THE ACTION OF PEROXIDASES WITH ENZYMICALLY GENERATED PEROXIDE IN THE PRESENCE OF CATALASE

by

W. E. KNOX*

Molteno Institute, Cambridge (England)

The oxidation of tryptophan in liver is the function of a peroxidase[26] which reacted under different conditions from those classically used with other peroxidases. Apparently unique properties of the tryptophan peroxidase were: 1. It was strictly specific for L-tryptophan; 2. the system could function in the presence of catalase; 3. its reaction was a "coupled oxidation" in two steps, one using oxygen and forming peroxide for the other; and 4. its reaction was inhibited by CO[28].

Other peroxidases have generally been studied with only a few, unnatural substrates, in the absence of catalase, and in the presence of a high peroxide concentration. What the other peroxidases normally do is not yet clear from such studies. It was therefore of interest to test their reactions under the conditions in which the physiological reaction of the tryptophan peroxidase occurred. It has been known since the work of THURLOW[40] who discovered this type of reaction, that peroxidases could act with enzymically generated H_2O_2, *i.e.*, as a coupled oxidation system. Her early advocacy of these reactions as physiologically appropriate models of those occurring normally has been borne out by the nature of the tryptophan reaction. The efficiency and substrate specificity of several other peroxidases have now been studied with peroxide generated by glucose oxidase and in the presence of catalase. The results show that all of them can react efficiently with these minimal peroxide concentrations which could occur physiologically, and that each possesses a considerable degree of substrate specificity. In comparison, the tryptophan peroxidase remains unique only in the degree of its specificity, and in its sensitivity to CO inhibition. Both of these properties can be attributed to its catalysis of two instead of one reaction steps, whereby it becomes a self-contained coupled oxidation. It is significant that one substrate, dihydroxymaleic acid, can be oxidized by the other peroxidases in two steps in a manner completely analogous to the oxidation of tryptophan by tryptophan peroxidase.

METHODS

Enzyme preparations and assays. The pure horse radish peroxidase and glucose-oxidase (notatin) preparations used have been described[24,25]. The catalase was a commercial preparation (Armours). Milk peroxidase was prepared from cow's milk by ammonium sulfate fractionation and heating at

* Present address: Cancer Research Institute, New England Deaconess Hospital, Boston, Mass.

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70° C, following the methods of ELLIOTT[18] and of THEORELL AND AKESON[38]. White blood cell peroxidase was isolated from the white cells of patients with chronic myelogenous leukemia, following the method of AGNER[2] through the alcohol fractionation step. Methemoglobin was prepared from rabbit hemoglobin[20] by oxidation with $K_3Fe(CN)_6$, followed by reprecipitation with $(NH_4)_2SO_4$ and dialysis. The tryptophan peroxidase used was the catalase-free, soluble pH 5 precipitate[26] of livers from rats which had been adapted previously to tryptophan[27]. In the manometric assays of the tryptophan peroxidase with different substrates, the heat inactivated enzyme (60° C for 5 min) and substrate were also present in the catalase-glucose oxidase control, to correct for nonspecific oxidation. Inhibitors were tested in the tryptophan peroxidase reaction by determination of the kynurenine formed[26], in the absence of added catalase and glucose-oxidase. Under these conditions the purified peroxidase utilized the peroxide generated by its own oxidase, and any effects due simply to action of the inhibitors on the catalase were avoided.

The coupled oxidation reaction. A glucose-glucose oxidase-catalase system was used to form and decompose H_2O_2. In a similar system plus a peroxidase and its substrate the H_2O_2 was used up, increasing the oxygen uptake. The peroxidase reaction was followed manometrically, by the difference in oxygen uptake between the systems with and without peroxidase. The peroxidase substrate was added to both to correct for its spontaneous or catalase-catalyzed oxidation. Only nitrite[22] and pyrogallol were oxidized in this way, and only to the extent of increasing the oxygen uptake about 10% above that referable to the glucose oxidation.

The reactions were run at 37° C, in 0.07 *M* phosphate, pH 7.0, except with methemoglobin, whose slow reaction at pH 7 necessitated measurements in 0.07 *M* acetate, pH 4.7. The components of the main compartment of the Warburg vessels are given in Fig. 1. The reactions were started after 10 min equilibration by tipping in 15 μmols of glucose. Measurements were made during the linear reaction period, lasting at least 30 min, and the H_2O_2 utilization was calculated from the extra oxygen consumption of the peroxidase-containing vessel ($\frac{1}{2}\ O_2 = H_2O_2$). From the observed rate of H_2O_2 utilization the activity of the peroxidase was calculated as μmols H_2O_2 used/mg enzyme/5 min. Comparisons of peroxidases acting on the same substrate were made with an amount of each peroxidase which caused about 1.5 times the oxygen uptake of the control.

Pyrogallol oxidation. The small spontaneous oxidation of pyrogallol was not included in the manometrically determined rates of the enzyme activities by the above method, but such oxidation during the equilibration as well as during the experimental period had to be avoided when purpurogallin formation was also determined. By running these reactions at pH 6.0 and 20° C, and by tipping in both glucose and the peroxidase after the equilibration period, the spontaneous formation of purpurogallin was practically eliminated. In this way the peroxide utilization was compared with the purpurogallin formation in the coupled oxidation system. Under these same conditions the coupled oxidation assay was compared with the purpurogallin assays using bottle-peroxide. Horse radish peroxidase was assayed by the method of SUMNER AND GJESSING[32], and milk peroxidase by the method of THEORELL AND AKESON[38], without extrapolation to higher peroxide concentration.

RESULTS

Peroxidase reactions in the coupled oxidation system. The ability of horse radish peroxidase to use immediately and completely the H_2O_2 generated by glucose oxidase *in the presence of catalase* is shown in Fig. 1. The complete system without peroxidase took up the theoretical $\frac{1}{2}\ O_2$ per molecule of glucose (curve 1). The peroxide formed was immediately decomposed by the excess catalase, and no oxidation of the tyrosine present occurred:

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2.$$

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + \tfrac{1}{2}\ O_2.$$

$$\text{Glucose} + \tfrac{1}{2}\ O_2 \longrightarrow \text{Gluconic acid} + H_2O.$$

When an excess of peroxidase was added (curve 4), it replaced the reaction of catalase. The oxygen uptake was exactly doubled (1 O_2 per molecule of glucose), and the yellow oxidation product of tyrosine appeared:

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$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2.$$

$$\text{Tyrosine} + H_2O_2 \xrightarrow{\text{Peroxidase}} \text{Yellow product} + H_2O.$$

$$\text{Glucose} + O_2 + \text{Tyrosine} \longrightarrow \text{Gluconic acid} + \text{Yellow product} + H_2O.$$

The rate, as well as the final amount of extra oxygen consumed, was approximately proportional to the peroxidase added (curves 2 and 3) when the total oxygen uptake was between 1.0 and 1.5 times the control. In this range, with utilization of as much

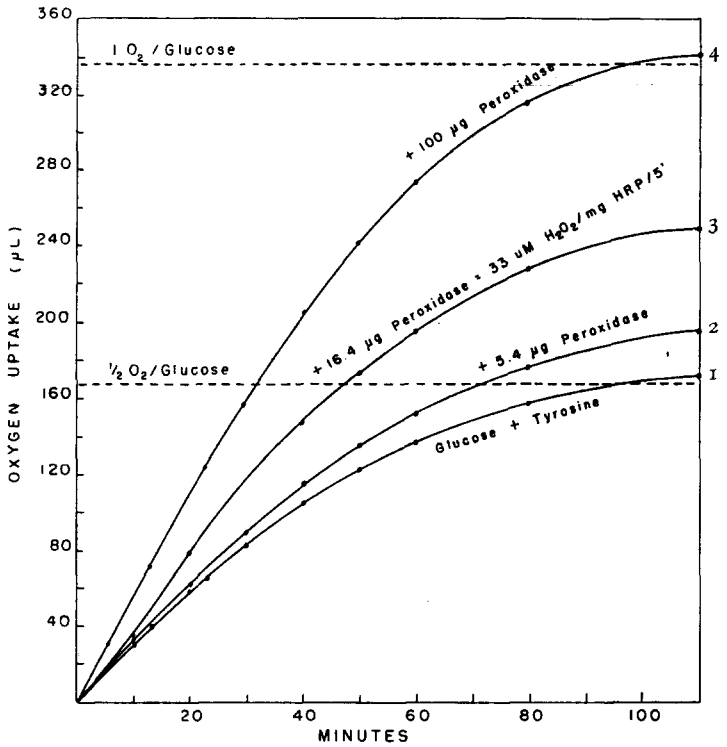

Fig. 1. The oxidation of L-tyrosine by horse radish peroxidase with the peroxide formed in a glucose oxidase-glucose-catalase system. Each vessel contained 1.0 ml 0.2 *M* phosphate, pH 7.0, 10 μg glucose oxidase, 0.5 mg catalase, 1 mg L-tyrosine, and in a side-arm, 15 μmols glucose. Total volume 3.0 ml, NaOH papers in center-wells, gas phase air, 37°. Horse radish peroxidase present: in curve 1, none; curve 2, 5.4 μg; curve 3, 16.4 μg; curve 4, 100 μg. The horizontal lines mark the theoretical oxygen uptakes of $^1/_2$ O_2/glucose (complete decomposition of peroxide by catalase) and 1 O_2/glucose (complete utilization of peroxide by peroxidase). The peroxidase activity calculated from curve 3 was 33 μmols H_2O_2/mg/5 min.

as half of the generated peroxide, the activity of a peroxidase preparation was reproducible within 20%, and was not significantly changed by altering the rate of glucose oxidation or the catalase concentration (Table I).

Substrate specificity of peroxidases. The rates at which a variety of compounds were oxidized by the different peroxidases were determined by experiments similar to that of Fig. 1. The total oxygen uptakes with excess peroxidase were never significantly more than 1 O_2 per glucose except with the tryptophan peroxidase plus tryptophan; *i.e.*, the other peroxidases did not act as oxidases. Since the different enzymes were not

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TABLE I

RATES OF OXIDATION OF TRYPTOPHAN BY HORSE RADISH PEROXIDASE IN THE COUPLED OXIDATION SYSTEM: REPRODUCIBILITY WITH DIFFERENT CONCENTRATIONS OF GLUCOSE OXIDASE AND OF CATALASE

	Peroxidase μg	*Glucose-oxidase* μg	*Catalase* mg	*Reaction rate* μmols H_2O_2/mg enzyme/5 min
1	100	10	0.5	3.4
2	200	10	0.5	3.2
3	200	10	0.5	2.9
4	200	10	0.15	3.0
5	200	10	1.5	2.8
6	200	20	0.5	3.1

of the same degree of purity, the rates at which they oxidized the various compounds have been given relative to the conveniently intermediate rate of tyrosine oxidation (Table II). The observed rates of tyrosine oxidation by each preparation are given in the footnote of the table. Tryptophan peroxidase in an amount which oxidized 8 μmols of L-tryptophan per hour did not oxidize any of the other test compounds at a significant rate and has therefore not been included in the table.

TABLE II

RELATIVE RATES OF OXIDATION OF COMPOUNDS IN THE COUPLED OXIDATION SYSTEM BY THREE DIFFERENT PEROXIDASES AND METHEMOGLOBIN

(The rate of tyrosine oxidation is set at 100 for each enzyme*. Each value represents the mean of three or more determinations. The reactions of methemoglobin were at pH 4.7, and the others at 7.0. The concentration of each of the compounds used in the test system is given in parentheses.)

	Horse radish peroxidase	*Milk peroxidase*	*White blood cell peroxidase*	*Methemoglobin*
L-tryptophan (0.0033 *M*)	7	18	2	210
$NaNO_2$ (0.016 *M*)	9	880	—	—
NaI (0.016 *M*)	24	31	—	—
L-tyrosine (0.0018 *M*)	100	100	100	100
Resorcinol (0.0033 *M*)	500	0	84	91
Ascorbic acid (0.008 *M*)	930	560	—	252
Pyrogallol (0.0067 *M*)	7600	3500	350	270

* The average absolute rates of peroxide utilization in the oxidation of L-tyrosine by the preparations used were: horse radish peroxidase, 40; milk peroxidase, 5.5; white blood cell peroxidase, 2.5; methemoglobin, 0.4 μmols H_2O_2/mg of enzyme/5 min.

The horse radish, milk and white blood cell peroxidases were able to oxidize most of the compounds tested, but did so at greatly differing rates. Each of the peroxidases was able to oxidize some substrates 100 or 1000 times more rapidly than it could others, while methemoglobin oxidized all the substrates tested at about the same rate. The absolute rate of tryptophan oxidation by horse radish peroxidase (its slowest reaction), was of the same order of magnitude as that of methemoglobin (0.8 and 2.9 μmols H_2O_2/mg protein/5 min). Both of these proteins were essentially pure. Methemoglobin also oxidized pyrogallol at a rate similar to tryptophan (1.0/mg/5 min) but horse radish peroxidase oxidized pyrogallol more than 1000 times faster than it did tryptophan, and so revealed a relative specialization for oxidation of the former substrate.

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Different and not necessarily optimal concentrations of the individual substrates were tested. However, each substrate was tested in the same concentration with the different peroxidases, so the pattern of rates shown by a peroxidase on the series of substrates could be compared to the patterns shown by the other enzymes. The strikingly different patterns pointed clearly to individual differences in the enzymes (compare the relative rates at which the enzymes oxidized nitrite, resorcinol and pyrogallol). The conclusion that substrate specificity, and not the inherent oxidizability of the compounds, determined the different rates at which a given enzyme would oxidize the compounds was supported by these differences between the enzymes. The same conclusion was drawn from the oxidation at widely different rates of closely related compounds. For example, horse radish peroxidase oxidized indolyl-proprionic acid 10 times more rapidly than tryptophan, and acetyl-tyrosine 27 times more rapidly than tyrosine. It oxidized L- and DL-tyrosine at the same rates. CHANCE also found no relation between the rates of oxidation of compounds and their oxidation-reduction potentials[9].

Nitrate, iodide, pyrogallol, tyrosine and tryptophan were oxidized respectively to nitrite, iodine, purpurogallin, a yellow and a brown product. The same product was formed in each case with each of the three peroxidases and methemoglobin in both the coupled oxidations and the enzyme reactions with bottle-peroxide (*cf.* ELLIOTT[19]). Tryptophan, in particular, was oxidized to a butanol-soluble, ninhydrin-negative, brown material which was readily distinguished from formyl-kynurenine or kynurenine produced by the tryptophan peroxidase[26].

Comparison of pyrogallol oxidation in the coupled oxidation and bottle-peroxide systems. The rates of pyrogallol oxidation at pH 6 and 20° C by horse radish peroxidase and milk peroxidase were determined with bottle-peroxide[32, 38] and in the coupled oxidation system (Table III). The rates of peroxide utilization determined manometrically were in good agreement with the amount of purpurogallin actually formed and extracted from the reaction mixtures in the coupled oxidation system (3 μmols H_2O_2 = 220 μg purpurogallin[41]). The rates with both horse radish and milk peroxidases in the coupled oxidation system were slower than in assays with added peroxide (1/50 and 1/2, respectively). The relative efficiency of milk peroxidase in the coupled oxidation could be attributed to its stability in this system, since it reacted at a linear rate for at least 30 min. Bottle-peroxide, even in low concentrations, progressively inactivated the milk-peroxidase during its assay (*cf.* THEORELL AND AKESON[38], CHANCE[10]). The white blood cell peroxidase was not directly compared in the two assay systems, but it, too, was stable in the coupled oxidation system and was inhibited by bottle-peroxide (*cf.* AGNER[2]).

TABLE III

RATES OF PYROGALLOL OXIDATION BY HORSE RADISH AND MILK PEROXIDASES IN THE COUPLED OXIDATION SYSTEM AND IN THE ASSAYS WITH BOTTLE-PEROXIDE

(Temp. 20°, pH 6.0).

	Horse radish	*Milk*
	(mg purpurogallin/mg enzyme/5 min)	
Coupled oxidation assays:		
calculated from H_2O_2 utilization	40.9	1.5
extracted purpurogallin	42.4	1.6
Classical P.N. assays:	2100	2.9

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Peroxidase inhibitors in the coupled oxidation reactions. The peroxidases retained their sensitivity to the typical peroxidase inhibitors in the coupled oxidation system. Table IV shows the effects of inhibitors on the tryptophan peroxidase, and the first five of these listed had entirely similar effects on the other enzymes, just as they do in other types of assays. Inhibition of the peroxidases was tested with tyrosine as substrate in the usual coupled oxidation reaction, but without catalase. The activity was determined colorimetrically by the formation of the yellow oxidation product of tyrosine.

TABLE IV

INHIBITION OF TRYPTOPHAN PEROXIDASE BY CARBON MONOXIDE AND BY TYPICAL PEROXIDASE INHIBITORS

Inhibitor	*Concentration M*	*Inhibition %*
Cyanide	10^{-5}	53
Hydroxylamine	10^{-4}	50
Azide	$5 \cdot 10^{-4}$	43
Fluoride	10^{-2}	23
Sulfide	10^{-3}	65
Copper sulfate	$5 \cdot 10^{-4}$	44
Carbon monoxide		
In dark	(CO 80% – O_2 20%)	96
In strong light	(CO 80% – O_2 20%)	100
In dark	(CO 40% – N_2 40% – O_2 20%)	40
In strong light	(CO 40% – N_2 40% – O_2 20%)	20

The tryptophan peroxidase was unique, however, in being inhibited by Cu^{+2} and by CO. Complete CO inhibition of this enzyme could not be reversed, but partial inhibition was lessened by light from a mercury vapour lamp. Light alone inactivated some of the enzyme and prevented more complete reversal. The other peroxidases were not inhibited by Cu^{+2} or CO. Horse radish peroxidase, for example, oxidized acetyl-tyrosine in the coupled oxidation system at a rate of 1080 μmols H_2O_2/mg enzyme/5 min, which was not changed in 10^{-3} *M* Cu^{+2}, or with 80% CO–20% O_2 in the dark.

DISCUSSION

Action of peroxidases with minimal peroxide concentration. The steady state concentration of peroxide available to the peroxidases in the coupled oxidation system was maintained by catalase at about 10^{-9} *M*[5]. This was less than that required for half-saturation of the enzyme-peroxide complexes (10^{-8} *M* for horse radish and milk peroxidases[8]), and was of a completely different order of magnitude than the high concentrations of bottle-peroxide used in most assays ($1.4 \cdot 10^{-3}$ *M* and $1.8 \cdot 10^{-4}$ *M* for horse radish and milk peroxidases, respectively). Nevertheless, all the peroxidases acted efficiently with this minimal peroxide concentration, and the usual instability of the milk and white blood cell peroxidases was not seen. It would appear that the general requirement of peroxidases for low concentrations of peroxide has been obscured by the exceptional resistance of horse radish peroxidase to high peroxide concentrations[33]. The inability of catalase to prevent the action of a peroxidase[31], and the use of a peroxidase to detect H_2O_2 not detectable by the catalase-alcohol system[17], were earlier indications of the efficiency of the peroxidases at minimal peroxide concentrations.

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The reaction of the peroxidases under these conditions demonstrates the possibility of their function *in vivo*, where they would also have to act with enzymically generated peroxide maintained at a very low concentration. Reactions of tryptophan peroxidase, and of catalase and cytochrome *c* peroxidase[14], are already known to occur *in vivo* under such conditions. There is no certainty, however, that the correct substrate has yet been tested with any of the other peroxidases. The qualitative difference between the type of reaction of the tryptophan peroxidase (peroxidase–oxidase) and the other enzymes suggests that the latter, too, might react in two steps with the appropriate substrate.

Substrate specificity of the peroxidases. The peroxidases have often been assumed to show little substrate specificity, particularly because the colorigenic assays used were not adapted to comparisons of the rates with different substrates. With the manometric method the oxidation rates of different substrates were easily compared and were found to vary widely and to point to individual specificities for each enzyme. Tryptophan was clearly the best substrate only for the tryptophan peroxidase. In contrast to this picture of the peroxidases as individually specialized for certain compounds, the pseudo-peroxidase, methemoglobin, showed no evidence of specificity by the same criteria.

Methemoglobin and horse radish peroxidase both contain the same protein-bound hemin and both oxidized certain compounds at similar rates. The much faster oxidation of other compounds by the horse radish peroxidase must be attributed to specialization of the enzyme for these particular reactions. An analogous effect of a specific protein in increasing the reaction of particular compounds was found in the copper catalyzed oxidations studied by DODDS[16]. Compounds related to ascorbic acid were oxidized by copper alone at very similar rates. When the copper was bound to the specific protein of ascorbic acid oxidase, some particular compounds like ascorbic acid itself were oxidized much more rapidly than were others.

Particular reactions studied here have previously been studied by other methods. The inability of milk peroxidase to oxidize resorcinol, discovered by ELLIOTT[19], was confirmed. With the less pure horse radish peroxidase used earlier, the relatively slow oxidation of nitrite and tryptophan[18,19], and of resorcinol[4], was apparently missed. The white blood cell peroxidase did not oxidize tyrosine in AGNER's experiments[2], though it did so readily in the present work. A number of comparisons between the reaction rates of one enzyme with different substrates, or of different enzymes with the same substrate, have been reported[2,4,7,10,11,18,19].

These conform with the present results where comparisons can be made and in general support the conclusion that the different peroxidases show substrate specificities. The same conclusion can be drawn from the peroxidatic actions of catalases from different sources[15].

Differences in the heme or heme-linked groups have been suggested as the basis of peroxidase specificities[37,10], but this alone could not account for the difference in the reactions of methemoglobin and horse radish peroxidase with the same heme. Studies of the reactions of the heme-peroxide complexes (sometimes called enzyme-substrate complexes) have not shown how they could replace real enzyme-substrate combinations as contended by HALDANE[21]. Specific combination of the substrate with the protein moiety of the enzyme was earlier suggested on kinetic grounds by MANN[30] and is assumed in the reactions of other types of enzymes. A similar more or less specific combination of enzyme protein and substrate would best explain the present results. Evidence for

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such a ternary complex of enzyme, cytochrome *c* peroxidase and cytochrome *c* has been given by CHANCE[6].

The unique two-step reaction of tryptophan peroxidase. The catalase-free tryptophan peroxidase with tryptophan consumed oxygen by itself, in the absence of glucose-oxidase, and formed its own peroxide. This oxidase function of one of the steps in the tryptophan reaction clearly differentiated it from the type of reaction of the other peroxidases. The occurrence of the reaction in two steps must contribute to its specificity, especially in determining the nature of the reaction product, and probably provided the basis for its inhibition by CO. Light reversible CO inhibition, when it occurs, is characteristic of a ferrous-enzyme. The action of the other inhibitors, and the peroxidatic function, are referable to a ferric-enzyme. Since the two steps of the tryptophan peroxidase have not been separated by fractionation, inhibitors, aging, or preparation from different sources, it is most likely that a single enzyme acts successively in its ferrous and ferric forms to catalyze both steps. The absence of recognizable amounts of an oxidation intermediate in the reaction is also consistent with this view.

The other peroxidases remain in the ferric form, have no oxidase function, and are not inhibited by CO, with one, or possibly two, exceptions. Cytochrome *c* peroxidase was reported to be completely inhibited in the dark by 87% CO–13% O_2, and its absorption spectrum was interpreted as being consistent with a ferrous form of the enzyme[1,3,23]. However, CHANCE has stated that the reaction of cytochrome *c* peroxidase was not inhibited by CO (personal communication, 1952).

The oxidation of dihydroxymaleic acid by horse radish peroxidase[36] and by milk peroxidase[38], discovered by THEORELL AND SWEDIN[34,35], is exactly analogous to the reaction of the tryptophan peroxidase. The responsible enzyme had previously been known as dihydroxymaleic acid oxidase before it was identified as the familiar, pure peroxidase. Oxygen was consumed in this reaction and addition of peroxide was not required. The intermediary formation of peroxide was demonstrated by inhibition of the reaction with catalase. The specific reversal of the catalase inhibition by adding enzymically generated peroxide, which was used to confirm the peroxide effect in the tryptophan peroxidase-oxidase system[26], was not attempted. The dihydroxymaleic acid reaction was inhibited by the usual ferric reagents[25,36], and like the tryptophan peroxidase reaction but in contrast to the classical peroxidase reactions, it also showed a light reversible CO inhibition and an inhibition by a low concentration of Cu^{+2}[34,36,38].

A scheme to explain dihydroxymaleic acid oxidation was advanced by LEMBERG AND LEGGE[29], but it did not account for the inhibition of the reaction by ferric reagents or the uptake of two atoms of oxygen per molecule of substrate. THEORELL discounted the scheme for the latter reason[39]. CHANCE also objected to the Lemberg scheme, and suggested a coupled oxidation type of reaction[12], although he did not find any CO inhibition of the reaction under his conditions[13].

The oxygen uptake, catalase inhibition, and CO and Cu^{+2} inhibitions are common to the oxidation of both tryptophan and dihydroxymaleic acid by their respective enzymes. Both reactions proceed through two 2-electron steps (1 O_2 per substrate molecule)[26,35]. Furthermore, a single enzyme is probably responsible for the tryptophan oxidation, and obviously only one is responsible for the oxidation of dihydroxymaleic acid by pure horse radish peroxidase. The very similar properties of these two systems can be most simply explained by a coupled oxidation (peroxidase-oxidase) type of reaction for both, like that originally postulated for the physiologically occurring

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tryptophan reaction[26], but in which the iron of the single enzyme changes valence as it catalyzes successively the peroxidase and the oxidase steps:

$$S + H_2O_2 \xrightarrow[\text{(peroxidase)}]{Fe^{+3}\text{-enzyme}} S\cdot(OH)_2$$

$$S\cdot(OH)_2 + O_2 \xrightarrow[\text{(oxidase)}]{Fe^{+3}\text{-enzyme}} S\cdot(O)_2 + H_2O_2$$

The specificity and function with low peroxide concentrations suggests that other peroxidases might also function in reactions of this type if the correct substrates were known.

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SUMMARY

Significantly rapid reactions of horse radish, milk and white blood cell peroxidases occurred in the presence of catalase with enzyme-generated peroxide.

The peroxidase reactions in this "coupled oxidation" system were followed manometrically. This method was used to compare the reaction rates of these enzymes and of the liver tryptophan peroxidase on a series of oxidizable substrates.

The enzymes showed individual substrate specificity, which was attributed to the different protein moieties of the several peroxidases. Methemoglobin, acting as a pseudo-peroxidase, showed no substrate specificity.

The tryptophan peroxidase, but not the other enzymes, was inhibited by Cu^{+2} and by CO, the latter reversible by light.

The oxidations of tryptophan and of dihydroxymaleic acid by their respective peroxidases were pointed out to be similar in regard to uptake of molecular oxygen, catalase inhibition, CO and Cu^{+2} inhibition, and catalysis of a two-step reaction by a single enzyme. A similar, physiologically appropriate, coupled oxidation mechanism for each was postulated.

RÉSUMÉ

Des réactions significatives rapides des pyroxydases du raifort, du lait et des leucocytes ont lieu en présence de catalase avec le peroxyde produit par les enzymes.

Les réactions peroxydasiques dans ce système d'"oxydation couplée" ont été suivies manométriquement. Cette méthode a été employée pour comparer les vitesses de réaction de ces enzymes et celle de la tryptophaneperoxydase du foie sur une série de substrats oxydables.

Chaque enzyme présente une spécificité de substrat qui lui est propre et qui peut être attribuée à son constituant protéique. La méthémoglobine, agissant comme une pseudoperoxydase, ne présente aucune spécificité.

La tryptophaneperoxydase, à la différence des autres enzymes est inhibée par Cu^{+2} et par CO; l'inhibition par CO est réversible à la lumière.

Les auteurs ont mis en évidence, entre l'oxydation du tryptophane et celle de l'acide dihydroxymaléique par leur peroxydase respective, des similitudes dans la consommation de l'oxygène moléculaire, l'inhibition par la catalase, l'inhibition par CO et par Cu^{+2}. Dans chaque cas il y a catalyse d'une réaction en deux étapes par un seul enzyme. Les auteurs supposent l'existence d'un mécanisme d'oxydation couplée, physiologiquement possible, semblable pour les deux enzymes.

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ZUSAMMENFASSUNG

Bezeichnend schnelle Reaktionen von Meerrettich-, Milch- und weissen Blutzellenperoxydasen treten mit enzymerzeugten Peroxyden in Gegenwart von Katalase auf.

Die Peroxydase-Reaktionen in diesem "gekuppelten Oxydations"-System wurden manometrisch verfolgt. Diese Methode wurde benutzt um die Reaktionsgeschwindigkeiten dieser Enzyme und der Lebertryptophanperoxydase auf eine Reihe oxydierbarer Substrate zu vergleichen.

Die Enzyme zeigten eine individuelle Substratspezifität, die den unterschiedlichen Proteinhälften der verschiedenen Peroxydasen zuzuschreiben war. Als Pseudoperoxydase auftretendes Methämoglobin zeigte keine Substratspezifität.

Die Tryptophanperoxydase, aber nicht die anderen Enzyme, wurde mit Cu^{+2} und CO gehemmt (im letzteren Fall reversibel im Licht).

Die Oxydationen von Tryptophan und Dioxymaleinsäure mit den dazugehörigen Peroxydasen erwiesen sich in Bezug auf die Aufnahme molekularen Sauerstoffs, Katalasehemmung, CO- und Cu^{+2}-Hemmung und der Katalyse einer Zweistufenreaktion mit einem Enzym als ähnlich. Ein ähnlicher, physiologisch passender, gekoppelter Oxydationsmechanismus für beide wurde postuliert.

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