THE OXIDATION OF HORSERADISH PEROXIDASE BY PERIODATE*

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Received March 7, 1968

A chemical mechanism for the action of horseradish peroxidase (HRP) has been proposed recently (Brill and Weinryb, 1967). One of the features of this mechanism is the association of an oxidizing equivalent with a methine bridge carbon atom in Compound I, in accord with earlier suggestions by Chance (1949) and Brill and Williams (1961). In particular, Brill and Williams (1961) suggested that one component of Compound I contains a porphyrin ring which has undergone oxidation at a methine bridge carbon atom. This idea has been criticized in the past on the grounds that such an oxidative attack would not be easily reversible. Recent Mossbauer spectroscopic studies on peroxidase by Maeda and Morita (1967) and Moss (1968) indicate renewed interest in the problem; their results are in agreement with the concept of Compound I employed by Brill and Weinryb (1967). The experiments of George (1953) strongly indicate that similar heme oxidation states are obtained whether peroxide or one of several other oxidants is reacted with HRP. This communication describes a spectrophotometric investigation of the oxidation of HRP by periodate, the results of which provide additional evidence for an oxidizing equivalent-methine bridge carbon atom association in Compound I.

METHODS AND RESULTS

Electrophoretically purified HRP (Worthington Biochemical Corp., Freehold, N.J.), approximately 20 μM, was incubated with 0.013 M sodium metaperiodate in acetate buffer, pH 4.6, at 5-6° C. in a brown glass bottle. Aliquots from the reaction mixture were removed at intervals and treated with lead (II)

* The opinions in this paper are those of the author and do not necessarily reflect the views of the Navy Department or the naval service at large.

acetate to destroy excess periodate. The resulting precipitate was centrifuged away and the supernatant was examined in a Cary Model 14 recording spectrophotometer over the visible wavelengths (400-700 mμ) region. The development of the spectrum of oxidized HRP with time revealed that the absorption band of free HRP at about 640 mμ shifts gradually to longer wavelengths until (after 3 hours oxidation) it is apparently centered at about 670 mμ. As the oxidation time increases, this band intensifies, until, after 89 hours oxidation, it is approximately half as intense as the HRP Soret band. By this time, a much smaller band at about 545 mμ has materialized, but the other bands in the 500-600 mμregion characteristic of HRP Compounds II and III are not distinguishable.

If the oxidation is conducted at 23-24° C. in a cuvet and monitored directly in the spectrophotometer, the spectral behavior observed is significantly different. The oxidation is much more rapid, and the absorption maximum in the near infrared has shifted to 675 mμ after oxidation for 70 min. Bands characteristic of Compound II appear in the 500-600 mμ region, in agreement with the results of George (1953). Another band, centered at about 620 mμ, appears immediately and persists until it is obscured by the rapidly intensifying absorption at 675 mμ. To the author's knowledge, a transition at about 620 mμ has not up to now been reported for an oxidized HRP derivative. This band is also present in the spectrum of periodate-oxidized HRP when the reaction is run at 5-6° C. in the spectrophotometer. Under these conditions, the absorption maximum in the 640 mμ region shifts only as far as 651-2 mμ, a transition typical of HRP Compound I (see, for example, Brill, 1966).

DISCUSSION

Recently it has become possible to prepare metallo-oxyporphyrins (Jackson et al, 1966) in which a methine bridge carbon atom has been oxidized. Their visible spectra show an intense band at about 675 mμ, exceeded in intensity in the visible region only by the Soret band. These considerations and the obser-

vations recorded above suggest that the basis of the 670-675 mμ band of oxidized HRP is the oxidation of a methine bridge carbon atom; and that, in Compound I, a reversible interaction between an oxidation equivalent and a methine bridge carbon atom may take place which results in an electronic structure which has a higher energy (lower wavelength) transition than is present in oxyporphyrins.

The significance of the 620 mμ band is not as yet clear. It is unlikely that this band arises from an impurity associated with the periodate, since the author has also observed this absorption band under conditions where HRP is oxidized with peroxide. A possible basis for the transition is suggested by the observation that metallo-oxyporphyrin-like structures show a like band as a shoulder at 625 mμ (Jackson et al, 1966). However, conclusions concerning the basis and significance of the HRP structures responsible for the 620 mμ band must ultimately rest on additional experimental work.

ACKNOWLEDGMENTS

This study was carried out in the Molecular Biophysics Department at Yale University, New Haven, Connecticut, while the author was a U.S.P.H.S. Predoctoral Fellow, and was supported by U.S.P.H.S. Research Grant GM-09256 to Prof. Arthur S. Brill, whom the author thanks for his continued interest.

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