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HORSERADISH PEROXIDASE. XXXVI.

ON THE DIFFERENCE BETWEEN PEROXIDASE AND METMYOGLOBIN

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SUMMARY: A mechanism for the reaction of hydrogen peroxide with horseradish peroxidase is proposed which involves the catalytic activity of the carboxylate side chain of aspartate residue 43. The corresponding residue in the active site of metmyoglobin is glycine E8, which explains the inability of metmyoglobin to form compound I. Certain aspects of the proposed peroxidase mechanism may be relevant to the catalytic triad for the serine proteases.

Considerable effort has been devoted to the determination of the mechanism of formation of compound I of the yeast and plant peroxidases (1-8). (For a summary of earlier work see ref. 8). In the case of horseradish peroxidase a group with a pK_a of 4.0-4.1 must be in the deprotonated form in order for compound I formation to occur and this is most likely a carboxylate anion (5). The possibility that a propionate side chain of the ferriprotoporphyrin IX is acting as a distal group has been explored and space filling models showed that the reaction might be feasible with the following steps occurring (5,9):

1. Simultaneous binding of one (α) oxygen atom of H_2O_2 to the heme ferric ion and release of the proton to the carboxylate anion. Each part of this overall step facilitates the other: oxygen binding facilitates proton release and proton release converts H_2O_2 into the much better nucleophile HO_2^-. A pK_a shift of at least 8 units must occur in order for the H_2O_2 to ionize and such a shift represents the essence of acid-base catalysis (10,11).
2. Rotation of the HO_2^- anion (bound to the ferric ion) through 180° around the Fe-O axis so that the second (β) oxygen atom is properly positioned for proton acceptance.

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3. Transfer of the carboxylic acid proton to the β oxygen atom of the HO_2^- thereby converting a potential ^-OH leaving group into the much better leaving group H_2O.
4. Departure of water leaving a Fe-O $^{\oplus}$ structure which may become Fe $^{\oplus}$=O.

These four steps outline a possible mechanism for only one part of compound I formation. They do not describe free radical formation at some other site and we shall continue to exclude this part of the overall process from our discussion.

There are certain problems in the above mechanism for Fe $^{\oplus}$=O formation. If one uses substituted perbenzoic acids in place of H_2O_2 compound I formation occurs even faster (12). There is considerable evidence that aromatic rings bind near the active site and their reactive side chains extend into the active site (13-15). The perbenzoic acids would be unable to react by the above mechanism because they would be unable to undergo the rotational process required in step 2. Work in our laboratory with peroxidases reconstituted with modified prosthetic groups also provides evidence against a propionate side chain acting as catalyst (T. Araiso and H.B. Dunford, unpublished results). Finally one may ask why metmyoglobin is unable to form compound I (16) and why it reacts so slowly with H_2O_2 (13) since it has the same prosthetic and proximal groups (18-20) as horseradish peroxidase.

A better mechanism would appear to be one in which the catalytic carboxylate group belongs to an amino acid residue. Table I shows part of the

Table I

Possible homologies in the active site regions of horseradish peroxidase and metmyoglobin (18-23)

	39	40	41	42	43	44	45	46
Horseradish peroxidase	Leu	His	Phe	His	Asp	Cys	Phe	Val
Metmyoglobin	Leu E4	Lys E5	Lys E6	His E7	Gly E8	Val E9	Thr E10	Val E11

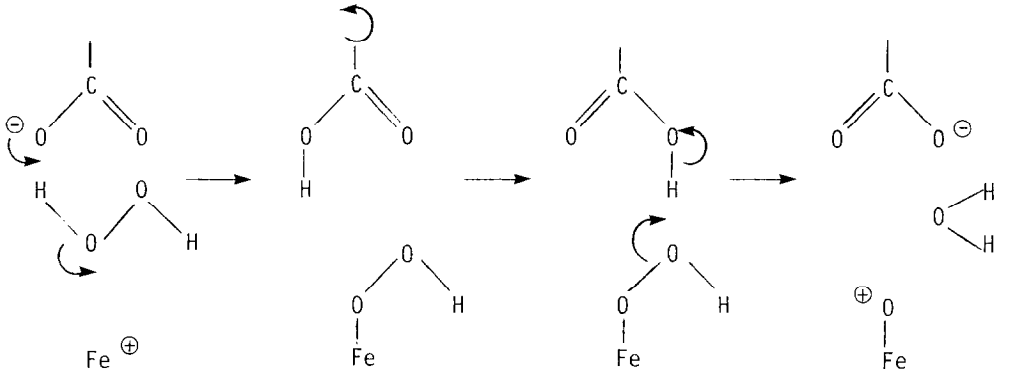

Fig. 1

primary structure of both horseradish peroxidase (19) and sperm whale myoglobin (21,22) in the active site region. Homologies in the two structures have been noted (23). His E7 is the distal group in metmyoglobin. There are only three histidines in peroxidase with histidine residue 170 the proximal group (18,19). There is chemical evidence for a distal histidine (24) and the homologies with myoglobin indicate that His 42 is a distal group in peroxidase. Thus although there are a total of about 70 glutamate and aspartate residues in horseradish peroxidase Asp 43 is an especially appealing candidate for the catalytic carboxylate group present in peroxidase but absent in myoglobin. The proposed mechanism is shown in Fig. 1.

The major change from the earlier mechanism (involving the propionate side chain) is that instead of rotating the HO_2^- group to facilitate the second proton transfer, the carboxylic acid group of asp 43 is rotated. The carboxylate side chain of the aspartate residue might be hydrogen-bonded to a neighbouring group. Proton addition from H_2O_2 to the carboxylate could break this hydrogen bond, facilitating the 180° rotation. At the completion of the process depicted in Fig. 1, the resonance symmetry of the carboxylate group effectively places it in its resting position.

The rates of the compound I formation reactions for the peroxidases listed in (1,2,4-7) correlate with the pK_a of the carboxylic acid group, with the weaker the acid, the faster the reaction (25). This could

indicate that the initial proton transfer to the carboxylate group (and oxygen binding to ferric ion) is rate limiting. Certain aspects of the mechanism for compound I formation may be relevant to the catalytic triad of carboxylate-imidazole-serine hydroxyl found in the serine proteases (26). In this regard the normal pK_a of the histidine residue of α-lytic protease recently proven using ^{15}N NMR spectroscopy (27) is relevant. The serine hydroxyl group with a pK_a of 15 requires more assistance to ionize than does H_2O_2 with a pK_a of about 12. An imidazolium ion with a pK_a of 7 is a weaker acid than a carboxylic acid group; hence imidazole is a stronger base than the buried carboxylate of the serine protease catalytic triad. Thus the imidazole group is better able to facilitate proton transfer from the serine hydroxyl in the initial stages of the nucleophilic attack on the carbonyl carbon atom of the substrate. When the tetrahedral intermediate is formed, then the imidazolium ion is then well positioned to protonate the leaving group (28). Whether the carboxylate anion is an intermediary proton acceptor or merely holds the histidine in the correct alignment is the essence of the "charge relay" debate. We merely note that if an abnormally protonated carboxylate group does exist in the serine proteases as in the peroxidases it is part of or near the transition state in a reaction coordinate diagram and rapidly loses its newly acquired proton in the next step of the reaction. It does not provide conflict with evidence for normal pK_a values in the resting enzymes.

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