HORSERADISH PEROXIDASE. XIX. A PHOTOCHEMICAL REACTION OF COMPOUND I AT 5°K

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SUMMARY: When HRP-I in a glycerol/water glass was irradiated with white light from a low power source at 5°K it was found that a new species was formed. Examination of the visible and Soret absorption spectra showed that the spectrum of the new species closely resembled that of HRP-II.

INTRODUCTION

It has been known for many years that light will dissociate carbon monoxy-hemoglobin (1). Other workers have shown that this property is shared by other ferroheme carbon monoxide compounds (2,3) and that photodissociation is a common property of all liganded ferrous derivatives of hemoglobin and myoglobin (4,5).

Photoreduction was demonstrated by Schuberth (6) for some hemoproteins using an ultraviolet light source. Recently flash photolysis has been used to measure the photodissociation of carbon monoxide complexes of hemes and hemoproteins frozen in a glassy matrix at 10°K (7).

It has been established (8,9,10) that the reactions of horseradish peroxidase normally proceed by the following mechanism

$$HRP + H_2O_2 \rightarrow HRP\text{-}I$$

$$HRP\text{-}I + AH_2 \rightarrow HRP\text{-}II + AH$$

$$HRP\text{-}II + AH_2 \rightarrow HRP + AH$$

where HRP represents the native enzyme, HRP-I and HRP-II are the oxidized forms of the enzyme, Compound I and Compound II.

Irradiation of HRP-I in a frozen glycerol/water glass at 5°K

with low power white light formed a different compound. There have been no previous reports on photochemical processes involving HRP-I.

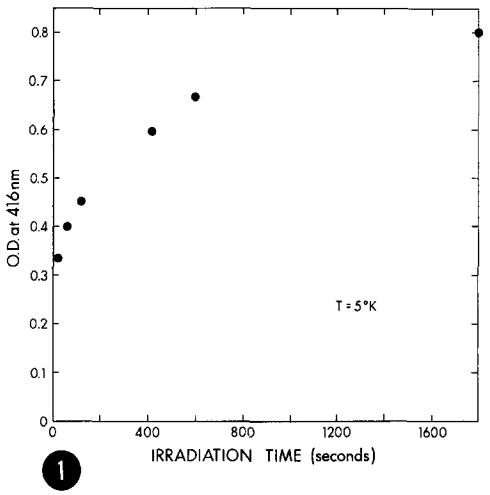
MATERIALS AND METHODS

Horseradish peroxidase (grade 1) was purchased from Boehringer Mannheim GmbH as a salt suspension batch number 7074125. The sample was prepared for use by extensive dialysis against triply distilled water. Glycerol used to prepare the low temperature glasses (11) was purchased from the Fisher Scientific Company and was certified A.C.S. grade.

A Cary 14 spectrophotometer was used to measure the absorption spectra. Samples for the low temperature spectra were placed in an Oxford Instruments Ltd. continuous flow cryostat using a carbon resistor thermometer and liquid helium as coolant. HRP-I was prepared in the laboratory at room temperature, loaded into a copper cell and immediately attached to the heat exchanger of the continuous flow cryostat held at 230°K in a nitrogen atmosphere. The cell cooled to 230°K within 3 minutes of preparation; cooling to 5°K took a further 13 minutes.

RESULTS AND DISCUSSION

The photochemical process was first observed for a dilute solution of HRP-I frozen in a 50% glycerol/water glass at 5°K. There were no changes in the spectrum of HRP-I in a frozen glass at 5°K in the region 750-250 nm in the absence of illumination over a period of five hours. Illumination of the entire cell with white light obtained from the infrared source of the Cary 14 spectrophotometer catalyzed the formation of a new species with a Soret band maximum at 416 nm compared with the Soret band maximum of HRP-I at 400 nm. After 1800 seconds of irradiation there was no further change in absorbance. Figure 1 shows a plot of the absorbance at 416 nm against the total irradiation time. In one experiment where the white light passed through the centre of the cell only, a brown region was formed which was surrounded by the green color of HRP-I. The rapid photochemical process at 5°K is in marked contrast to the extremely slow spontaneous decay of HRP-I at room temperature which is biphasic (12)



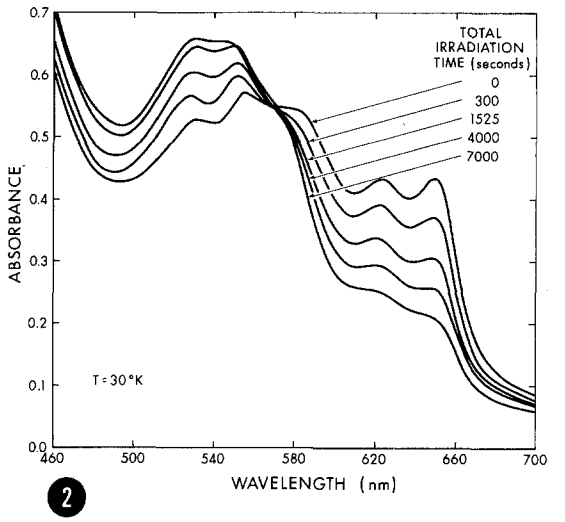


Figure 1: The change in absorbance at 416 nm when a glycerol/water glass containing HRP-I at 5°K is irradiated with polychromatic light for different lengths of time. The HRP-I was prepared by the addition of 2 molar equivalents of hydrogen peroxide to a solution of HRP in 50% glycerol/water.

Figure 2: The change in the absorption spectrum when a glycerol/water glass containing HRP-I at 30°K is irradiated with polychromatic light. The HRP-I was prepared as in Figure 1. The trace at (t = 0) is the absorption spectrum of HRP-I at 30°K in this region.

producing mainly HRP-II and then free enzyme. The Soret band maximum at 416 nm of the species formed by irradiation can be compared to that of HRP-II at 5°K, 419 nm [a] and that of HRP-II at room temperature, 420 nm (13).

The visible spectra of HRP, HRP-I and HRP-II are completely different and allow characterization of each species (12,13). Figure 2 shows the low temperature visible spectrum of HRP-I before irradiation has taken place (t = 0) and the development of the new species for increasing times of illumination with an isosbestic point at 566 nm. In this case the lamp power was

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

reduced to avoid annealing the glass through an increase in temperature and the spectra were recorded at 30°K where greater temperature stability could be maintained during both the illumination and dark periods. In the final trace (t = 7000 s), band maxima are observed at 530, 546, 572, 620 and 650 nm. Like the Soret band, the visible band maxima are not exactly coincident with HRP-II band maxima which have been observed at 77°K (14) and 5°K [(a)]. Qualitatively, however, the spectrum generated in the photochemical process has the major characteristics of the HRP-II spectrum (Figure 2). Furthermore, a similar isosbestic point at 566 nm is observed in the spontaneous decay of HRP-I to HRP-II at room temperature (12). There appears little doubt therefore, that the reaction product is HRP-II. Conformational strain of the porphyrin of the HRP-II generated photochemically from HRP-I in a glassy matrix may alter the ground and excited states resulting in slightly different transition energies from those of HRP-II prepared at room temperature and then cooled.

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