Horseradish Peroxidase Catalysed Sulfoxidation is Enantioselective

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Horseradish peroxidase catalysed oxidation of aryl methyl sulfides by H_2O_2 preferentially gives (−)-(*S*)-sulfoxides, enantiomeric excess 30–68%.

Peroxidases are haemoproteins that catalyse the oxidation of a large number of organic and inorganic substrates.[1] Among them, horseradish peroxidase (donor: hydrogen peroxide oxidoreductase, EC 1.11.1.7) has been the most thoroughly investigated.[2] There are a few reactions promoted by peroxidases that involve the incorporation of oxygen into the product but the origin of the oxygen is generally not H_2O_2.[3] The only exception seems to be the sulfoxidation of thioanisoles, for which partial oxygen incorporation from peroxide has been found.[4] According to these authors, horseradish peroxidase (HRP) is, however, unable to induce chirality in the oxidation with H_2O_2 of *p*-tolyl methyl sulfide to the corresponding sulfoxide.[4] Instead, we report that asymmetric sulfoxidations catalysed by HRP are possible under appropriate reaction conditions, eqn. (1).

$$\text{Ar–S–Me} \xrightarrow{\text{HRP, } H_2O_2} \text{Ar–SO*–Me} \qquad (1)$$

Substituted phenyl methyl sulfides were chosen as model substrates since the stereocentre of the corresponding sulfoxides is optically stable. In order to verify the generality of this type of asymmetric synthesis we also investigated the behaviour of benzyl methyl and 2-pyridyl methyl sulfide. Yields, absolute configurations and enantiomeric excess (e.e.) values are reported in Table 1. The data show that asymmetric induction takes place only with phenyl methyl sulfide and *para*-substituted phenyl methyl sulfides.† No asymmetric synthesis was observed with *ortho*-substituted phenyl methyl sulfides, benzyl methyl sulfide and 2-pyridyl methyl sulfide. In the cases examined the prevailing sulfoxide had the (*S*) absolute configuration. Interestingly, this stereochemical course is opposite to the course we observed with another haemoprotein, chloroperoxidase.[5] Also soybean hydroperoxide-dependent oxygenase leads preferentially to the (*S*) sulfoxides.[6] Therefore, in the sulfoxidation reaction catalysed by different haem monoxygenases, the differences in the active site environment, which controls the mode of binding of the sulfides, have dramatic consequences on the stereochemistry of the process.

It should be pointed out that in all cases the HRP catalysed oxidation of sulfides is in competition with their spontaneous oxidation by H_2O_2 at a rate dependent on the concentration and nature of the substrate. Since the catalytic activity of HRP in the oxygenation of sulfides in the presence of H_2O_2 is low (*e.g.* the k_{cat} of HRP is 7000 times lower than that of chloroperoxidase with thioanisole)[7] a high (1 : 31) enzyme : substrate ratio was used to maximize the contribution of the catalysed reaction.

The data relative to the catalysed and uncatalysed oxidations are shown in Table 1. The e.e. values reported for the enzymatic reaction thus refer to the sulfoxides resulting from the two competitive processes; in other words, they reflect the minimum contribution to the enzymatic pathway. When taking into account the contribution of the non-enzymatic process, the stereoselectivity for the HRP catalysed oxygenation alone would be higher. Unfortunately, this cannot be done in an accurate way because of the different actual concentration of the oxidant in the catalysed and uncatalysed reaction. In fact, in the presence of HRP the oxidant is used by the enzyme not only for the oxidation of the sulfides but it is also consumed in a disproportionation reaction of a catalase type.[8] Therefore, it is likely that the average concentration of the oxidant in the catalysed reaction is lower than in the uncatalysed one. This explains why significant enantioselectivity is obtained in some cases with HRP in spite of the high spontaneous conversions observed in control experiments performed under apparently comparable conditions.

In the case of methyl *p*-tolyl sulfide the time course of the oxidation was also monitored. The chemical yield of the sulfoxide reached an approximate 50% value after 1 h (equivalent to 18 aliquots of H_2O_2, see legend in Table 1) and then it remained almost constant. The e.e. of the sulfoxide did not significantly change for the entire reaction period. HRP did not appreciably oxidize racemic *p*-tolyl methyl sulfoxide to the corresponding sulfone (GC measurements).

The stereochemical studies, which indicated that the stereoselectivity of HRP was dependent on the structure of the substrate, did not find a correlation with the kinetic studies which showed that both the K_m and k_{cat} values did not markedly differ for the various substrates, with the exception of 2-pyridyl methyl sulfide, which was a very poor substrate for HRP (Table 2). Thus, factors other than the mere catalytic efficiency must play a major role in dictating the stereochemistry of the reaction. The data in Table 2 also indicates that the oxidation of sulfides by horseradish peroxidase is a true enzymatic process, which follows Michaelis–Menten kinetics.

In conclusion, we have observed for the first time a substantial enantioselectivity in the horseradish catalysed

Table 1 Horseradish peroxidase catalysed oxidation of sulfides to sulfoxides by H_2O_2[a]

Sulfide[b]	Yield (%)	E.e. (%)[c]	Yield (%) of the uncatalysed oxidation[d]
PhSMe	95	46	40
p-MeC_6H_4SMe	47	68	23
p-$MeOC_6H_4SMe$	84	30	28
o-MeC_6H_4SMe	50	0	37
o-ClC_6H_4SMe	50	0	18
$PhCH_2SMe$	55	0	35
2-pyridyl-SMe	40	0	30

[a] The sulfide (36 μmol) and HRP (Boehringer, Grade I) (1.16 μmol) were magnetically stirred in 3.5 ml of 0.05 mol dm^{-3} phosphate buffer, pH 6, at 25 °C for 5 min. H_2O_2 (72 μmol) in 480 μl of buffer, pH 6, was added in 18 aliquots at 3 min interval (titration showed that H_2O_2 was almost completely consumed in the 3 min interval). The reaction was quenched with sodium sulfite 1 h after the first addition of H_2O_2, rapidly extracted with diethyl ether and dried. The product was analysed by HPLC on a Chiralcel OB column employing a chiral stationary phase (Daicel) using n-hexane–propan-2-ol (6 : 1 v/v) as the mobile phase. All the sulfides and the corresponding sulfoxides were base-line separated. [b] The sulfides were prepared as previously described.[5] [c] The absolute configuration of the sulfoxides, deduced by the elution order on Chiralcel OB, was (*S*). [d] The reactions were carried out in the absence of HRP.

† Horseradish peroxidase recovered at the end of the process had 10–15% of the original activity and, when reused, very poor or no asymmetric synthesis was found. This indicates that inactive protein cannot induce enantioselectivity.

Table 2 Kinetic parameters for the horseradish peroxidase catalysed oxidation of sulfides by H_2O_2[a]

Sulfide	K_m/μmol dm^{-3}	k_{cat}/min^{-1}
PhSMe	133	0.27
p-MeC_6H_4SMe	113	0.75
p-$MeOC_6H_4SMe$	106	0.61
o-MeC_6H_4SMe	210	0.31
o-ClC_6H_4SMe	260	0.23
$PhCH_2SMe$	196	0.61
2-pyridyl-SMe		0.05[b]

[a] The kinetic experiments were carried out in 0.05 mol dm^{-3} phosphate buffer, pH 6 at 25 °C, in 1 ml cuvettes, 1 cm path length. The reaction mixtures contained 12.5 μmol dm^{-3} HRP, 300 μmol dm^{-3} H_2O_2 (the K_m of HRP for H_2O_2, using *p*-MeC_6H_4SMe as the substrate was 38 μmol dm^{-3}) and 20–400 μmol dm^{-3} sulfide. The oxidation of sulfides to sulfoxides was spectrophotometrically monitored using the following ε values (dm^3 mol^{-1} cm^{-1}): PhSMe, 5875 at 272 nm; *p*-MeC_6H_4SMe, 8040 at 253 nm; *p*-$MeOC_6H_4SMe$, 8150 at 256 nm; *o*-MeC_6H_4SMe, 8350 at 253 nm; *o*-ClC_6H_4SMe, 9524 at 250 nm; $PhCH_2SMe$, 3090 at 225 nm; 2-pyridyl-SMe, 13600 at 246 nm. The K_m and k_{cat} values were obtained from the initial rate measurements using ENZFITTER (R. J. Leatherbarrow, *Enzfitter a Non-Linear Regression Data Analysis Program for IBM PC*, Elsevier Biosoft, Cambridge, 1987). [b] Determined at 400 μmol dm^{-3} substrate.

oxidation of some aryl methyl sulfides with hydrogen peroxide. This, together with ^{18}O-labelling studies[4] and sulfide binding studies to HRP,[9] can be considered as an evidence that aryl methyl sulfides bind near the haem active site, even if the actual mechanism of the S-oxygenation is not fully understood.

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