REVERSIBLE CONVERSION OF HORSERADISH PARAPEROXIDASE (LOW SPIN) INTO NORMAL PEROXIDASE (HIGH SPIN) BY SULFHYDRYL REAGENTS

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Horseradish paraperoxidase (or peroxidase I) was first discovered by Theorell in 1940, who later concluded (Theorell, 1942) that paraperoxidase is a conversion product of peroxidase. Paraperoxidase was thus considered to be a derivative of normal peroxidase (or peroxidase II) formed by a change in the formation of the peroxidase protein, thus bringing a suitable group into proximity with the iron in order to form a loose bond (Theorell, 1947). This idea may be consistent with the results of Keilin and Hartree (1951) in which they reported always obtaining a typical true peroxidase with the characteristic methaemoglobin-like absorption spectrum. On several occasions a solution of highly purified true peroxidase left for a considerable time in an ice chest gradually turned its methaemoglobin-like absorption spectrum into that of parahematin type. Paul (1958) has also observed that horseradish normal peroxidase showed a partial conversion to paraperoxidase after a few hours in the refrigerator at pH 9. However, the reproducible conversion of normal peroxidase to paraperoxidase has not yet been observed.

Paraperoxidases have been also isolated from sweet potato (Kondo and Morita, 1952), broad beans (Morita, 1954), Japanese radish (Yamazaki and Fujinaga, 1956 and Morita and Kameda, 1957), and wheat germ (Hagihara et al., 1958). Paraperoxidases are present in these tissues in variable but always lower concentrations than those of normal peroxidase. The relationship between these two types of peroxidase has not been elucidated.

Yamazaki and Fujinaga (1956) observed that the addition of small amounts of Cu^{2+} with certain reductants converted Japanese radish paraperoxidase into "normal" peroxidase. This conversion was also the same for the horseradish paraperoxidase. Recently, it was found that the conversion of paraperoxidase into "normal" peroxidase was caused by the addition of sulfhydryl reagents such as Ag^{+}, Hg^{2+}, and p-chloromercuribenzoate (CMB) and was easily reversed with a stoichiometric amount of cysteine as shown in Fig. 1.

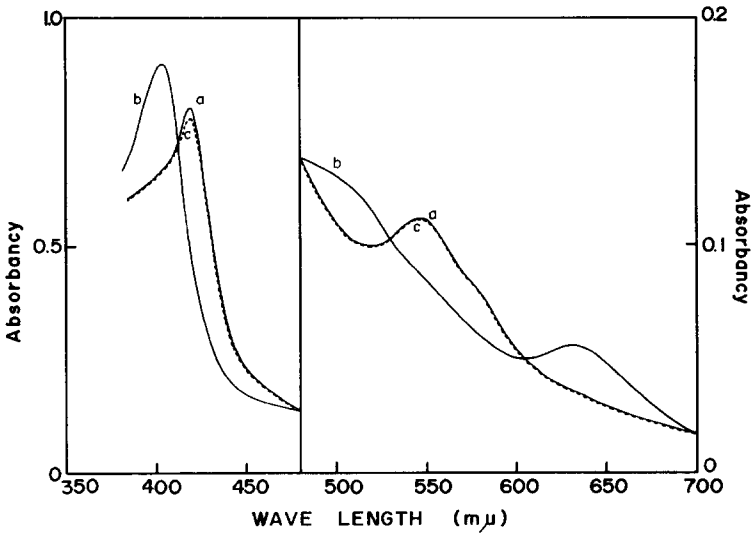

Fig. 1. Reversible conversion of horseradish paraperoxidase into "normal" peroxidase by sulfhydryl reagents. 0.05 M phosphate, pH 7.0. a. paraperoxidase b. a + 5 μM $HgCl_2$ c. b + 20 μM cysteine. All the transformation occurred instantaneously. Addition of too much cysteine should be avoided since cysteine is autoxidized to produce hydrogen peroxide.

Titration of paraperoxidase with Hg^{2+} and CMB showed clear stoichiometric increase in the absorbance at 403 mμ, which is shown in Fig. 2. Assuming that the molar extinction coefficient at 403 mμ of normal peroxidase is 107.7 mM^{-1} cm^{-1} (Paul, 1963) and that one mole of CMB reacts with one mole of paraperoxidase, one can roughly estimate that the present paraperoxidase preparation consists of 68°/o pure paraperoxidase and 32°/o normal peroxidase. These enzymes were adsorbed on carboxymethyl cellulose and cation-exchange resin such as CG-50. However, the further purification of paraperoxidase from the contaminated normal peroxidase by chromatography has so far been unsuccessful. NEM and iodoacetate did not react with paraperoxidase under ordinary conditions. Paraperoxidase and its Hg-modified peroxidase had the same peroxidase activity for the guaiacol oxidation in the presence of a sufficient amount of hydrogen peroxide. However, it should be noted that each enzyme may have different kinetics of peroxidase-oxidase reaction, a point which is now under investigation.

As can be seen in Fig. 2, one mole of Hg^{2+} reacted with two moles of paraperoxidase. This does not mean the formation of a dimer. When

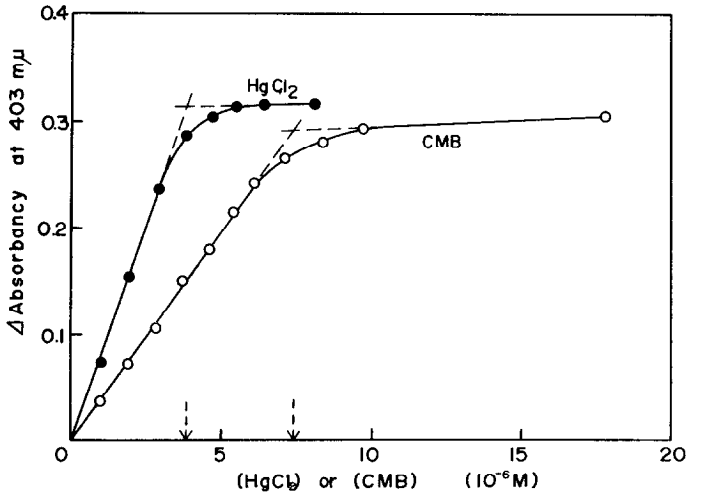

Fig. 2. Titration of paraperoxidase with $HgCl_2$ and p-chloromercuribenzoate (CMB). 0.05 M phosphate, pH 7.0 Initial absorbance at 420 mμ of paraperoxidase was 0.925. Total concentration of protoheme was found to be 11.1 μM. Stoichiometric concentrations of $HgCl_2$ and CMB which reacted with paraperoxidase were 3.77 and 7.36 μM, respectively.

Hg-modified peroxidase was isolated from the solution by ammonium sulfate fractionation, this peroxidase was never reversed to the original paraperoxidase by the addition of cysteine. By the addition of both cysteine and filtrate (ammonium sulfate solution) to the peroxidase, a restoration of the absorption spectrum to paraperoxidase was clearly observed. It may be concluded that certain sulfur compounds are responsible for the conversion between paraperoxidase and normal peroxidase. It was found that this sulfur compound did not react with the normal peroxidase originally existing in this form but reacted with the normal peroxidase which was formed from paraperoxidase after reaction with Hg^{2+} or CMB.

Paraperoxidase was a red, low-spin hemoprotein and gave typical hemochromogen bands lying at 565 and 535 mμ on reduction with $Na_2S_2O_4$. By addition of Hg^{2+}, the paraperoxidase was turned to a brown, high-spin hemoprotein and, like a metmyoglobin, gave a strong band at 557 mμ and a narrow, faint band at 594 mμ on reduction with $Na_2S_2O_4$. The existence of certain sulfur compounds which are responsible for this transformation is very interesting and the detailed results will be reported elsewhere.

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