Photochemical Reactions of Horseradish Peroxidase Compounds I and II at Room Temperature and 10°K†

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ABSTRACT: Some photochemical reactions of horseradish peroxidase compounds I and II (HRP-I and HRP-II, respectively) have been studied by electronic absorption spectroscopy over the temperature range 297°K–10°K. In glassy matrices below 80°K HRP-I is rapidly converted to HRP-II when irradiated with low power white light. The native enzyme and HRP-II are not photochemically active at these temperatures with low power irradiation. At room temperature the spontaneous decay of both HRP-I and HRP-II is catalyzed by irradiation with white light. It is shown that the photolysis is dependent upon light in the region 450–320 nm. It is concluded that the HRP-I and HRP-II conformations are closely related with only a low transition energy in the presence of electrons generated by the light. The conversion of HRP-II to HRP is accompanied by large conformational changes and so is inhibited at low temperatures.

There have been many reports on the photochemistry of hemoprotein systems. The first reports dealt with the photosensitivity of carbon monoxide hemochromagens (Haldane and Smith, 1895; Warburg et al., 1929; Bücher and Negelein, 1942). The cytochrome oxidase–carbon monoxide system was also studied and the photosensitivity well defined (Warburg and Negelein, 1929; Chance, 1953; Yonetani and Kidder, 1963). Later it was found that other compounds such as ferroperoxidase cyanide and myoglobin cyanide (Keilin and Hartree, 1955) and oxygen, nitric oxide, and ethyl isocyanide compounds of hemoglobin (Gibson and Ainsworth, 1957) were also photosensitive. At this time it was thought that photosensitivity was a property only of ferroheme compounds. Later, however, Schuberth (1960) reported the results of many experiments and showed that both ferri and ferro forms of some hemoproteins can undergo photochemical reactions.

In general these workers used high power polychromatic light (Gibson and Ainsworth, 1957) or mercury arc lamps, which exhibit strong emission at 254 nm (Schuberth, 1960; Bücher and Kaspers, 1947); for example, Gibson and Ainsworth report that a continuous light source of 1.2×10^3 kW would be required to replace their flash lamp to observe the spectral changes in the myoglobin and hemoglobin compounds which they studied. It has been suggested (Schuberth, 1960) that the photochemical process is initiated by absorption into the protein bands which are located in the 250–280-nm region, followed by energy transfer to the porphyrin ring. However, Noble et al. (1967) have reported that the 546-nm line of the Hg lamp will cause photodissociation of carbon monoxide from normal and modified myoglobin, hemoglobin subunits, and hemoglobin. Detailed photochemical action spectra of both cytochrome and lactate oxidase systems resemble the absorption spectra of typical heme proteins (Warburg and Negelein, 1929; Chance, 1953; Yonetani and Kidder, 1963). More recently flash photolysis has been used to measure the photodissociation of carbon monoxide complexes of hemes and hemoproteins (Yonetani et al., 1973; Austin et al., 1974; Hasinoff, 1974; Caldin and Hasinoff, 1975).

Intermediate compounds I and II of horseradish peroxidase have been known for many years (Keilin and Mann, 1937; Theorell, 1941) and the oxidation–reduction cycle is well established (Chance, 1952; George, 1952, 1953)

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

where HRP refers to the native enzyme and HRP-I and HRP-II are compounds I and II containing two and one oxidizing equivalents, respectively, in excess of that of HRP.

Recent studies on HRP and its intermediates aimed at elucidating the electronic structure of the iron-porphyrin moiety have included resonance Raman (Rakshit and Spiro, 1974) of HRP, Mossbauer (Moss et al., 1969) and electron paramagnetic resonance (Blumberg et al., 1968; Aasa et al., 1975) of HRP, HRP-I, and HRP-II. Absorption spectra of HRP, HRP-I, and HRP-II as frozen glasses have been measured at 77°K (Blumberg et al., 1968) while Douzou et al. (1970) have developed a technique for studying this and other enzymatic reactions between room temperature and −67° using solvents which remain fluid over this temperature range. However, there have been no previous reports of photochemical processes involving either the native HRP or its compounds I and II.

During a study of the low temperature spectrum of compound I of horseradish peroxidase it was found that irradiation with low power polychromatic light of wavelengths greater than 330 nm catalyzed the conversion of HRP-I to HRP-II. It was also found that irradiation with this light source greatly enhanced the conversion of HRP-I → HRP-II → HRP at room temperature. HRP and HRP-II were not photosensitive under the experimental conditions described below at low temperature. A preliminary report of our findings for HRP-I at 5°K has been published (Stillman et al., 1975).

Experimental Section

Materials. Horseradish peroxidase (grade 1) was purchased from Boehringer Mannheim GmbH as a salt suspen-

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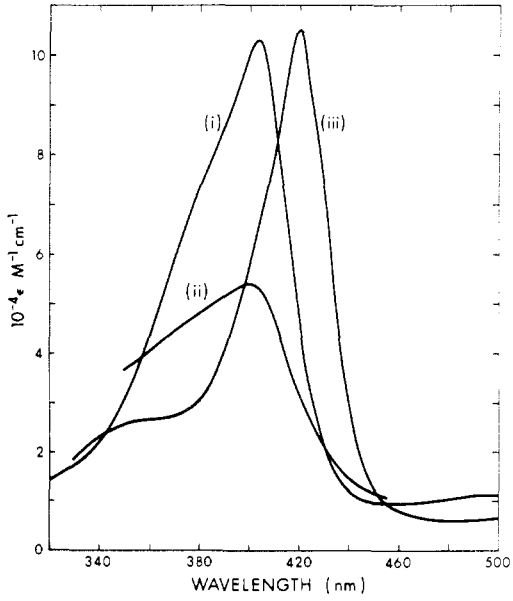

FIGURE 1: The Soret spectra at room temperature of HRP(i) and its intermediate compounds HRP-I(ii) and HRP-II(iii). Molar absorptivities for HRP in this region were taken from Schonbaum and Lo (1972), for HRP-I, Roman and Dunford (1972), and HRP-II, footnote 1.

sion, batch number 7074125, and was prepared for use by extensive dialysis against triply distilled water. The solution was concentrated where necessary by vacuum dialysis. Glycerol used to prepare the low temperature glasses was purchased from the Fisher Scientific Company and was certified A.C.S. grade.

Spectra. Glycerol is one of the few glassing agents for use with aqueous solutions at temperatures below 100°K. It has been extensively used in the measurement of low temperature optical spectra (Keilin and Hartree, 1949; Blumberg et al., 1968). Other compounds have been used for subambient temperature spectroscopy although these act as anti-freeze agents allowing the solvent to remain fluid (Douzou, 1970). These do not extend below −67°.

All absorption spectra were recorded on a Cary 14 spectrophotometer using 0.0–1.0 and 0.0–0.1 optical density slidewires.

An Oxford Instruments Ltd., Oxford, England (O.I.L.), continuous flow cryostat (C.F.C.) was used with liquid helium to cool a copper sample cell held under vacuum (10^{-5} Torr). A carbon resistor and a cryogenic linear temperature sensor attached to the heat exchanger were used for thermometry. Temperature control was achieved by varying the transfer rate of coolant liquid with a gas flow controller (O.I.L. VC 30) monitored with a Matheson mass flow meter and by applying heat to the exchanger from a Harwell temperature controller (O.I.L.). The temperature was held constant to within 0.5°K between 5 and 40°K and to within 2°K above 40°K.

In a typical run the C.F.C. was cooled to 230°K in a nitrogen atmosphere. HRP-I was made in the laboratory at room temperature by injecting the required amount of hydrogen peroxide into a 50% glycerol–50% H_2O solution of HRP. This solution was injected into the sample cell at room temperature which was then bolted to the cold heat exchanger. The cell rapidly cooled to 230°K (30 sec). The sample compartment was evacuated and a temperature of 10°K reached within 16 min.

The spectrum of the sample was recorded before irradiation using low-power monochromatic light over the region 760–250 nm. No reference was used; the effect of the restricted C.F.C. aperture was equivalent to approximately 1 OD unit and was readily offset using the balance control.

The sample was then illuminated with white light from the Cary infrared source for varying lengths of time and spectra were recorded after each illumination period. Only representative spectra are shown here.

There was no change in the spectra recorded at both 10 and 80°K over a period of 4 hr in the absence of white light. Spectra recorded at 5°K before and after illumination in 10-sec intervals up to a total time of 1 min were identical with those recorded at 10°K. In one series of experiments Corning sharp cut-off filters were used to restrict the frequency of the source radiation.

Results

The absorption spectra at room temperature of the three compounds observed in the HRP cycle, HRP → HRP-I → HRP-II → HRP, shown in Figure 1, illustrate the relative Soret band maxima at 403 nm for HRP, 400 nm for HRP-I, and 420 nm for HRP-II.

The spontaneous decay of HRP-I at room temperature prepared by the addition of 1 molar equiv of H_2O_2 shows a single isosbestic point at about 390 nm; after 1 hr formation of HRP results in the loss of the isosbestic point. This biphasic course of the decay of compound I has been reported by Schonbaum and Lo (1972). The spectra taken after 1 hr clearly show that the HRP-I concentration is still much greater than HRP-II or HRP. The decay of HRP-I at room temperature is considerably enhanced by irradiation with light. After an hour total elapsed time, including 30 min illumination with light, the decay is complete and native HRP is quantitatively regenerated. In the absence of light it takes over 24 hr to obtain HRP through the spontaneous decay of HRP-I.

The spontaneous decay of HRP-I at room temperature which had been prepared by injecting 2 molar equiv of hydrogen peroxide into an HRP solution in 50% glycerol–water exhibits only a slight difference compared with the rate of decay of HRP-I in water alone, demonstrating that neither the excess peroxide nor glycerol is appreciably modifying the decay rate. Irradiation of the solution of HRP-I in 50% glycerol–water (Figure 2(i)) has the same effect as irradiation of the HRP-I water solution.

The initial spectrum of HRP-I recorded at 10°K in a 50% glycerol–water glass is shown in Figure 3 marked as $t = 0$. In the glass the HRP-I does not decay unless the sample is irradiated with light. Figure 3 shows the effect of irradiation on the absorption spectrum of HRP-I. Decay rates measured for irradiation at 10 and 80°K were essentially the same. Production of a single new compound with an absorption maximum at 416 nm is suggested by the isosbestic point at 397 nm. This spectrum was invariant to further light after 1-hr irradiation at both 10 and 80°K and it appears to represent the absorption spectrum in the Soret region of the final product.

At room temperature HRP-II also undergoes photochemical changes. Figure 2(ii) shows the enhanced rate of decay when a sample of HRP-II which had been prepared from HRP-I by the addition of *p*-cresol was irradiated with light. The final product with and without irradiation is the native enzyme. It can be seen that when the sample has been exposed to white light for a total of 60 min within 78

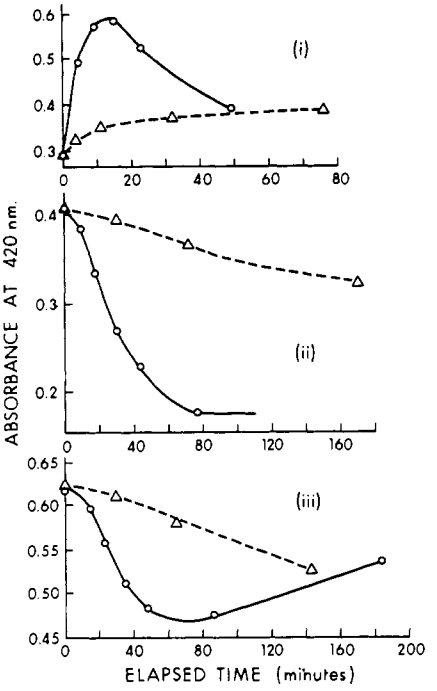

FIGURE 2: The spontaneous decay of HRP-I and HRP-II at room temperature in the absence (△) and presence (○) of irradiation. (i) HRP-I in 50% v/v glycerol–water solution. Absorbances were taken from spectra recorded after total irradiation times of 0, 1, 2, 4, 10, and 30 min for the upper curve (○) at the elapsed time shown on the graph. Both steps in the decays HRP-I → HPR-II and HRP-II → HRP are catalyzed by the light. (ii) HRP-II formed by the addition of *p*-cresol to HRP-I in water. Absorbances for the irradiation curve (○) were taken after total irradiation times of 0, 5, 10, 20, 30 and 60 min. (iii) HRP-II formed by the addition of ferrocyanide to HRP-I in water. Absorbances for the irradiation curve (○) were taken after total irradiation times of 0, 5, 10, 20, 30, 63, and 154 min. Increased absorbance beyond 80 min is caused by the formation of the HRP–CN complex. The lines connecting the data points have no theoretical significance.

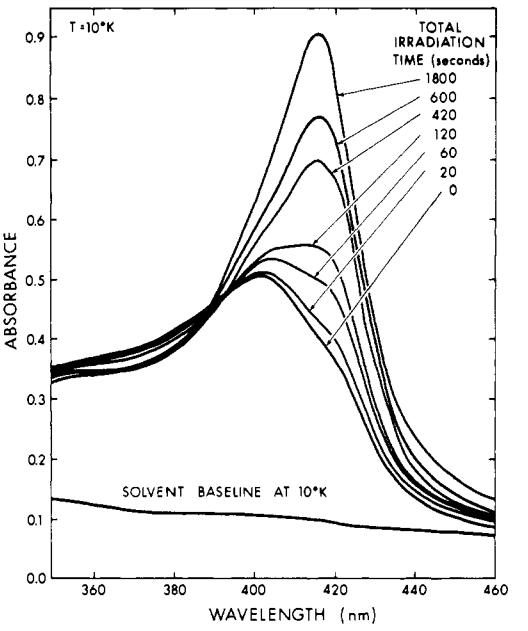

FIGURE 3: The effect of irradiation with light on a 50% glycerol–water glass containing HRP-I at 10°K. The spectrum at t = 1800 sec is that of the product.

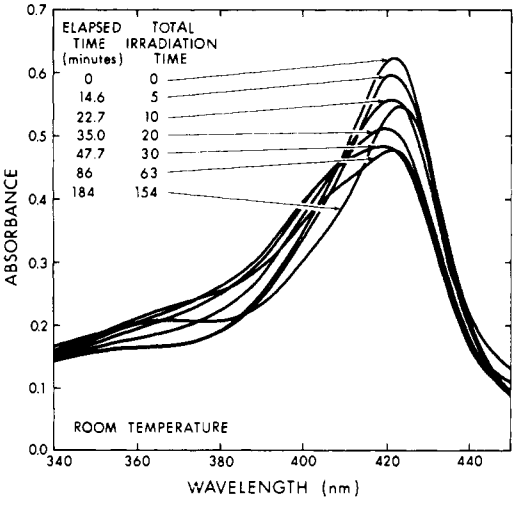

FIGURE 4: The effect of light on the spontaneous decay of HRP-II at room temperature. HRP-II was prepared by the addition of 1.2 molar equiv of hydrogen peroxide in the presence of 1.0 molar equiv of potassium ferrocyanide. The conversions HRP-II → HRP followed by HRP + CN^- → HRP-CN are catalyzed by the light.

min of elapsed time, there is no further change in the spectrum whereas the normal spontaneous decay to native enzyme takes over 24 hr.

In the case of a sample of HRP-II prepared from HRP-I by the addition of potassium ferrocyanide the decay pattern when the sample was irradiated differed markedly from that of HRP-II prepared from *p*-cresol (Figure 2(iii), Figure 4). The spectrum of the final product is that of ferri-HRP-CN (Willick et al., 1969) and not that of HRP which is the product of the spontaneous decay without irradiation (Hasinoff and Dunford, 1970). The spectrum of the final product after irradiation remained constant for at least another 24 hr. Figure 2 summarizes part of a large amount of spectral data obtained in the Soret region, which is available from the authors upon request.

When a 50% glycerol–water glass of HRP-II at 5°K which had been prepared by the addition of hydrogen peroxide and potassium ferrocyanide to HRP was irradiated, there was no change in the absorption spectrum.

Previous workers (Schuberth, 1960) have reported that illumination of several myoglobin compounds with ultraviolet light from a mercury arc lamp resulted in absorption of light by the protein bands in the 240–270-nm region which in turn caused the occurrence of photochemical processes. This possibility was examined by filtering out all light below 300 nm with a glass plate (cut-off at 330 nm) and then illuminating a sample of HRP-I at 5°K. There was no noticeable difference in the efficiency of the formation of HRP-II when similar samples were irradiated with and without the filter

Further experiments were carried out to determine the spectral region giving rise to the greatest photochemical efficiency for the conversion of HRP-I to HRP-II in a glassy matrix at 80°K. Using combinations of cut-off filters samples were irradiated with light of a restricted frequency range. It was found that there was negligible conversion of HRP-I (<1%) following irradiation with light in the 530–2000-nm region after a total of 2000-sec irradiation (Figure 5(i)). When the source spectral range was extended from 530 to 475 nm some product formation was apparent after 2400-sec irradiation. A further extension to 450 nm did not increase the decay rate significantly (Figure 5(iii)). Finally the sample was irradiated with light of a spectral range 330–450 nm. The efficiency of product formation reached a

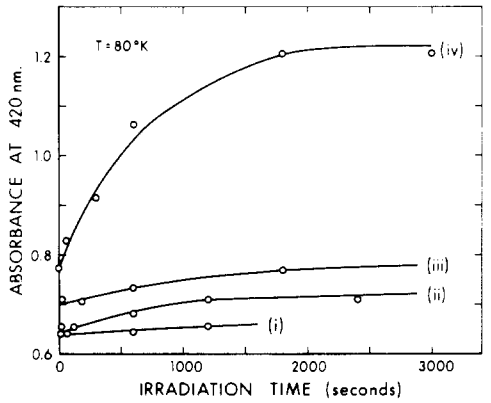

FIGURE 5: Formation of HRP-II from the decay of HRP-I under the same condition as for Figure 3, as a function of the frequency of the irradiation light. Curves are for formation following irradiation with light of wavelengths: (i) 530–1000 nm; (ii) 475–1000 nm; (iii) 460–1000 nm; (iv) 320–1000 nm. The lines connecting the data points have no theoretical significance.

Table I

Temp	Time Sample was Illuminated	Reaction	Time Required to Complete Reaction
RT	0 min	HRP-I → HRP-II → HRP	>24 hr[f] >14 hr[f]
RT	60 min	HRP-I → HRP-II → HRP	~76 min[a,b]
10°K	1800 sec	HRP-I → product with a Soret λ_{max} at 416 nm	1800 sec[b]
80°K	1800 sec	HRP-I → product with a Soret λ_{max} at 416 nm	1800 sec[b]
RT	0 min	HRP-II → HRP	>14 hr[f]
RT	60 min	HRP-II → HRP[c]	~76 min[b,d]
RT	150 min	HRP-II → HRP·CN[e]	~184 min[b]
10°K	5 hr	HRP-II → no reaction	
80°K	5 hr	HRP-II → no reaction	

[a] This time includes the irradiation time. [b] Dependent on lamp voltage. [c] HRP-II prepared from HRP-I using *p*-cresol. [d] Some destruction of HRP takes place. [e] HRP-II prepared from HRP-I using $K_4Fe(CN)_6$. [f] Conditions described in the text.

maximum and the conversion was complete within 2000 sec of irradiation.

Discussion

Approximate rates of the reactions shown in the spectra are summarized in Table I. The similarity of the spectral changes during the first 10 min at room temperature with those accompanying the reaction of HRP-I + $h\nu$ at low temperature (Figure 3) suggest that the same initial product is formed in both cases. The existence of an isosbestic point at about 395 nm, the near doubling in intensity of the Soret band, the unsplit character of the band, and the presence of the broad shoulder centered on 360 nm (Chance, 1952) all support the characterization of the low temperature product as HRP-II.

The visible spectrum of the photochemical product at low temperatures (Stillman et al., 1975) closely resembles that of HRP-II at room temperature. The major peaks in the photochemical product are at 530 and 554 nm; at room temperature peaks occur at 528 and 553 nm and at 10°K at 527 and 555 nm.[1]

It is well known that electron configuration changes due to the central ion (e.g., Fe^{2+} high spin → Fe^{2+} low spin) will greatly affect the observed spectrum in the visible region in all hemoproteins (Smith and Williams, 1970; Bolard and Garnier, 1972). This region is extremely sensitive to the nature of the sixth position ligand and to the oxidation state of the iron.

The band center of the product in the Soret region in all the low temperature spectra obtained by the irradiation of compound I is at 416 nm.

The sharp and well-resolved nature of the Soret band suggests that this absorption spectrum represents that of a single species. It seems reasonable to postulate therefore that the changes to the HRP-I following irradiation result in a product with an electronic configuration closely resembling that of HRP-II at room temperature. However, the constraints of the frozen matrix do not allow the porphyrin ring, the central iron atom, and the axial ligands to fully adopt the conformation of HRP-II at room temperature. Thus the band center shift of 4 nm must reflect the strain imposed by the frozen matrix on to the new HRP-II-like porphyrin group.

The results of the frequency dependence for the photolysis reaction show that the greatest efficiency occurs when the sample is illuminated with light of the same frequency as the Soret region of HRP-I. A similar result was found with the more detailed action spectra recorded for the photodissociation of carbon monoxide in other systems (for example, Yonetani and Kidder, 1963).

It would appear reasonable that the electron production process should be the same for both compounds I and II in the low temperature glass matrices. Therefore changes in the rate of reaction of HRP-I → HRP-II and HRP-II → HRP as a function of temperature must reflect the differences in activation energy of these steps. Collisional processes due to scavanger molecules become important only above 30°K (Yonetani et al., 1973). Since the rates of formation of HRP-II are similar at 80 and 10°K it is unlikely that collisional processes are important. It appears therefore that the configurational and conformational changes required in the reaction HRP-I + e occur with a very low energy barrier and that the conformational changes that are required for the reaction HRP-II + e → HRP are large enough that at low temperature in a glassy matrix using a low power lamp this reaction does not take place. It has been shown recently that electron tunneling is a facile process in aqueous glasses at low temperatures (Tachiya and Mozunder, 1974). However, since the photochemical enhancement of the decay of HRP-I occurs in both solution and solid phases it seems unlikely that tunneling is an important factor in the low temperature glass. It appears that the photochemical process is initiated by absorption of a photon into the iron porphyrin. The energy of the photon corresponds to the energy of the transition observed in the Soret region.

Ehrenberg (1962) has interpreted his magnetic susceptibility data as Fe(V) for HRP-I and Fe(IV) for HRP-II. It has been shown that oxygen from an organic peroxidase is incorporated into HRP-I (Schonbaum and Lo, 1972) and labeling experiments by Hager et al. (1972) show that one oxygen from the peroxide is used to form one molecule of compound I in the closely related chloroperoxidase. Steady-state cyanide inhibition experiments (Cotton et al., 1973) also indicate that the fifth and sixth coordination positions

[1] M. J. Stillman, J. S. Stillman, and H. B. Dunford, unpublished results.

are blocked in compounds I and II. These experiments all support a ferryl type of structure for HRP-I which is retained in HRP-II (George, 1952).

Moss et al. (1969) have reported that there is no major change in the Mössbauer parameters in going from HRP-I to HRP-II and suggest that there is little difference in the iron configuration in these two compounds. Their data do indicate a major change in iron configuration in going from native enzyme to HRP-I and HRP-II and suggest that the extra oxidizing equivalent of compound I is unlikely to be found in a Fe(IV) configuration. Therefore the additional unpaired electron should be localized at a site other than iron. Dolphin et al. (1971) have demonstrated the stability of metalloporphyrin π cation radicals and the similarity of the optical spectra of the π cation radical $[Co(III)(Et)_8P]^{2+}\cdot 2Br^-$ (where $(Et)_8P$ = octaethylporphyrin) with compound I has led them to suggest that compound I contains a π cation radical. Recently Aasa et al. (1975) have obtained in low temperature electron paramagnetic resonance experiments a small signal which had free radical properties and is proportional to the amount of compound I present. They suggest that there is a free radical present which is relaxed by the nearby iron. It thus appears that HRP-I may be similar to complex I of cytochrome *c* peroxidase (Yonetani et al., 1966), the main difference being that the free radical of HRP-I is located so close to the Fe(IV) that its electron paramagnetic resonance signal is almost obliterated by the paramagnetic center. Preliminary magnetic circular dichroism experiments[2] indicate large electronic changes in the vicinity of the iron of HRP-I compared with either native HRP or HRP-II.

It is probable, therefore, that the porphyrin ring is active in stabilizing the normally unstable electron configuration found in the iron in HRP-I and HRP-II. Spectroscopic studies of a range of metal phthalocyanines (Stillman and Thomson, 1974) have shown that when there is interaction between metal d electrons and the phthalocyanine ring π electrons there is a considerable reduction in intensity of the main visible band of the $\pi \rightarrow \pi^*$ spectrum. This band is considered to be equivalent to the Soret band in the porphyrin system (Zerner et al., 1966). Similar changes are observed in the HRP-I Soret band compared with HRP and HRP-II (Figure 1).

The photochemical reactions clearly demonstrate that the reaction of HRP-I $\rightarrow$ HRP-II is via a low activation energy process and that no conformational changes are required for the reaction to proceed.

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Active Site Directed Inactivators of Mouse Submaxillary Renin†

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ABSTRACT: The following active site directed inactivators for the pressor enzyme renin were synthesized: L-α-bromoisocaproyl(BIC)-Leu-Val-Tyr-Ser-OH, L-BIC-Val-Tyr-Ser-OH, L-BIC-Leu-Val-OCH_3, L-BIC-Leu-Val-OH, L-BIC-Val-Tyr-NH_2, L-BIC-Val-Tyr-OCH_3, L-BIC-Val-Tyr-OH, L-BIC-Leu-OCH_3, L-BIC-Val-OCH_3, and L-BIC-OCH_3. The rate of inactivation of mouse submaxillary gland renin by these reagents was studied under a variety of conditions. L-α-Bromoisocaproyl-Val-Tyr-Ser-OH and L-α-bromoisocaproyl-Leu-Val-Tyr-Ser-OH were the most efficent inactivators followed by L-α-bromoisocaproyl-Val-Tyr-NH_2. The rates of inactivation by the first two peptides were strongly dependent on pH, being most efficient at low pH, least efficient at pH near 5.6, and becoming efficient again at netural pH. The rate of the inactivation by L-α-bromoisocaproyl-Val-Tyr-NH_2, in which the C-terminal carboxyl group is blocked, was only slightly dependent on pH. Complete inactivation was achieved by these three reagents. The inactivation was accompanied by incorporation of a stoichiometric quantity of the radiolabeled reagents. Based on these findings it was concluded that the inactivators reacted with a carboxyl group(s) in the active site of the renin molecule to form an esteric linkage. These data also suggest that a carboxyl group(s) may constitute part of the catalytically essential functional groups in renin action. D-α-Bromoisocaproyl derivatives of the various peptides mentioned above were also prepared. These compounds were much less active than the L isomers indicating that the inactivation by the L-α-bromoisocaproyl peptides was a specific reaction.

Renin (EC 3.4.4.15) plays a central role in the homeostatic control of blood pressure by producing angiotensin I through the limited proteolysis of the unique leucylleucine peptide bond of the plasma renin substrate. It also cleaves the same peptide bond in the tetradecapeptide renin substrate H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-*Leu-Leu*-Val-Tyr-Ser-OH (Skeggs et al., 1957). The mechanism of such a highly selective proteolysis is not understood. Experiments designed to characterize renin action using specific inactivators of proteases such as diisopropyl phosphorofluoridate (Pickens et al., 1965; Reinharz et al., 1971), *p*-hydroxymercuribenzoate (Reinharz et al., 1971), or EDTA (Pickens et al., 1965) have indicated that renin does not belong to a known class of protease such as the serine, cysteine, or metallo protease.

Systematic studies of Kokubu et al. (1968) of competitive inhibitors possessing part of the structure of the tetradecapeptide substrate (Skeggs et al., 1957) have indicated that the C-terminal portion Leu-Leu-Val-Tyr is the specific determinant for the binding to the active site of hog renal renin. Our preliminary studies support these findings. Furthermore, we have observed that the N-terminal portion of the tetradecapeptide such as angiotensin I has little affinity for renin.[1]

Although renins from different sources may be different to a certain extent, the extrarenal renin from the mouse submaxillary gland has been shown to have properties closely resembling renal renins (Cohen et al., 1972; Inagami et al., 1974). Furthermore, antibodies against the mouse submaxillary gland renin were found to cross-react with mouse renal renin (Michelakis et al., 1974). Thus, information obtained from studies of renal renin and submaxillary gland enzyme was interchangeably utilized in designing a series of active site directed inactivators possessing the general structure of α-bromoisocaproyl oligopeptide. The isocaproic acid possesses the hydrocarbon side chain identical with that of leucine, thus it can function as one of the leucine residues of the unique leucylleucine structure in directing the inactivators to the active site. These reagents were applied to the pure and stable preparation of renin from the mouse submaxillary gland (Cohen et al., 1972) for the purpose of characterizing its active site and as a method of specific inactivation of renin.

Materials and Methods

Materials. The peptides L-valyl-L-tyrosine, L-valyl-L-tyrosinamide, and L-leucyl-L-valine were obtained from Cyclo Chemical Co.; L-valyl-L-tyrosyl-L-serine and L-leucyl-L-valyl-L-tyrosyl-L-serine were purchased from Fox

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[1] Unpublished observation of Murakami and Inagami.