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Tandem peroxidase–glucose oxidase catalysed enantioselective sulfoxidation of thioanisoles

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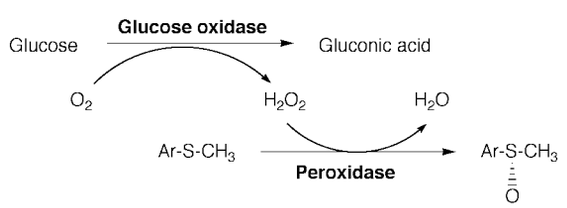
Chiral sulfoxides were synthesised from sulfides with high levels of chemical conversion and asymmetric induction using the oxygen transfer propensity of a peroxidase–glucose oxidase bienzymatic system.

Peroxidases (POD) are potentially attractive biocatalysts for selective oxidative transformations which meet industrial and ecological preferences. Indeed, numerous peroxidases have been shown to catalyse enantioselective sulfoxidation, epoxidation, benzylic hydroxylation and oxidation of alcohols and indole.[1] The mechanism of peroxidase oxygenation of organic compounds involves the transfer of two electrons at the expense of H_2O_2. However, the synthetic utility of this reaction is limited by the fact that haem-POD shows low stability towards added H_2O_2 or other hydroperoxides (oxidative haem inactivation, oxidation of the porphyrin ring).[1,2] Furthermore, the enantioselective oxidation of various substrates by POD is often in competition with their spontaneous oxidation by H_2O_2. Slow addition of the oxidant is then essential for successful stereoselective biotransformation. Numerous processes for keeping a low hydroperoxide concentration through the continuous addition of oxidant (use of a syringe pump,[2] of an H_2O_2-stat;[3] use of *t*-BuOOH[4] or other alkyl hydroperoxides[5] or urea–H_2O_2[6]) have been proposed. Recently, chloroperoxidase (CPO) catalysed oxidations assisted by dihydroxyfumaric acid or ascorbic acid and O_2 have been published.[7,8] However, formation of H_2O_2 as an intermediate in these transformations was considered doubtful by the authors. Furthermore, only non-enzymatic oxidation was observed in the presence of horseradish peroxidase (HRP).[8] We have investigated the oxygen transfer propensity of a glucose oxidase–peroxidase system in which H_2O_2 is progressively generated *in situ* at the expense of glucose, H_2O and O_2 (Scheme 1).



Scheme 1

A standard method for determination of glucose concentration in biological fluids is based on glucose oxidase (GOD) catalysed formation of hydrogen peroxide and its concomitant titration through oxidation of a chromogenic substrate by a peroxidase[9] (available as commercial kits). However, this procedure has not been applied to preparative peroxidase mediated oxidations (only one patent—disclaimed—reports the preparation of propylene oxide *via* a halohydrin formed by the combined action of a chloroperoxidase and an oxidase[10]).

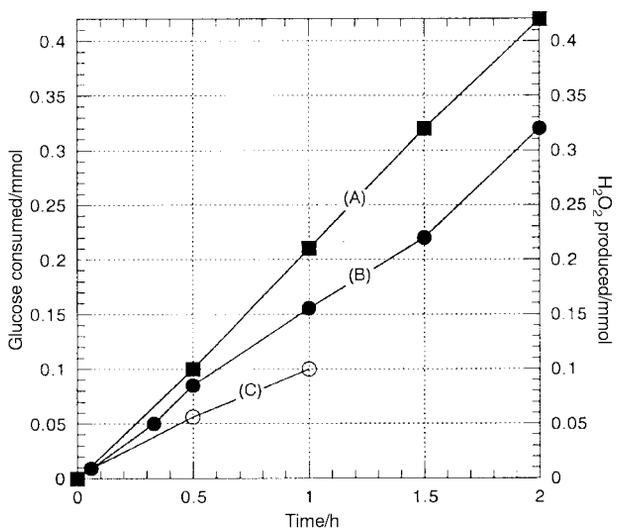


Fig. 1 GOD catalysed production of gluconic acid from glucose (2 mmol/10 ml, pH 7) at 25 (A) and 40 °C (B), monitored by pH-stat controlled addition of 2 M NaOH. Concentration of hydrogen peroxide in the medium was estimated spectrophotometrically at 240 nm (C).

The well documented transformation of sulfides to sulfoxides[1,4,11–13] was taken as a model in our work. A plant peroxidase from *Coprinus cinereus* (Cip: EC 1.11.1.7), recently studied in the oxidation of methyl phenyl sulfide, was selected.[2,5] Cip is a robust enzyme employed in a mixture with lipases (BioCip Novo Nordisk) as a cleaning agent and is now available from Roche Molecular Biochemicals (Mannheim).

Glucose oxidase (GOD: EC 1.1.3.4) from *Aspergilius niger* was chosen as the standard catalyst for the oxidation of glucose. It is a commercially available, inexpensive enzyme which is produced for food or diagnostic use.[9] Commercial GOD (here Gluzyme BG from Novo Nordisk) can be used without any purification. The kinetics of the formation of gluconic acid by GOD were monitored at 20 and 40 °C in an aqueous solution of glucose maintained at pH 7 by addition of NaOH using a pH-stat. Simultaneous H_2O_2 formation was checked by UV-spectrophotometry[2] (Fig. 1). A mixture of substrate and Cip was added at the same time to GOD in glucose solution. The pH of the reaction medium was held at 7.[2] The sulfide oxidation was monitored by GC (Fig. 2) and the enantiomeric excess of the sulfoxide was measured by HPLC on a chiral column.

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Table 1 Oxidation of aryl methyl sulfides **1a**–**e** to aryl methyl sulfoxides **2a**–**e** catalysed by GOD–POD systems

Substrate (0.5 mmol)	Time/h	Temp./°C	Volume/ml	GOD/mg	POD	Conversion (%)[a]	Ee (%)[b]
1a	3	25	20	4	—	90	0
1a	4	25	5	2	Cip[c]	95	75
1a	4	25	10	2	Cip[d]	85	79
1a	4	25	10	2	HRP[e]	80	64
1b	3.5	25	20	2	Cip[c]	90	88
1c	12	40	10	2	Cip[c]	91	90
1d	4	40	20	4	Cip[c]	65	57
1d	15	25	10	2	Cip[c]	95	58
1e	20	40	20	4	Cip[c]	0	—

[a] Estimated by GC. [b] Estimated by chiral HPLC. [c] NOVO SP 676 (20 mg). [d] From Roche (5 ml). [e] Sigma, type II (40 mg).

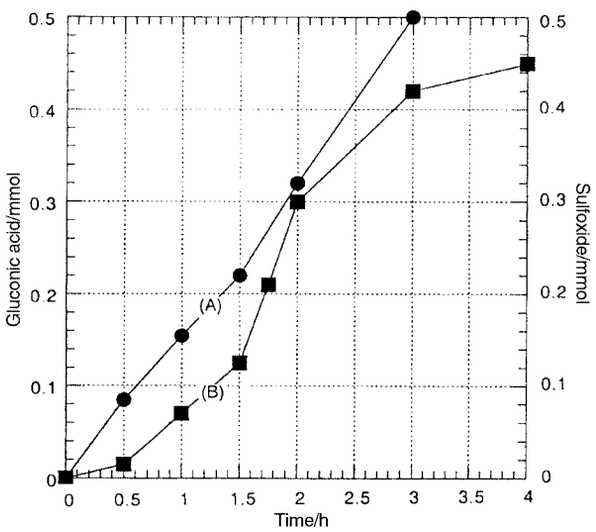

Fig. 2 Simultaneous production of gluconic acid from glucose (1 mmol) (A) and of phenyl methyl sulfoxide (estimated by GC) from phenyl methyl sulfide (0.5 mmol) (B) catalysed by a GOD–Cip system at 25 °C, pH 7 (GOD: 2 mg; Cip: 20 mg).

Aryl methyl sulfides were oxidised as a suspension in water at a concentration of 0.05–0.1 mol l^{-1}. The optimal Cip to substrate molar ratio was approximately 1:1400. At lower ratios, spontaneous (non-stereoselective) thioanisole oxidation by H_2O_2 could be observed. Two mole equivalents of glucose ensured the continuous formation of H_2O_2 at a flux of 100 μmol h^{-1}. The activity of the Cip–GOD tandem stays stable at 20, 40 and 50 °C in terms of the yield and enantioselectivity of the reaction.

After consumption of 1.2 eq. of NaOH the reaction mixture was quenched with sodium sulfite, extracted with ethyl acetate and the product was isolated with a yield >90% (Table 1). Under these conditions, thioanisole **1a** gave the corresponding sulfoxide **2a** with an ee of 75%, In the absence of peroxidase, oxidation was complete, but as expected, was without asymmetric induction.

Methyl *p*-tolyl sulfide **1b** gave **2b** with 88% ee and 2-naphthyl methyl sulfide **1c** gave **2c** (at 40 °C) with 90% ee. *p*-Chlorophenyl methyl sulfide **1d** gave **2d** with only 57% ee. This modest value could not be improved by doing the reaction at various temperatures (4–40 °C). *p*-Nitrophenyl methyl sulfide **1e** was not oxidised at all, probably as a result of the deactivation of the sulfide toward oxidation due to the electron withdrawing substituent. These last two results are in keeping with the literature.[5,13] Under the conditions described, a GOD–HRP system oxidised **1a** to **2a** with an ee of 64% at 80% conversion. For both Cip and HRP, in accordance with the literature,[2,12] the (*S*)-sulfoxides were formed in contrast to the sulfoxidation catalysed by CPO, which gives the (*R*)-enantiomer.[1,11]

In conclusion, we have demonstrated that GOD–POD mediated preparative oxidation of sulfides provides an improved process that takes advantage of the progressive generation of H_2O_2 from the cheap and environmentally friendly precursors glucose and oxygen. The substrates and the enzymes can be mixed at once, which avoids the cumbersome, slow continuous addition of the oxygen donor. Chemical yields and enantiomeric excesses are in keeping with the highest values reported in the literature for the tested enzymes.

Experimental

Cip was obtained from Novo Nordisk (Novo SP 676) as a freeze-dried powder, containing 639 mg of protein per g of solid, with a specific activity of 2675 kPODU g^{-1} (one PODU converts 1 μmol of hydrogen peroxide per min in a system where 2,2′-azinobis(3-ethyl-1,3-benzothiazoline-6-sulfonate) is oxidised), and from Roche Molecular Biochemicals (Mannheim) as an aqueous solution with a specific activity of 10 kU per g of solution. HRP (type II) was from Sigma. GOD was obtained from Novo Nordisk (Gluzyme 10000 BG) as a solid, with a specific activity of 10 kU g^{-1}. Sulfides **1a**, **1b** and **1e** are commercially available. Sulfides **1c** and **1d** were prepared according to described methods.[14,15]

Typical procedure

Thioanisole (0.5 mmol) and Cip (20 mg, 53 000 units) were added to a solution of GOD (2 mg, 20 units) and glucose (1 mmol) in 10 ml of water. The mixture was gently stirred, and the pH was maintained at 7 by continuous addition of 2 M sodium hydroxide using a pH-stat (Metrohm). After 4 hours, the reaction was quenched by addition of sodium sulfite, and the product was extracted with ethyl acetate. The formed sulfoxides are all known in optically active form. The physical and spectroscopic properties of our specimen were in agreement with those reported. Enantiomeric excesses were determined by HPLC on Chiralcel OD, using pentane–propan-2-ol 95:5 as the eluent. The degree of conversion of the sulfides and the chemical purity of the sulfoxide (>98%) were determined by gas-chromatography on a CP.SIL 19 CB capillary column. The concentration of hydrogen peroxide during the GOD catalysed oxidation of glucose was monitored spectrophotometrically[2] at 240 nm ($\varepsilon = 43.6$ M^{-1} cm^{-1}).

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