Kinetic Study of the H_2O_2 Oxidation of Phenols, Naphthols and Anilines Catalysed by the Haem Octapeptide Microperoxidase-8

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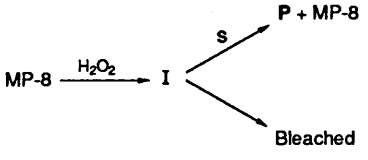
A range of phenols, naphthols and anilines has been oxidised by hydrogen peroxide using microperoxidase-8 (MP-8) as a catalyst. The rate of the reaction is independent of the nature and concentration of the substrate over a limited range, confirming that oxidation of the catalyst is the rate-limiting step. The efficiency of the catalytic oxidation, as opposed to deactivation, has been defined in terms of relative catalytic efficiency; this is dependent on the nature, but not the concentration, of the substrate. This has been interpreted as kinetic evidence for a 'substrate involved' MP-8 decomposition pathway.

The peroxidases are a class of enzymes that catalyse the oxidation of a range of substrates by hydrogen peroxide.[1] The active site of this and many related oxidising enzymes such as myoglobin, haemoglobin, catalase and the cytochromes, contains an iron porphyrin. Over the years many simpler iron porphyrins have been used as model compounds for such systems.[2] In particular, Traylor[3] has studied the peroxidase reaction in organic solvents using organic hydroperoxides and a series of simple haem catalysts having attached oligopeptide chains. Most workers have concentrated on organic solvent systems, although Jones has observed potent peroxidasic activity for a simple haem in aqueous solution.[4] Recently Pratt *et al.* have prepared[5a] a water-soluble haem octapeptide microperoxidase-8 (MP-8) and have shown that this compound exhibits peroxidase behaviour.[5b] Further aspects of the peroxidase activity have been investigated by Adams.[6] Traylor[3a] (for catalysis by a haem compound), and Pratt[5b] and Adams[6] (for MP-8), have proposed a sequence of events as outlined in Scheme 1, a scheme related to that proposed for the



Scheme 1

peroxidase enzyme, in which the oxidised haem intermediate (**I**), generated in the rate-limiting step, can either react with substrate (**S**) to produce the product (**P**) and (ultimately) to regenerate MP-8, or undergo deactivation, not involving **S**, resulting in bleaching of the catalyst. It is generally assumed that the intermediate (**I**) is an analogue of the peroxidase compound **I** (*vide infra*); the evidence for this in aqueous solution is limited, but there is some spectroscopic evidence for such analogues in the cases of coprohaem[4a] and MP-8.[6a]

Most studies have concentrated on the interaction of the catalyst with the hydrogen peroxide, rather than the oxidation of the organic substrate. Dunford and Job[7] have measured the rates of reaction of a range of phenols and anilines with the oxidised horseradish peroxidase intermediate, commonly called compound **I**, while Traylor[3a] has attempted a qualitative comparison of substrate reactivity towards an oxidised form of a haem catalyst. We were interested in making a quantitative study of how a range of readily oxidised aromatic substrates are oxidised by the hydrogen peroxide/MP-8 system. Since the substrate is involved in a step which occurs subsequent to the rate-limiting step, this portion of the reaction was studied by a comparison of product formation (and regeneration of the MP-8) with destruction of the catalyst. Experimentally this is reflected in the 'relative catalytic efficiency' which is defined in the Experimental section.

Experimental

Materials.—The H_2O_2 (60%) was of Analar grade and was diluted with water to yield stock solutions. Concentrations of H_2O_2 in the stock solutions were determined spectrophotometrically (ε_{240} = 39.4 dm^3 mol^{-1} cm^{-1}).[8] Microperoxidase-8 was prepared from horse heart cytochrome-c following a literature procedure;[5a] the concentrations of stock solutions (in 20% methanol–water) were measured spectrophotometrically (ε_{397} = 1.56 × 10^5 dm^3 mol^{-1} cm^{-1}). Phosphate buffers (0.10 mol dm^{-3}) were prepared using the sodium salts. Substrates **2**–**10** were commercially available and were purified by recrystallisation prior to use. Diguaiacol (**1**) was prepared according to Booth and Saunders.[9] Cyclopropanone hydrate (**11**)[10] was prepared as a stock solution in water by hydrolysis of 1-ethoxycyclopropanol[11] which was prepared from 1-ethoxy-1-trimethylsiloxycyclopropane.[12]

Kinetics.—Reaction rates were measured by monitoring the change in absorbance using a Philips PU 8720 or PU 8740 UV–VIS spectrophotometer and thermostatically controlled cuvettes. In general, reaction solutions were prepared by injecting an appropriate volume of stock substrate (**1**–**10**), followed by an appropriate volume of stock MP-8 solution into a 0.1 mol dm^{-3} phosphate buffer solution. Reactions were generally initiated by injection of a stock solution of aqueous H_2O_2. First-order rate constants were obtained from plots of ln ($A_{inf} - A$) against t; A_{inf} was determined after about ten half lives and values of A used in the calculation were those obtained over about one half-life. Initial rate values of A were generally obtained during the first 5% of the reaction, but for faster reactions or where the total absorbance change was small, during the first 10% of the reaction. For initial rates standard deviations over several determinations of $dA/dt_{t=0}$ were $< \pm 8\%$; deviations in values of pseudo-first-order rate constants were $< \pm 3\%$.

Determination of $\Delta A/[H_2O_2]_{consumed}$.—The term $\Delta A/[H_2O_2]_{consumed}$ was determined for each substrate either: (*a*) by measuring $A_{inf} - A_{t=0}$, where a known initial amount of H_2O_2 was allowed to react rapidly and to completion in an excess of organic substrate under conditions where MP-8 was not ap-

Table 1 Second-order rate constants k, obtained by the initial-rate method, for the MP-8 catalysed reaction of substrates **1–8** with H_2O_2 in aqueous buffer of pH 7

Compound	$c/10^{-4}$ mol dm^{-3} [a]	$(\Delta A/[H_2O_2]_{con})/10^3$ mol^{-1} [b]	$k/10^3$ dm^3 mol^{-1} s^{-1}
1 Diguaiacol	0.6–2.0	—	6.4 ± 1.8
2 4-Methoxy-1-naphthol	0.5–1.0	4.3 (620)	4.9 ± 0.5
3 4-Methoxyphenol	0.1–10	2.7 (315)	5.4 ± 0.7
4 Guaiacol	0.5–100	5.2 (470)	5.5 ± 0.4
5 2,4-Dimethoxyaniline	0.1–0.6	5.0 (500)	5.9 ± 0.1
6 Ferrocyanide	0.5–1000	2.2 (420)	5.9 ± 1.4
7 4-Methoxyaniline	0.1–0.6	5.1 (494)	5.1 ± 0.3
8 1-Naphthol	1.0–10	2.0 (450)	4.5 ± 0.4
9 2-Naphthol	—	1.9 (450)	—
10 2,4,6-Tri-*tert*-butylphenol[c]	—	9.2 (300)	—

[a] Range of substrate concentration over which the rate of reaction is substrate-independent. [b] λ/nm is given in parentheses. [c] In methanol.

preciably consumed during the reaction; or (*b*) by measuring $A_{inf} - A_{t=0}$ under similar conditions, but using the HRP enzyme which, unlike MP-8, is much less prone to destruction. A typical determination *via* (*a*) was as follows for a reaction involving 4-methoxyaniline (**7**). Reaction of **7** (5×10^{-4} mol dm^{-3}), H_2O_2 (0.86×10^{-4} mol dm^{-3}) and MP-8 (6.7×10^{-7} mol dm^{-3}) in phosphate buffer (pH 7) led to an increase in absorbance to 0.44 (at 494 nm) which was complete after 30 min; that reaction had ceased due to complete consumption of H_2O_2 was shown by the observation that addition of further H_2O_2 caused the reaction to recommence. Thus, a value of $\Delta A/[H_2O_2]_{consumed}$ was calculated to be 5116 mol^{-1}. A typical determination *via* (*b*) for guaiacol (**4**) was as follows. Reaction of **4** (1.09×10^{-3} mol dm^{-3}), H_2O_2 (0.50×10^{-4} mol dm^{-3}) and HRP (1×10^{-8} mol dm^{-3}) led to an increase in absorbance to 0.257 (at 470 nm) which was complete after 75 s; that reaction had ceased due to complete consumption of H_2O_2 was shown by the observation that addition of further H_2O_2 caused the reaction to recommence. The value of $\Delta A/[H_2O_2]_{consumed}$ was calculated to be 5140 mol^{-1}.

Relative Catalytic Efficiency (*RCE*).—A typical determination of this term, defined as $RCE = [H_2O_2]_{consumed}/[MP\text{-}8]_{deactivated}$ was as follows for guaiacol (**4**). Reaction of guaiacol (0.20 mmol dm^{-3}), H_2O_2 (0.50 mmol dm^{-3}) and MP-8 (1.4×10^{-4} mmol dm^{-3}) in phosphate buffer (pH 7) at 25 °C resulted in an increase in absorbance at 470 nm. After 5 min the absorbance at 470 nm had increased from 0 to 0.162; no further increase occurred, but addition of further MP-8 caused the reaction to resume. The change in absorbance corresponds to the consumption of 0.031 mmol of H_2O_2; this in turn accompanied the inactivation of all the 1.4×10^{-4} mmol of MP-8 leading to an RCE value of 221.

Results

The range of substrates (**1–10**) is listed in Table 1. In addition to phenols, naphthols and anilines, it includes potassium ferrocyanide [$K_4Fe(CN)_6$] (**6**). Guaiacol (**4**) is 2-methoxyphenol and diguaiacol (**1**) is 2,2′-dihydroxy-3,3′-dimethoxybiphenyl.

Rate Equation.—The dependence of the rate on MP-8 concentration and on H_2O_2 concentration was determined for diguaiacol (**1**), 4-methoxy-1-naphthol (**2**), and for 4-methoxyphenol (**3**), by monitoring the initial rate of absorbance change at a suitable wavelength, over a range of initial concentrations of MP-8 and H_2O_2, and was found to be of the form $dA/dt_{t=0} = k'_{obs}[MP\text{-}8]_0[H_2O_2]_0$. A similar rate equation has been found for guaiacol (**4**)[5b] and by Adams for 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphononic acid) (ABTS).[6] When the change in absorbance was monitored for an extended period, curvature due to depletion of H_2O_2 and/or MP-8 (*vide infra*) was observed. In the case of the substrate 2,4-dimethoxyaniline (**5**), where destruction of the MP-8 was negligible and the aniline was in excess, pseudo-first-order kinetics were observed for the loss of H_2O_2. Conversely, in the case of the substrate **3**, where the phenol and the H_2O_2 were in excess, pseudo-first-order kinetics were observed for the loss of MP-8.

Although the organic products giving rise to the 'product absorbance' were often complex mixtures, the change in absorbance can be used as a quantitative measure of the extent of H_2O_2 consumption at any stage during the reaction. Thus, for each substrate we defined a term $\Delta A/[H_2O_2]_{consumed}$. This was determined for each substrate by one or both of the two methods described in the experimental section; values for compounds **2–10** are given in Table 1. There is a degree of uncertainty in either method (for example many of the products are rather unstable); for most substrates, values of this term showed deviations over several determinations of $< \pm 5\%$, but for those substrates where the 'amount' of product formed was small (**8** and **9**) deviations were $< \pm 10\%$. Uncertainties in the value of this term are a major source of uncertainty in those parameters derived using it.

The observed rate constant from the initial rates method, k'_{obs}, can be converted into a pseudo-second-order rate constant k by dividing by $\Delta A/[H_2O_2]_{consumed}$ and the experimentally determined rate equation can be written in terms of concentrations as $-d[H_2O_2]/dt = k[H_2O_2][MP\text{-}8]$.

Variation of Initial Rate with Organic Substrate.—The variation of the initial rate $dA/dt_{t=0}$ with the concentration of the organic substrate was found to be complex. Substrates **1–8** all showed a region in which the rate was independent of substrate concentration, but a steady fall-off was observed at higher concentrations. The region of substrate-independent rate for each substrate (**1–8**), along with the value of the second-order rate constant k in this region, is given in Table 1. Quoted uncertainties are standard deviations over determinations at several concentrations within the range.

Destruction of MP-8.—Where small amounts of the catalyst MP-8 were used, it was found that the reaction terminated early owing to destruction of the MP-8. This was reflected in a value of A_{inf} substantially lower than that predicted were the reaction to have proceeded to completion, based on the known amounts of H_2O_2 and substrate added. Furthermore, the finding was confirmed, in such cases, by the fact that no reaction was observed upon addition of further H_2O_2 or further substrate, while addition of further MP-8 caused the reaction to recommence. Successive additions of MP-8 were found to cause the A_{inf} value to approach the predicted value.

In those reactions where the MP-8 was destroyed completely, the actual amount of H_2O_2 consumed was determined from $A_{inf} - A_{t=0}$ using the $\Delta A/[H_2O_2]_{consumed}$ term, defined previously.

We have defined RCE as equal to $[H_2O_2]_{consumed}/[MP\text{-}8]_{deactivated}$; this term can be viewed as a quantitative reflection of the relative efficiency of an MP-8/H_2O_2/substrate interaction which leads to product and regeneration of the catalyst, as compared with an interaction which leads to destruction (as defined by deactivation) of the catalyst. For ease of analysis we have concentrated on those reactions in which all the MP-8 is destroyed and therefore the RCE term becomes $[H_2O_2]_{consumed}/[MP\text{-}8]_{t=0}$. The RCE was determined at various substrate concentrations [$(1\text{–}30) \times 10^{-4}$ mol dm^{-3}] for a range of substrates. In general, the RCE was found to be invariant (or

Table 2 Relative catalytic efficiency (RCE) for the MP-8-catalysed reaction of substrates **2**–**10** with H_2O_2 in aqueous buffer of pH 7 along with the range of substrate concentration over which it was measured

Compound	$[MP\text{-}8]_0$ /10^{-7} mol dm^{-3}	$[H_2O_2]_0$ /10^{-4} mol dm^{-3}	$[substrate]_0$ /10^{-4} mol dm^{-3}	RCE
2	2.2	0.50	1	151 ± 30
3	1.5	5.1	1–10	415 ± 15
4	1.5	5.0	1–10	232 ± 24
5	1.4	7.5	10	1170 ± 160
6	0.6	0.9–1.9	1–10	580–1032
7	2.0	0.9–2.2	2–10	470 ± 90
8	6.5	0.51	1–30	16 ± 3
9	6.5	0.51	1–10	15 ± 2
10	2.5	0.53	1–10[a]	112 ± 12

[a] In methanol.

Table 3 The variation of the RCE with initial MP-8 concentration for the MP-8 catalysed H_2O_2 oxidation of **3** and **5**

$[MP\text{-}8]_0$ /10^{-7} mol dm^{-3}	RCE
4-Methoxyphenol (**3**)[a]	
0.3	645
0.65	675
1.0	675
1.7	645
3.1	560
5.1	480
8.0	450
10.3	470
2,4-Dimethoxyaniline (**5**)[b]	
0.2	910
0.4	1335
1.4	1098
3.0	875
3.1	715
4.1	600

[a] $[3]_0 = 1.03 \times 10^{-3}$ mol dm^{-3}, $[H_2O_2]_0 = 0.99 \times 10^{-3}$ mol dm^{-3}. [b] $[5]_0 = 1.0 \times 10^{-3}$ mol dm^{-3}, $[H_2O_2]_0 = 7.5 \times 10^{-4}$ mol dm^{-3}.

Table 4 The variation of the RCE with initial H_2O_2 concentration at given concentrations of **4** and MP-8 for the MP-8 catalysed H_2O_2 oxidation of **4**[a]

$[H_2O_2]_0$ /10^{-4} mol dm^{-3}	$[4]_0$ /10^{-3} mol dm^{-3}	RCE
1.0	1.0	250
5.0	1.0	200
0.50	2.0	190
5.0	2.0	225
0.50	5.0	200
5.0	5.0	265

[a] $[MP\text{-}8]_0 = 1.4 \times 10^{-7}$ mol dm^{-3}.

to vary only slightly in the case of **6**) over a five- to ten-fold change in substrate concentration, but to vary significantly with the nature of the substrate. The values of the RCE, along with the substrate concentration range over which it was measured are given, for a range of substrates, in Table 2. Quoted uncertainties are standard deviations over determinations at several concentrations within the range, but being derived using the $\Delta A/[H_2O_2]_{consumed}$ term, uncertainties in individual values are $< \pm 10\%$ (*vide supra*).

In order to ensure that there was complete consumption of MP-8 within a reasonable reaction time in all cases where the RCE was determined, it was necessary to use different initial amounts of MP-8 and H_2O_2 for different compounds as shown in Table 2; in the cases of **6** and **7** it was necessary to use a different concentration of H_2O_2 for each different substrate concentration. We have made a limited examination of the effect on the RCE of varying both MP-8 concentration (Table 3) and the peroxide concentration (Table 4); in general the effect is rather small, particularly for peroxide.

Variation of Pseudo-first-order Rate Constant for Loss of MP-8.—Under conditions where $[H_2O_2]_0 = 9.3 \times 10^{-4}$ mol dm^{-3}, $[MP\text{-}8]_0 = 2.2 \times 10^{-7}$ mol dm^{-3}, and $[substrate]_0 = (2 - 10) \times 10^{-4}$ mol dm^{-3}, the absorbance change for the reaction of 4-methoxyphenol (**3**) was found to follow first-order kinetics. Initial-rate experiments (see values for $dA/dt_{t=0}$ in Table 5) showed that the rate was independent of substrate concentration, and the total absorbance change showed that $< 10\%$ of the H_2O_2 had reacted by termination; furthermore, addition of further MP-8 caused the reaction to recommence. Thus, it was clear that the first-order change in absorbance reflected a first-order loss of MP-8. The values of the pseudo-first-order rate constants for the loss of MP-8 at various concentrations of **3** are given in Table 5.

Kinetics of Loss of MP-8 *in the Presence of* 1-*Naphthol.*—When 1-naphthol (**8**) was used as a substrate, relatively rapid deactivation of the MP-8 occurred along with formation of unidentified materials absorbing above 400 nm in the UV–VIS region of the spectrum. During the reaction the MP-8 peak at 397 nm was transformed into a peak at 408 nm. The material represented by this peak was stable over several hours and was catalytically inactive. No evidence of a similar peak was obtained when MP-8 was allowed to 'bleach' on treatment with H_2O_2, and then treated with naphthol. The conversion of the peak due to MP-8 at 397 nm into that at 408 nm was found to follow first-order kinetics with excess H_2O_2. The observed rate constant k_{obs} for this conversion was found to be linear in H_2O_2, and to vary only slightly on going from a naphthol concentration of 10^{-4} to 10^{-3} mol dm^{-3}. The results are collected in Table 6.

Reaction in the Presence of Cyclopropanone Hydrate.—Values of the RCE for the oxidation of guaiacol (**4**) in the presence of $(0.12–12) \times 10^{-3}$ mol dm^{-3} cyclopropanone hydrate (**11**) were determined, and the results are collected in Table 7.

Discussion

Given the degree of uncertainty in the value of $\Delta A/[H_2O_2]_{consumed}$ used in the calculation of the second-order rate constants k of Table 1, the values of k can be considered to be constant for all the substrates provided that the reaction is examined over a limited range of substrate concentration. This observation, in addition to the first-order dependence of rate on

Table 5 Initial rate of change of absorbance, along with values of k_{obs}, k and RCE_{calc}, for the MP-8 catalysed H_2O_2 oxidation of 4-methoxyphenol (**3**) in aqueous buffer of pH 7[a]

[**3**]/10^{-4} mol dm^{-3}	$(dA/dt)/10^{-3}$ s^{-1}	$k_{obs}/10^{-3}$ s^{-1}	k/dm^3 mol^{-1} s^{-1}	RCE_{calc}
2.0	1.95	9.24	3668	369
4.0	2.60	8.16	4675	533
6.0	2.45	8.67	4718	506
8.0	2.40	7.77	4725	566

[a] $[H_2O_2]_0 = 9.3 \times 10^{-4}$ mol dm^{-3}, $[MP\text{-}8]_0 = 2.2 \times 10^{-7}$ mol dm^{-3}.

Table 6 Values of k_{obs} along with concentrations of H_2O_2, **8** and RCE_{calc} for the MP-8 catalysed H_2O_2 oxidation of **8** in aqueous buffer of pH 7[a]

[**8**] /10^{-4} mol dm^{-3}	[H_2O_2] /10^{-4} mol dm^{-3}	k_{obs}/s^{-1}	RCE_{calc}
1.00	0.26	0.022	5
1.00	0.52	0.047	5
1.00	0.85	0.072	5
1.00	1.11	0.085	6
1.00	0.58	0.054	5
10.00	0.58	0.044	6

[a] $[MP\text{-}8]_0 = 13 \times 10^{-7}$ mol dm^{-3}.

Table 7 RCE values along with concentrations of cyclopropanone hydrate (**11**), for the MP-8 catalysed H_2O_2 oxidation of guaiacol (**4**) in aqueous buffer of pH 7[a]

[**11**]/10^{-3} mol dm^{-3}	RCE
0.00	260
0.12	248
0.59	218
1.17	175
5.87	120
11.7	83

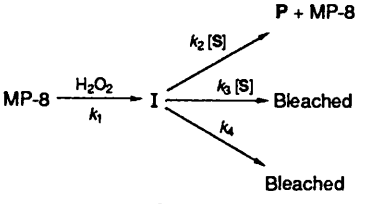
[a] $[H_2O_2]_0 = 5.15 \times 10^{-4}$ mol dm^{-3}, $[\mathbf{4}]_0 = 5.0 \times 10^{-4}$ mol dm^{-3}, $[MP\text{-}8]_0 = 7.0 \times 10^{-7}$ mol dm^{-3}.

[H_2O_2] and on [MP-8], supports an initial rate-limiting reaction of H_2O_2 and MP-8. The mean value of k is 5500 ± 600 dm^3 mol^{-1} s^{-1}, which agrees well with that of Pratt[5b] (5290) and that found by Adams[6b] (4778).

At substrate concentrations higher than the range given in Table 1 the initial rate was found to fall. A similar result has been found by Adams[6b] using 2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonate) (ABTS) as the substrate and has been attributed to competitive formation of an MP-8-ABTS adduct. We believe that something similar is occurring in our case, and intend to deal with this aspect of the work in a later publication. However, most of the results discussed here relate to the region where the initial rate is substrate independent and where substrate is not involved in the rate-limiting step.

For Scheme 1 the RCE as defined above, should increase more or less linearly with increasing substrate concentration for a given initial concentration of MP-8. Indeed an examination of Traylor's[3a] work shows that for a protohemin-catalysed *m*-chloroperbenzoic acid oxidation of **10**, the amount of product produced for a given amount of catalyst increases approximately linearly with substrate concentration up to a maximum determined by the amount of *m*-chloroperbenzoic acid present.

An examination of the results in Table 2 shows that such a simple scheme is not appropriate in the present case, since the RCE for most substrates hardly varies at all over a large range of substrate concentration. We have already noted the effect on the RCE of varying the initial concentrations of MP-8 or H_2O_2, but in the context of the analysis of the results (*vide infra*) and given the large range of values in Table 2, we do not consider this effect to be significant. The implications of the invariance of the RCE are that either: (*a*) both the 'product-forming' and the 'destructive' reactions of the intermediate (**I**) are independent of **S**; or (*b*) there is a second, but 'destructive', pathway involving **S** leading to deactivation of the MP-8; this must also be the predominant destructive pathway. The fact that the RCE depends on the nature, but not on the quantity of the substrate seems to exclude (*a*), and we therefore propose Scheme 2.



Scheme 2

For such a scheme, assuming the intermediate (**I** in Scheme 2) to be in 'steady state', we can derive equations for the initial rate v [eqn. (1)], the rate of loss of MP-8 (d[MP-8]/dt) (eqn. (2)] and the RCE [eqn. (3)].

$$v = \frac{k_1 k_2 [MP\text{-}8]_0 [H_2O_2]_0}{k_2[S]_0 + k_3[S]_0 + k_4} \times [S]_0 \quad (1)$$

$$\frac{d[MP\text{-}8]}{dt} = -k_1[MP\text{-}8][H_2O_2] \times \frac{k_3[S] + k_4}{k_2[S] + k_3[S] + k_4} \quad (2)$$

$$RCE = \frac{k_2[S]}{k_3[S] + k_4} \quad (3)$$

The expression for the RCE is derived by dividing $[H_2O_2]_{consumed}$ by $[MP\text{-}8]_{deactivated}$; the former quantity is obtained from the integrated form of $-d[H_2O_2]/dt$ (equivalent to v at any time) and the latter by integrating eqn. (2) from $t = 0$ to infinity. If it is assumed that [S] varies little over the course of the reaction, the terms involving **S** can be taken outside the integral terms which then cancel (the expression for the experimentally determined RCE, $[H_2O_2]_{consumed}/[MP\text{-}8]_{t=0}$ is a special case of the above when all MP-8 is consumed). It can be seen that our experimental results are consistent with the proposed Scheme 2 by consideration of the following analysis. For all substrates examined, there is considerable catalytic activity; this is reflected in the RCE which is never less than five. More specifically this implies that the product forming reaction of **I** is generally much faster than the sum of the 'destructive' reactions. In our scheme this implies that $k_2[S] > k_3[S] + k_4$ and therefore eqn. (1) simplifies to eqn. (4), which corresponds to the form observed experimentally, and eqn. (2) simplifies to eqn. (5).

$$v = k_1[MP\text{-}8]_0[H_2O_2]_0 \quad (4)$$

$$\frac{d[MP\text{-}8]}{dt} = -k_1[MP\text{-}8][H_2O_2] \times \frac{k_3[S] + k_4}{k_2[S]} \quad (5)$$

We have found, in agreement with others,[5,6] that decomposition of **I** occurs in the absence of an oxidisable substrate, and this is represented in our scheme by k_4. The substrate concentration independence of the RCE can be rationalised in terms of Scheme 2 if we assume that $k_3[S] > k_4$; this implies that deactivation of **I** is predominantly due to the 'substrate-involved' path and that the k_4 route only becomes apparent in the absence of substrate. To an approximation, therefore, eqn. (3) simplifies to eqn. (6) and the RCE is dependent on the nature, but not the concentration, of substrate as observed.

$$RCE = k_2/k_3 \quad (6)$$

$$d[MP\text{-}8]/dt = -k_1[MP\text{-}8][H_2O_2]/RCE \quad (7)$$

Eqn. (5) can be further simplified by substituting in eqn. (3) to give eqn. (7). Therefore, if the rate of loss of MP-8 can be measured the values of the RCE calculated [using k_1 from eqn. (4)] in this way can be checked for consistency with those obtained directly. As stated in the results section the data in Table 5 relate to the first-order loss of the MP-8 catalyst and according to eqn. (7) $k_{obs} = k_1[H_2O_2]_0/RCE$. Values of k_1 (equal to k in Table 5) have been determined from initial-rate measurements and values of the RCE calculated using k_1 and k_{obs} are given in Table 5. As can be seen from Tables 2 and 5, values of the RCE agree reasonably well.

At present we have only kinetic evidence for the 'substrate-involved' deactivation of the MP-8. In general the deactivated catalyst is bleached, and shows no absorbance in the region where MP-8 absorbs. However, a deactivated form of the catalyst, showing a peak at 408 nm was observed when naphthol was used as substrate. The form of the rate equation for conversion of the MP-8 into the deactivated material at 408 nm can be seen from the results in Table 6 to be consistent with the form of eqn. (7). As above, a value of the RCE can be calculated using k_{obs} and k_1 (equal to k in Table 1). In particular it should be noted that it does not increase significantly as the concentration of naphthol is increased ten-fold; the value of the RCE obtained in this set of experiments is rather lower than that in Table 2, but in view of the very low extent of product production reflecting the low value of the RCE the agreement is reasonable.

We examined the literature to search for evidence to support the proposition of 'substrate-involved' deactivation, and found that cyclopropanone hydrate (**11**) can act as a 'suicide inhibitor' toward the peroxidase enzyme.[10] There is evidence[10a] that the mode of deactivation for this substrate involves attack on the haem portion of the catalyst. Addition of increasing amounts of this inhibitor to the MP-8 catalysed H_2O_2 oxidation of guaiacol (**4**) led to a steady decrease in the RCE (Table 7) suggesting that **11** is competing with **4** for reaction with **I** and enhancing deactivation of the MP-8. No non-bleached deactivated catalyst could be detected by UV at the end of the reaction suggesting that any deactivated form of MP-8 to which the inhibitor is bonded is transient.

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Paper 1/02074I
Received 1*st May* 1991
Accepted 24*th July* 1991