxidase Compound I†

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ABSTRACT: Horseradish peroxidase (HRP) compound I is photolabile at all temperatures between room temperature and 4 K. The photoredox reaction has been studied in frozen glassy solutions by using optical absorption and magnetic circular dichroism spectra following photolysis of HRP compound I with visible-wavelength light at 4.2 and 77 K. The photochemical process is characterized as a concerted two-electron transfer reaction which results in the conversion of the Fe(IV) heme π-cation radical species of HRP compound I into a low-spin Fe(III) heme species. This reaction occurs even when photolysis is carried out at 4.2 K. Spectra recorded between 4.2 and 80 K for the low-spin ferric hydroxide complex of HRP closely resemble the data measured for the photochemical product. The proposed mechanism for the photoreaction is

$$\underset{\text{HRP I}}{[\text{O=Fe(IV) heme}^{\bullet+}]} \xrightarrow{h\nu\ (+2e^-)} \underset{\text{low-spin ferric HRP}}{[\text{Fe(III) (OH}^-\text{) heme}^0]}$$

No evidence is found for the formation of an Fe(II) heme at these temperatures.

Horseradish peroxidase (HRP) (EC 1.11.1.7) catalyzes the oxidation of various substrates in the presence of hydrogen peroxide by forming, sequentially, two intermediates, namely, compounds I and II (HRP I and HRP II) (Dunford & Stillman, 1976), according to the following scheme:

$$\text{HRP} + \text{H}_2\text{O}_2 \rightarrow \text{HRP I}$$

$$\text{HRP I} + \text{RH} \rightarrow \text{HRP II} + \text{R}^{\bullet}$$

$$\text{HRP II} + \text{RH} \rightarrow \text{HRP} + \text{R}^{\bullet}$$

An almost complete picture has emerged with respect to the electronic environment of the heme in the native enzyme, as well as in the compound I and compound II species, from the collation of information from a variety of spectroscopic techniques (Dolphin et al., 1973; Fajer et al., 1973; Schulz et al., 1979, 1984; Browett & Stillman, 1981a,b; Chance et al., 1984, 1986; Van Wart & Zimmer, 1985; Browett et al., 1988). Currently, the heme iron in compound I is assigned as a ferryl [Fe(IV)=O] species, and the porphyrin group is assigned as a π-cation radical structure; with this arrangement the ground state of HRP compound I will have three unpaired electrons. The formation of HRP compound II involves a one-electron reduction of the porphyrin π-cation radical to the neutral porphyrin group, leaving the heme iron with the ferryl structure and $S = 1$ spin state.

Much of the success of these assignments arises from previous studies in which comparisons were made between spectroscopic data for the protein and model compounds that mimic a specific part of the heme group. Thus, the first suggestions that the heme π ring was oxidized to a 17-electron system came from a series of studies on the optical and electron paramagnetic resonance (EPR) properties of synthetic porphyrins by Dolphin and Fajer (Dolphin et al., 1973; Fajer et al., 1973). Further detailed support for the existence of a heme π-cation radical species was obtained by comparing the magnetic circular dichroism (MCD) spectra of the compounds I and II of catalase and HRP (Browett & Stillman, 1981a) with a series of model porphyrin compounds (Browett & Stillman, 1981b). Model iron porphyrin compounds Fe(IV)P$^{\bullet+}$, have also been used successfully to mimic the electronic structure and chemical properties of the iron component of HRP I (Groves et al., 1981; Boso et al., 1983) and confirm the existence of the ferryl structure for the HRP I iron heme complex. A similar electronic structure has been proposed for compounds I and II of catalase (Browett & Stillman, 1980, 1981a).

HRP compound I has been shown to be photolabile both at room temperature and in the frozen solution (Stillman et al., 1975a,b). This photochemical reaction appeared to provide the first example of a light-induced redox reaction of a hemeprotein complex. The photoaction spectrum matched that of the HRP I heme absorption, suggesting that the reaction involved the π system of the porphyrin ring. The optical absorption spectrum of the photolytic product was similar to that measured for HRP II at all temperatures (4–100 K) of photolysis. At room temperature in solution, the photolysis exhibits complicated kinetics, and the native HRP is recovered 24 h following the photolysis. This highly efficient photolysis of HRP I with visible light presents a serious problem in the spectroscopic analysis of the HRP I. Recent resonance Raman studies of frozen solutions of HPR I at −5 °C (Van Wart & Zimmer, 1985) have confirmed the original reports (Stillman et al., 1975a,b) that compound I undergoes photoreduction upon laser irradiation and that this process is strongly dependent on the wavelength of the light used. The resonance Raman spectra of the photolyzed, frozen (−5 °C) samples of HRP I (Van Wart & Zimmer, 1985) have shown resonances that have been ascribed to the presence of both ferric and ferrous forms of the enzyme. The appearance of the ferrous

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form of the enzyme means that the photoreduction of HRP I at −5 °C would involve the transfer of as many as three electrons.

EPR measurements of the photolyzed HRP I below 100 K (McIntosh & Stillman, 1977; Chu et al., 1977; Nadezhdin & Dunford, 1979) have shown the presence of an intense signal that was first believed to be due to radicals located on the protein rather than on the heme group. An EPR signal, as strong as was observed in these studies at $g = 2.0025$, can arise from either a porphyrin π-cation radical or a nonspecific, protein-based radical. These results clearly suggested that the species formed at the end of the photolysis was quite different from HRP II. The EPR signal characteristics were found (McIntosh & Stillman, 1977) to be sensitive to the temperature at which the photolysis took place; photolysis at liquid helium temperatures resulted in sharper lines that changed to a broader signal upon annealing to 77 K. However, despite the availability of these optical and EPR spectra, the exact electronic structure of the photochemical product has not been determined. A closer examination of the optical absorption spectrum of the photochemical product recorded in low-temperature glasses (Law, 1978) indicated that the spectrum of the photochemical product did not match spectra previously reported for various oxidized states of HRP; in particular, the spectrum did not match that of HRP II closely enough for HRP II to be the final product.

In a series of studies, we have demonstrated that MCD spectroscopy is a powerful tool for the characterization of porphyrin π-cation radical species (Browett & Stillman, 1981a,b). In particular, we have shown that the MCD spectrum of the porphyrin π-cation radical species can be readily used to identify the ground-state configuration ($^2A_{1u}$ or $^2A_{2u}$) of the radicals (Browett & Stillman, 1981a,b). We have also used this technique to identify products of the photolytic reactions of porphyrins and phthalocyanines (Gasyna et al., 1984; Nyokong et al., 1987). The MCD technique offers a sensitive means of identifying the electronic configuration in the photolytic product. It has been well documented that the temperature dependence of the MCD spectrum can be used as an important tool in identifying Fe(III) porphyrin species (Browett, 1980; Browett et al., 1983a). In our studies on heme enzymes (Browett & Stillman, 1981a; Browett et al., 1983b–d), we have reported the low-temperature MCD spectra of a number of HRP compounds. In this present work, variable-temperature MCD spectroscopy is applied to study the spectral properties of the product of the photolysis of HRP compound I and a model low-spin ferric heme compound. The temperature and magnetic field dependent spectra give an insight into the details of the electronic structure of the photochemical product.

Experimental Procedures

Materials and Methods. Freeze-dried horseradish peroxidase (HRP) (Boehringer Mannheim; grade I, purity number $A_{403}/A_{280} = 3.3$) was prepared for use in a 1:1 v/v solution of 3 times distilled water and glycerol (Fisher). The HRP solution was pretreated with H_2O_2 (1.2:1 H_2O_2 to protein mole ratio) and allowed to stand at room temperature for 12–24 h before use. HRP compound I was made at 273 K by injecting a second aliquot of H_2O_2 in a 1.2:1 ratio into the solution. HRP compound II was prepared by the addition of 2 molar equiv of H_2O_2 to the HRP solution in H_2O–glycerol (1:1 v/v) containing 2 molar equiv of *p*-cresol at pH 10. The alkaline form of HRP in H_2O–glycerol was prepared by treating the solution with a pellet of KOH (Law, 1978). Magnesium octaethylporphyrin (MgOEP) was a gift from Dr. J. Fajer. Solutions of metalloporphyrins in CH_2Cl_2 were oxidized chemically by adding aliquots of Br_2 in CCl_4.

Solutions of HRP I and II were transferred into the optical cells immediately after preparation and were glassed by plunging the cells into liquid nitrogen. Compound I was photolyzed at either 77 (in liquid nitrogen) or 4.2 K (in liquid helium) by using white light (from a 300-W tungsten–halogen, Kodak projector lamp) that was passed through water in a glass cell to remove both IR and UV wavelengths.

Absorption spectra were obtained with CARY Model 219 and Model 17 spectrophotometers. The glassed sample was transferred into an Oxford Instruments CF204 optical cryostat precooled to 100 K. The temperature was monitored by using an Oxford Instruments CLTS temperature sensor. The MCD spectra were obtained on a circular dichroism (CD) spectrometer built in this laboratory (W. R. Browett and M. J. Stillman, unpublished results), and the magnetic field was obtained from an Oxford Instruments Model SM4 magnet at temperatures from 1.7 to 100 K. Temperatures in the range of 1.7–4.2 K were obtained by immersing the sample in a bath of liquid helium at a constant reduced pressure that was stabilized by using an Oxford Instruments manostat. The temperature was measured by using a calibrated carbon–glass sensor (Lake Shore Cryotronics Inc.). Depolarization of the circularly polarized light by the glassy samples was assessed by measuring the CD of an aqueous solution of dextro[tris(ethylenediamine)cobalt(III)] trihydrate which was placed before and after the sample. The magnetic field strength and sign were calibrated by using the visible-region band of aqueous $CoSO_4$, for which a value of $\Delta\epsilon_M = -1.9 \times 10^{-2}$ L mol^{-1} cm^{-1} T^{-1} at 510 nm was calculated.

Analysis of the Absorption and MCD Data. Digitized spectra were recorded. Each spectrum has had the appropriate base lines subtracted. Absorption spectra have been recalculated in units of ϵ (cm^{-1} M^{-1}), and the MCD spectra are presented as $\Delta\epsilon_M$ (cm^{-1} M^{-1} T^{-1}). Computer programs developed in this laboratory for the IBM S9001 laboratory microcomputer were used for the analysis of the spectra (Browett & Stillman, 1987a) and subsequent plotting (Browett & Stillman, 1987b). MCD saturation measurements were carried out by monitoring the MCD intensity as a function of magnetic field at fixed temperatures. The data were recalculated as plots of MCD intensity vs $\beta B/2kT$, where β is the Bohr magneton, B is the magnetic flux density, k is the Boltzmann constant, and T is the absolute temperature. The theoretical MCD saturation expressions for allowed electronic transitions in axial molecules (Schatz et al., 1978) were used with the extensions of Thomson and Johnson (1980) for molecules with rhombic symmetry and the $S = {}^1/_2$ ground state. The parameters m_z and m_+, quoted in the theoretical magnetization data, are measures of the polarization of the transitions and refer to the transition dipole moments along z axis and in the x,y plane for an axial chromophore, respectively.

Results and Discussion

MCD Spectra of HRP I and the Photolysis Product. Figure 1 shows the MCD spectra of HRP I and the product of the photolysis of HRP I at 4.2 K, for increasing irradiation times. Line 1 is the initial HRP I spectrum, line 2 is after 0.5 min, and line 3 is after 2 min of photolysis in the SM4 magnet. This photolysis was not continued to completion. Figure 2 shows the 4.2 K MCD spectrum of the photolytic product prepared by exhaustively photolyzing the HRP I at 77 K (line 2, dashed), plotted together with the original 4.2 K spectrum of HRP I (line 1, solid). In these spectra, the loss of HRP I that

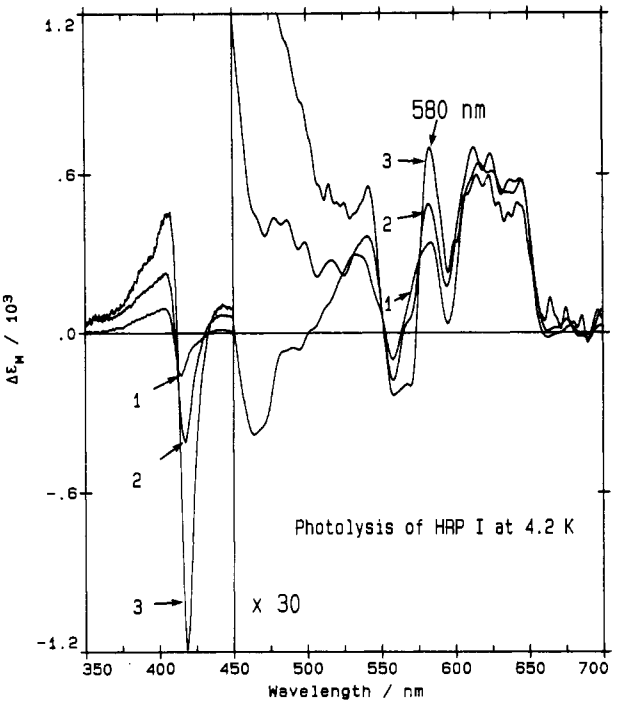

FIGURE 1: Change in the MCD spectrum recorded at 4.2 K for HRP I (1.1×10^{-4} mol/L) upon photolysis at 4.2 K. The photolysis was carried out with water-filtered white light from a 300-W tungsten–halogen lamp for 0.5 and 2 min. The glassy solution was prepared in a H_2O–glycerol (1:1 v/v) solvent mixture.

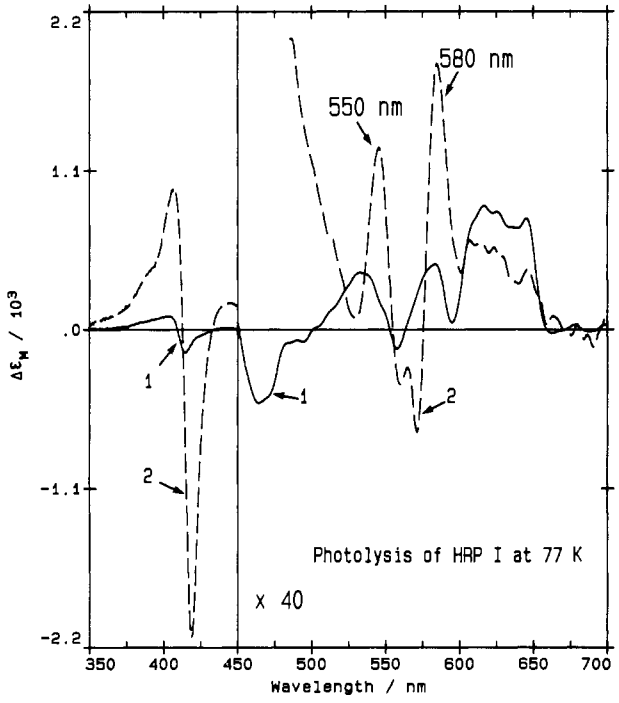

FIGURE 2: MCD spectrum recorded at 4.2 K for HRP I (solid line) and of the exhaustively photolyzed sample of HRP I at 77 K (dashed line).

occurs upon photolysis is indicated by an intensity decrease in the temperature-dependent, HRP I specific MCD bands between 600 and 650 nm (Browett et al., 1988). Formation of the photolytic product, either at 4.2 K or at 77 K, is characterized by the appearance of a strong, temperature-dependent Soret band (a positive, derivative-shaped MCD band) and by bands at 550 and 580 nm. The MCD spectrum recorded at 4.2 K and below (to 1.7 K) for the photolytic product prepared by photolysis of HRP I at 77 K reveals that

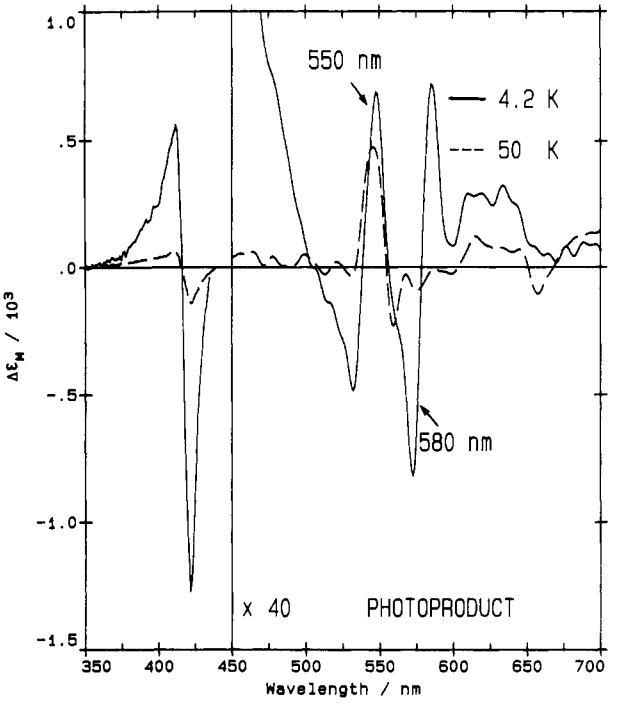

FIGURE 3: MCD spectra measured at 4.2 K (solid line) and at 50 K (dashed line) for the photochemical product obtained by exhaustive photolysis of HRP I with water-filtered white light at 4.2 K in the SM-4 magnet.

both the MCD band in the visible region at 580 nm and that in the Soret region are highly temperature dependent.

Figure 3 shows the MCD spectra of the photolytic product at 4.2 and 50 K. The MCD spectrum of the photolytic product is strongly temperature dependent. The Soret band MCD intensity shows a near-linear relationship with the inverse temperature (Browett et al., 1983c). This is an indication that in the photolytic compounds the lowest level of the ground-state manifold is degenerate. However, closer examination of these spectra shows that the band at 550 nm is less temperature sensitive than the band at 580 nm. When MCD spectra in the visible region of the photolytic product and HRP II are compared at temperatures between 50 and 100 K, we find that both exhibit this 550-nm band as the most intense feature in the visible region and that the envelope in these two sets of spectra are very similar. However, the bands in the HRP II spectrum appear to be also almost temperature independent between 1.7 and 100 K (Browett et al., 1988).

Figure 4 shows absorption spectra of HRP I, HRP II, and the photochemical product. Despite the apparent similarity between the absorption spectra of the compound II species and the photolytic product, the difference in the temperature dependence of the two MCD spectra clearly discriminates between these two species. Indeed, the magnitude of the MCD spectrum of the photolytic product is similar to the intensity of the spectrum of native HRP, a ferric heme species. Moreover, the MCD spectra of the photochemical product exhibit close similarity to the MCD spectra of low-spin ferric HRP, as is evident from Figure 5, which shows the MCD spectra at 4.2, 50, and 80 K of alkaline HRP prepared in H_2O–glycerol (1:1 v/v) solution.

Because so many bands overlap in the spectral region between 300 and 720 nm, absorption spectra of the compound I species of peroxidases and catalases have proven to be of little utility in assignments. Measurements of MCD spectra greatly improved the situation. Figure 6 shows the absorption and MCD spectra of two molecules that act as models for the

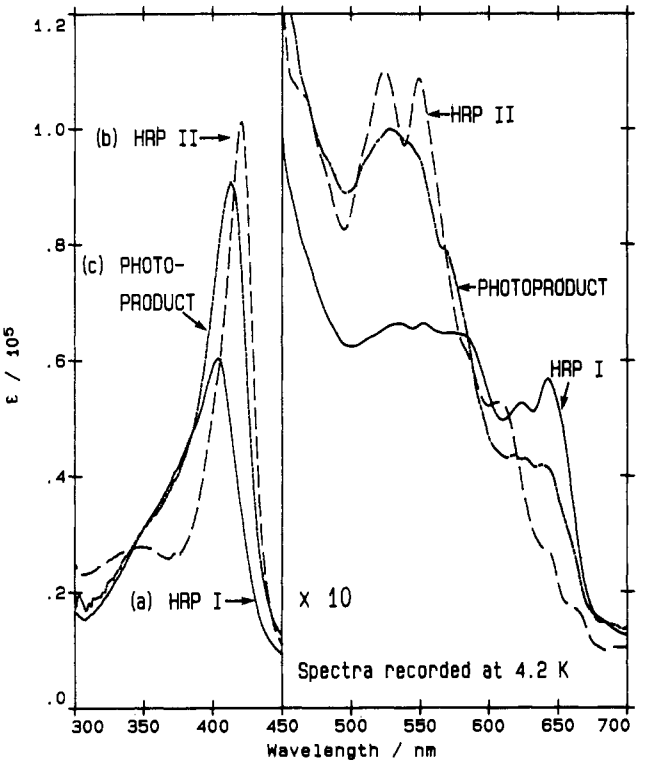

FIGURE 4: Optical absorption spectra recorded at 4.2 K for (a) HRP I, (b) HRP II, and (c) the product of the photolysis of HRP I; the photolysis was carried out at 77 K.

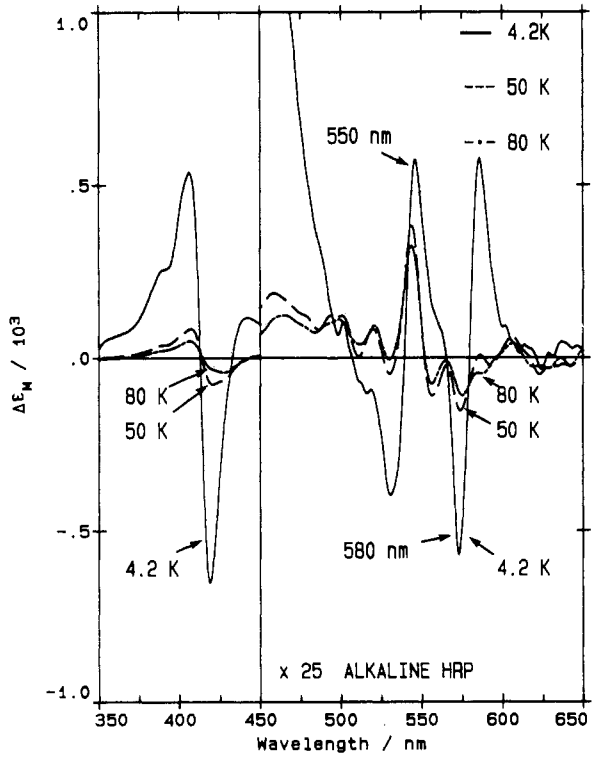

FIGURE 5: MCD spectra recorded for alkaline HRP in a H_2O–glycerol mixture (1:1 v/v) at 4.2 (—), 50 (– –), and 80 K (–·–).

π-cation radical heme, zinc tetraphenylporphyrin ($ZnTPP^{\cdot+}$) and $MgOEP^{\cdot+}$, with ground states of $^2A_{2u}$ and $^2A_{1u}$, respectively. The model compounds exhibit temperature-independent MCD signals. We have found these same spectral features in all the model compounds we have examined, including those with paramagnetic metals. For catalase and HRP compound I at room temperature, the MCD spectra appear to be a superposition of the spectra of these two extreme cases. [We should note that this $MP^{\cdot+}$ MCD spectrum is quite unlike the MCD spectrum observed for either the HRP compound II species (Stillman et al., 1976) or compound I of cytochrome *c* peroxidase (Stillman and Laframboise, unpublished data), where the resolved *A* terms measured under the α and Soret bands are reminiscent of the carbonmonoxy complexes of low-spin ferrous hemes (Vickery et al., 1976).]

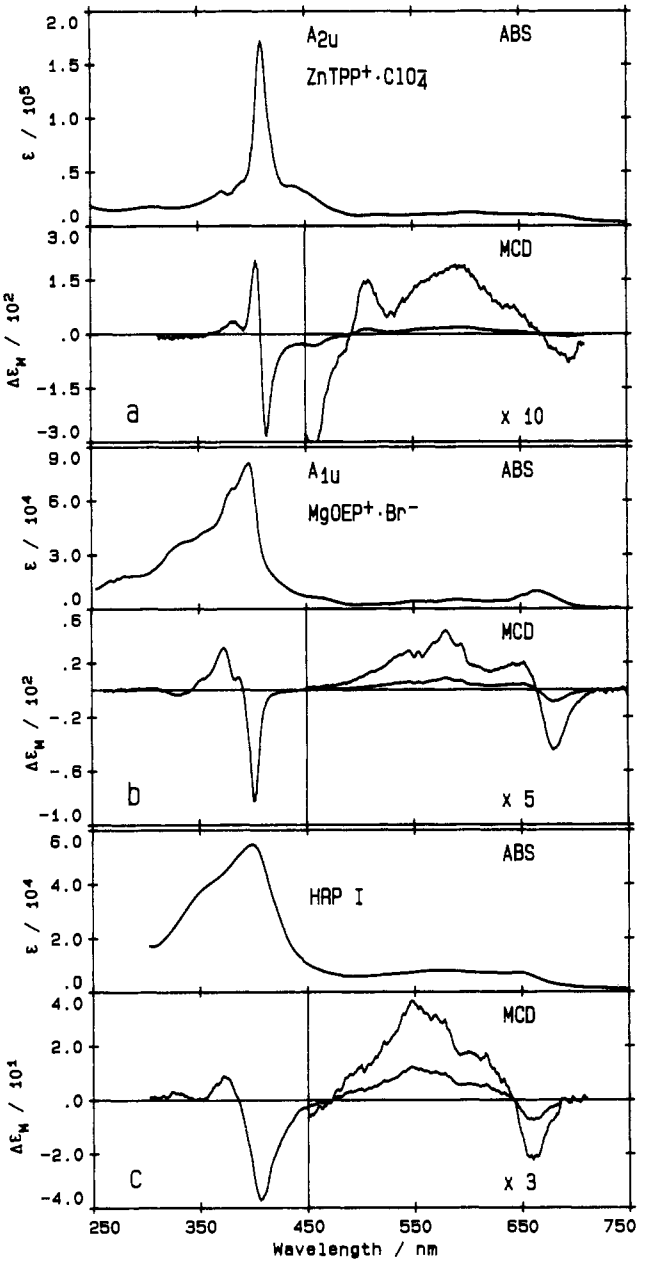

FIGURE 6: Absorption and MCD spectra recorded at room temperature for (a) $ZnTPP^{\cdot+}ClO_4^-$, a species with a $^2A_{2u}$ ground state [data replotted from Browett and Stillman (1981b)], (b) $MgOEP^{\cdot+}Br^-$, a species with a $^2A_{1u}$ ground state, and (c) HRP compound I [data replotted from Browett and Stillman (1980)].

Loss of the specific spectral features of the HRP I complex as photolysis proceeds is convincing evidence that the ring is being reduced from a 17 π (heme)$^{\cdot+}$ to an 18 π (heme)^0. The 4.2 K MCD spectra of several HRP-based compounds are presented in Figure 7 for comparison. The spectral similarities of the photolysis product and the HRP II, which are found in the absorption spectra, are not as strong in the MCD spectra, spectra c and d, respectively, of Figure 7. The MCD spectrum of the photolysis product, Figure 7c, most closely

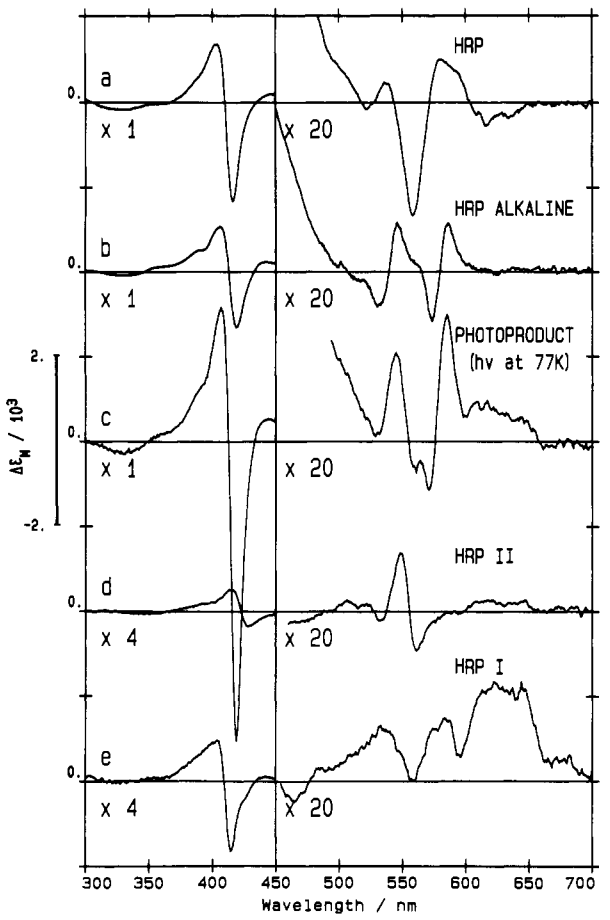

FIGURE 7: MCD spectra recorded at 4.2 K for (a) native HRP (pH 5.4), (b) alkaline HRP, (c) the product of the photolysis of HRP I (the photolysis was carried out at 77 K), (d) HRP II, and (e) HRP I. The MCD spectra are arranged in this figure in such a way that the relative intensities of the Soret and α/β band sets may be compared directly. For most data, the ordinate scale is $\pm 2.0 \times 10^3$ L mol^{-1} cm^{-1} T^{-1}; this scale is indicated by the length of the bar drawn by the y axis label. Specific exceptions are for all the α/β regions where the scale is $\pm 0.1 \times 10^3$ (as indicated by "×20") and in the Soret region for HRP II (d) and I (e), where the ordinate scale is $\pm 0.5 \times 10^3$ ("×4").

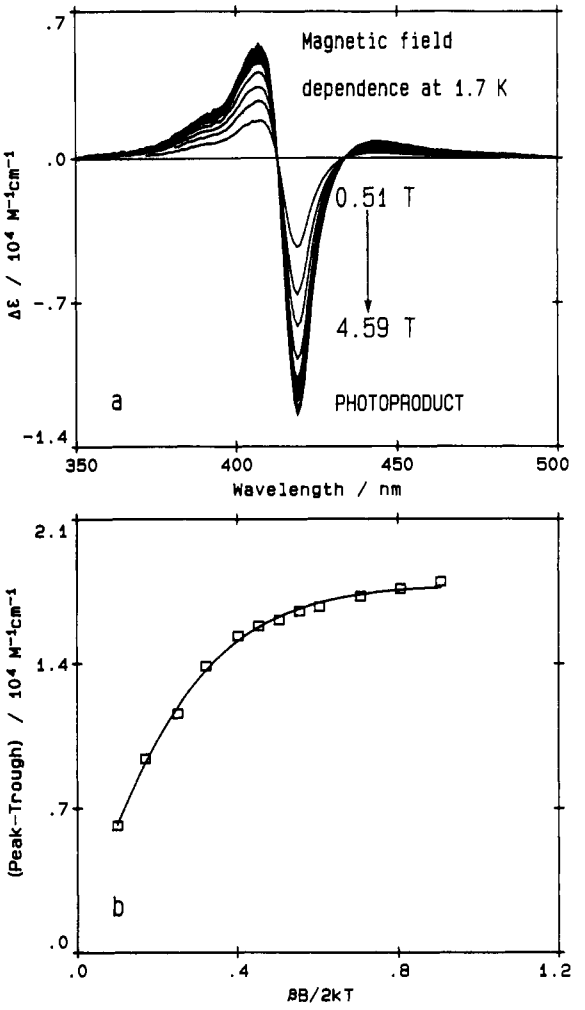

FIGURE 8: (a) Effect of magnetic field on the Soret region MCD spectrum recorded at 1.7 K of the photolysis product of HRP I (the photolysis was carried out at 77 K). Spectra were taken at magnetic flux densities of 0.51, 0.87, 1.28, 1.63, 2.04, 2.30, 2.55, 2.81, 3.06, 3.57, 4.08, and 4.59 T. (b) Magnetization curve for the Soret band region of the MCD spectrum of the photolysis product (the MCD intensity used was taken from the Soret region recorded at 1.7 K, by calculating the magnitude of the 407-nm peak to 419-nm trough); the intensity is plotted vs $\beta B/2kT$ for the experimental data (points) and a fitted line (solid line) based on a ground state with an $S = {}^1/_2$ spin system, $g_\parallel = 3.9$, $g_\perp = 1.6$, $m_+ = 60$, and $m_z = 1.2$.

resembles the low-spin Fe(III) spectrum, Figure 7b. However, if the photolysis of HRP I results in the formation of a mixture of a compound II like species and a low-spin Fe(III) species, at 4.2 K, the strong temperature dependence of the Fe(III) spectrum would be expected to obscure the much less temperature dependent spectrum of the compound II like species (Browett et al., 1988) that may be present in low concentration. The appearance of bands in the 600–650-nm region of the photochemical product spectrum, Figure 7c, suggests that there is some residual HRP I in the sample.

The MCD spectra of porphyrin complexes are dominated by transitions of the π ring. Interaction between the central metal and the π system is normally observed by both the appearance of additional bands, assigned as charge-transfer transitions, and temperature dependence in the MCD intensity that arises because of coupling between the orbitally nondegenerate ground state of the porphyrin ring and an orbitally degenerate central metal. Recently, we have shown that coupling between the ring and the paramagnetic Fe(IV) in HRP compound I or compound II is relatively weak in comparison to the coupling that occurs between the ring and the low-spin Fe(III) of ferric hemes (Browett et al., 1983b, 1988). Thus, the MCD spectral intensity of a low-spin ferric heme is extremely temperature dependent, exhibiting intensification of strong derivative signals in both the α and Soret band regions as the temperature falls to 4.2 K (Springall et al., 1976). Orbital degeneracy in the ground state of the heme complexes results in Faraday C terms in the MCD spectrum that have an intensity dependent on both the temperature and magnetic field (Schatz et al., 1978). Comparison of the MCD spectral intensities of Fe(III) and Fe(IV) species of HRP with the photolysis product indicates that the strong temperature dependence of the photochemical product closely resembles the strong temperature dependence that is observed in the Fe(III) native HRP and low-spin alkaline HRP spectra rather than the weak HRP I and very weak HRP II temperature dependence. The saturation limit of the photochemical product, Figure 8b, of approximately 19×10^3 cm^{-1} M^{-1} is much closer to that of low-spin Fe(III) complexes (Thomson et al., 1981) than the 1.9×10^3 cm^{-1} M^{-1} of HRP I (Browett et al., 1988).

Figure 8a shows the effect of the magnetic field on the Soret band MCD intensity of the photochemical product (obtained by photolysis of HRP I at 77 K) for MCD spectra recorded at 1.7 K. The magnetization curve in Figure 8b can be reproduced by using theoretical expressions (Schatz et al., 1978;

Thomson & Johnson, 1980) for an iron porphyrin $S = {}^1/_2$ spin system with corresponding g values and polarizations of the Soret band transition: $g_\parallel = 3.9$, $g_\perp = 1.6$, $m_+ = 60$, and $m_z = 1.2$. These data may be compared to the respective EPR parameters determined for alkaline ferric HRP ($g_\parallel = 2.94$, $g_\perp = 1.87$) and the ferric HRP–CN^- complex ($g_\parallel = 3.05$, $g_\perp = 1.71$) (Dunford & Stillman, 1976), and with the EPR signal observed recently for the photolytic product of HRP I ($g_\parallel = 2.94$) (Foote et al., 1987).

It is clear from the MCD spectral envelope characteristics, the spectral temperature dependences, and the magnetic field properties that the final, photochemically produced product is a low-spin Fe(III) complex. Some HRP II may be present in the glassy solution at low temperatures following photolysis of HRP I, but at temperatures below 77 K this species cannot be discerned. A major advantage of the MCD and absorption techniques for monitoring photochemical reactions is that very low intensity light is used in the measurement of the spectra, so that we can obtain spectra of HRP compound I without contamination from the photochemical product (Teraoka et al., 1982; Oertling & Babcock, 1985), and second, we can obtain spectra of the selective levels of reduction to the photochemical product.

The photoreduction of the HRP I species to the low-spin Fe(III) heme requires a two-electron transfer. The mechanism could be either a concerted two-electron reduction or a series of two, consecutive electron-transfer reactions, which pass through the intermediate HRP compound II, as illustrated in the reaction pathways:

$$\underset{\text{HRP I}}{[O{=}Fe(IV)\ heme^{\bullet+}]} \xrightarrow{h\nu\ (+2e^-)} [Fe(III)\ (OH^-)\ heme^0]$$

or

$$\underset{\text{HRP I}}{[O{=}Fe(IV)\ heme^{\bullet+}]} \xrightarrow{h\nu\ (+e^-)} \underset{\text{HRP II}}{[O{=}Fe(IV)\ heme^0]} \xrightarrow{h\nu\ (+e^-)} [Fe(III)\ (OH^-)\ heme^0]$$

Recent resonance Raman studies of the HRP compound I (Van Wart & Zimmer, 1985) show that formation of a distinct photochemical product occurs when HRP compound I is exposed to the laser light used in the resonance Raman experiment. Van Wart and Zimmer (1985) reported that frozen samples of both compound I and compound II undergo photoreduction to a ferrous heme species upon laser irradiation. It was suggested that photoreduction occurs via HRP II as an intermediate, such that:

$$[O{=}Fe(IV)\ heme^{\bullet+}]\ (\text{HRP I}) \xrightarrow{h\nu} [O{=}Fe(IV)\ heme^0]\ (\text{HRP II})$$

Then

$$[O{=}Fe(IV)\ heme^0] \xrightarrow{h\nu} [Fe(III)\ heme^0] \xrightarrow{h\nu} [Fe(II)\ heme^0]$$

There is no evidence in our data for the presence of a ferrous peroxidase species, in either a "nonequilibrated" form, which can be obtained by reduction of Fe(III) peroxidase in frozen solution at 77 K (Magonov et al., 1977, 1978), or the normal deoxy, high-spin ($S = 2$) form. The nonequilibrated form of the Fe(II) peroxidase, which results in the optical absorption and MCD spectral characteristics in the visible region of a low-spin Fe(II) heme center, shows the typical MCD spectra of the high-spin Fe(II) in the Soret region (Magonov et al., 1977, 1978). The characteristic MCD feature for the high-spin Fe(II) is a negative A term with a crossover near 440 nm (Springall et al., 1976). Therefore, the presence of either a high-spin ($S = 2$) Fe(II) or the nonequilibrated form of Fe(II) in HRP would result in an optical absorption band at 440 nm and a "negative" MCD A term in the Soret region (Springall et al., 1976; Sharonov et al., 1978). Both these features are absent in our spectra.

Resonance Raman data for the Mb–H_2O_2 complex indicate (Campbell et al., 1980) that this iron Fe(IV) heme complex, which has a t^4_{2g} low-spin configuration and is analogous to the HRP II species, undergoes a two-electron photoreduction to a high-spin $t^4_{2g}e^2_g$ Fe(II) heme complex without any evidence of the intermediate formation of the Fe(III) heme. Therefore, photoreduction appears to be a general property of oxidized forms of heme proteins, and the two-electron process may be quite common. If two sequential electron-transfer steps are involved in the HRP I photolysis, then the intermediate species will likely be an unstable HRP II like compound, which is reduced, then, in a faster process to the Fe(III) heme.

Conclusions

The photolysis of HRP I results in the reduction of the HRP I heme group with two electrons. This intramolecular electron-transfer reaction results in the conversion of HRP I to a low-spin Fe(III) heme protein, probably through an intermediate HRP II species. The two-electron photoreduction of HRP compound I occurs with visible light at 77 K and at 4.2 K. No evidence is found for the formation of the Fe(II) heme at these temperatures.

Registry No. Peroxidase, 9003-99-0.

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Femtosecond Charge Separation in Organized Assemblies: Free-Radical Reactions with Pyridine Nucleotides in Micelles

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ABSTRACT: Femtosecond laser UV pulse-induced charge separation and electron transfer across a polar interface have been investigated in anionic aqueous micelles (sodium lauryl sulfate) containing an aromatic hydrocarbon (phenothiazine). The early events of the photoejection of the electron from the micellized chromophore and subsequent reaction of electron with the aqueous perimicellar phase have been studied by ultrafast infrared and visible absorption spectroscopy. The charge separation (chromophore^{+}···e^{-}) inside the micelle occurs in less than 10^{-13} s (100 fs). The subsequent thermalization and localization of the photoelectron in the aqueous phase are reached in 250 fs. This results in the appearance of an infrared band assigned to a nonrelaxed solvated electron (presolvated state). This transient species relaxes toward the fully solvated state of the electron in 270 fs. In anionic aqueous micelles containing pyridine dinucleotides at high concentration (0.025–0.103 M), a single electron transfer can be initiated by femtosecond photoionization of phenothiazine. The one-electron reduction of the oxidized pyridine dinucleotide leads to the formation of a free pyridinyl radical. The bimolecular rate constant of this electron transfer depends on both the pH of the micellar system and the concentration of oxidized acceptor. The free-radical reaction is analyzed in terms of the time dependence of a diffusion-controlled process. In the first 2 ps following the femtosecond photoionization of PTH inside the micelle, an early formation of a free pyridinyl radical is observed. This suggests that an ultrafast free-radical reaction with an oxidized form of pyridine nucleotide can be triggered by a single electron transfer in less than 5×10^{11} s^{-1}.

Intensive studies have produced a substantial body of information concerning reactions between solvated electron and chemical or biological acceptors in homogeneous aqueous solutions (Anbar & Neta, 1967, 1968; Land & Swallow, 1968; Anbar, 1969; Lichtin et al., 1973; Dainton, 1975; Klapper & Faraggi, 1983). The knowledge of absolute rate constant is of prime importance for a proper understanding of the chemical reactions. In particular, the studies of one-electron-transfer reactions in concentrated solutions of biomolecules (coenzymes, amino acids) should permit apprehension of the mechanisms

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