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Enantioselective synthesis of sulfoxides catalysed by an oxidase–peroxidase bienzymatic system

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Abstract—We have devised a new bienzymatic oxidase–peroxidase system in which hydrogen peroxide is produced from air by a D-amino acid oxidase. The generated hydrogen peroxide is instantly used for asymmetric oxygenation of sulphides by a peroxidase. In the absence of peroxidase no spontaneous oxidation is observed, indicating that hydrogen peroxide production by the D-amino acid oxidase is fully controlled. Arylmethyl sulfoxides can be synthesised on a preparative scale with high yields and good enantiomeric excess. © 2002 Published by Elsevier Science Ltd.

1. Introduction

There is an ever increasing demand for more selective and clean oxidation catalysts, especially for the production of enantiomerically pure compounds. Peroxidases are widely distributed enzymes which have a considerable scope for undertaking potentially useful transformations in organic synthesis and their applications have been extensively reviewed.[1–3] However, there is no example of their use as catalysts in industrial organic synthesis. Limited commercial availability, high price and low operational stability have hampered their application. Nowadays, new 'ready to use' inexpensive industrial peroxidases have appeared on the market. Nevertheless, one major problem is the inhibition of these heme-containing enzymes by an excess of hydrogen peroxide, their substrate. In addition to oxidation of usual substrates (phenols, aromatic amines) involving single electron transfers, peroxidases can also catalyse the enantioselective transfer of oxygen from hydrogen peroxide (sulfoxidation, epoxidation).[1–3] Improvement of both operational stability of peroxidase and enantioselectivity of the oxygen transfer by maintaining concentration of hydrogen peroxide at a low level can be achieved either by step-wise or continuous feed on demand, or in situ generation of hydrogen peroxide.[1]

Bienzymatic systems coupling an oxidase producing H_2O_2 and a peroxidase can allow 'in vivo like' oxidations by atmospheric oxygen.

In our previous work,[4] we devised a bienzymatic system in which hydrogen peroxide is progressively generated in situ at the expense of glucose and oxygen (air) by glucose oxidase to effect the preparative enantioselective oxidation of thioanisoles by a peroxidase from *Coprinus cinereus*. This procedure has also been attempted with chlorperoxidase.[5,6]

In those systems, the pH of the reaction medium has to be kept close to the optimum pH of the enzymes (5.5–7 for glucose oxidase); the use of a pH-stat is therefore required, since the production of hydrogen peroxide is concomitant to the synthesis of gluconic acid. Glucose oxidase (GOD, specific activity: 10 U/mg) is hardly inhibited by H_2O_2. As a result, an excess of hydrogen peroxide can be produced if the peroxidase becomes deactivated during the process, and non-enzymatic substrate oxidation may take place.

In principle, other oxidases can be used in this approach which allow a fine tuning of the oxidase–peroxidase combination in terms of substrate, optimum pH and controlled H_2O_2 production. Therefore, we investigated the in situ H_2O_2 production by D-amino acid oxidase (DAO; EC 1.4.3.3), a flavin-containing enzyme which catalyses the oxidative deamination of D-amino acids to the corresponding alpha-ketoacids. Reoxidation of the flavin coenzyme by molecular oxy-

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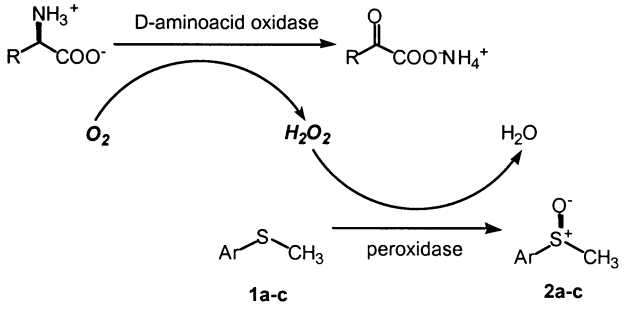
gen is accompanied by the release of H_2O_2. DAO can catalyse the deamination of a wide spectrum of D-amino acids. D-Alanine is a good substrate, but inexpensive DL-alanine can be used as well, leading to L-alanine and ammonium pyruvate as side products. Data from the literature indicate deactivation of DAO occurs in the presence of hydrogen peroxide, but this can be avoided by catalase addition.[7–9] Our preliminary experiments have shown that only limited amount of H_2O_2 can be produced by DAO in the absence of peroxidase. Thus, low-cost substrate, constancy of reaction medium pH and limitation of hydrogen peroxide overproduction make DAO an interesting alternative for a new bienzymatic oxidase–peroxidase system. Moreover, DAO optimum pH is 7 to 9. In terms of pH, the DAO–peroxidase system is then complementary to the glucose oxidase–peroxidase couple previously described. For our study, the well documented oxidation of thioanisoles was adopted as a model (Scheme 1).



a: Ar = phenyl, **b:** Ar = p-tolyl, **c:** Ar = 2-naphthyl

Scheme 1.

2. Results and discussion

Two DAOs were tested: from Porcine Kidney (PKDAO; Sigma, 0.15 U/mg) and from yeast *Trigonopsis variabilis* (TvDAO; Fluka, 28 U/g). Unfortunately, PKDAO was found to have too low operational stability, and to be inhibited by the substrates and products of peroxidase. DAO from *Trigonopsis variabilis* has its principal industrial application in the preparation of semisynthetic cephalosporin antibiotics.[10] It has also been used for the kinetic resolution of DL-amino acids, the production of α-ketoacids[7] and in analytical chemistry.[11] This enzyme is commercially available from Fluka in an immobilised form on polymeric support. Fungal peroxidase from *Coprinus cinereus* (CiP; EC. 1.11.1.7) is a low cost industrial enzyme produced by Novozyme as an additive for laundry detergent (liquid preparation; specific activity: 231 kU/ml). CiP has excellent thermostability and a broad pH optimum (5 to 9). It has been investigated as a catalyst in sulfoxidation of thioanisoles[4,12,13] and epoxidation of styrene derivatives (on a microscale).[14]

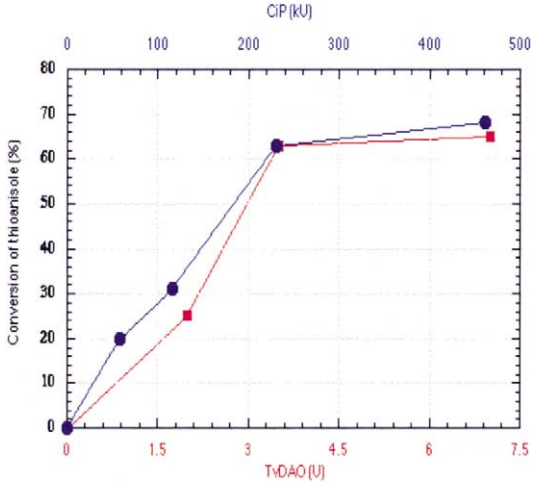


Figure 1. Optimisation of DAO/CiP ratio in sulfoxidation of **1a** (1 mmol) in H_2O pH 8.5 at rt. Reaction time 4 h. ■ TvDAO in the presence of 231 kU of CiP, ● CiP in the presence of 3.5 U of TvDAO.

The oxidation rate of thioanisole was sensitive to the amount of both TvDAO and CiP used (Fig. 1). In the DAO/Catalase-mediated resolution of DL-phenylalanine authors have recommended an enzyme ratio of 1 U/2.6 kU.[8] Similarly, in our experiments, satisfactory rates of sulfoxidation could be obtained only when a large amount of peroxidase was used. Optimisation of the reaction led us to use a TvDAO/CiP ratio of 1 U/66 kU. This value was chosen for all our investigations. Under these conditions only a small excess of D-amino acid was necessary. The yield of sulfoxide relative to the starting alanine was greater than 65%, indicating a low catalase activity in the reaction medium. The flux of hydrogen peroxide produced by the DAO-mediated oxidation of DL-alanine could be evaluated by titration of the pyruvate formed (Fig. 2). The kinetics of pyruvate formation was monitored spectrophotometrically using an L-lactate dehydrogenase-catalysed oxidation of NADH.[15]

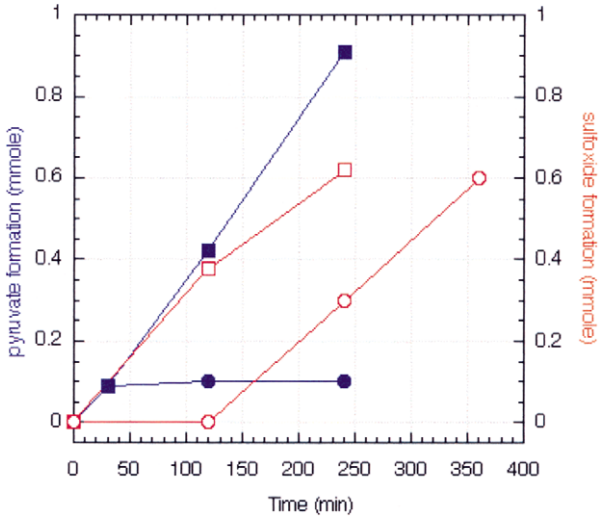


Figure 2. Formation of pyruvate from DL-alanine (2.8 mmol) and of methylphenyl sulfoxide from thianisole (1 mmol) catalysed by the TvDAO/CiP system ■: pyruvate in the presence of CiP; □: sulfoxide in the presence of CiP; ●: pyruvate in the absence of CiP ○: sulfoxide formation when CiP was added after 120 min.

In the absence of CiP, formation of pyruvate from DL-alanine (2.8 mmol) and conversion of sulphide was less than 7%, and conversion of sulphide (1 mmol) to less than 2%. This is probably a consequence of retroinhibition of DAO by H_2O_2 and/or hydrogen peroxide oxidation of pyruvate.[16] The normal rate of sulfoxidation can be restored even after 2 h by addition of CiP (Fig. 2, Table 1).

At optimum TvDAO/CiP ratio, the rate of sulfoxide formation can be influenced by temperature and choice of amino acid. (Fig. 3)

The best result was obtained with D-alanine as a substrate of TvDAO. Only a slightly lower rate was observed with the more economical DL-alanine. Slower reaction with glycine can be interesting for difficult peroxidase-catalysed oxygenation like epoxidation, where a lower flux of H_2O_2 is required.[14]

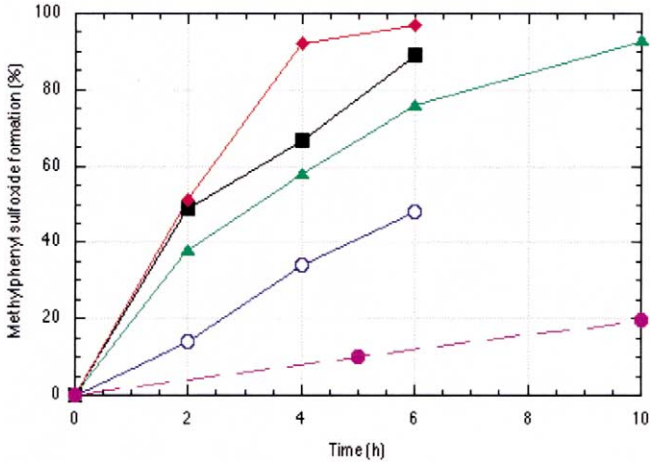


Figure 3. Formation of methylphenyl sulfoxide using various substrates of TvDAO ◆: DL-alanine at 40°C, ■: D-alanine at 25°C, ▲: DL-alanine at 25°C, ○: D-phenylalanine at 25°C, ●: glycine at 25°C.

Three arylmethyl sulphides were tested as substrates of the peroxidase in various conditions (Table 1). TvDAO was added at once to thioanisole dispersed in aqueous solution of CiP and amino acid at pH 8.5. The mixture was mechanically stirred under atmospheric air or in a closed vessel under oxygen.[17] Reaction medium pH is constant during the process, thus avoiding any buffer utilisation. All obtained chiral arylmethyl sulfoxides have the ***S*** absolute configuration.[4,12]

Compared with the GOD-mediated oxidation of **1a** (entry 1), where 43% of racemic **2a** is formed in the absence of peroxidase, the reaction catalysed by TvDAO in the same conditions (entry 3) leads to less than 2% of **2a**. Under the optimal oxygenation conditions (entry 9–12) conversion is >90% and enantiomeric excesses of sulfoxides are the best reported for CiP-catalysed sulfoxidation. The enantiomeric purities of sulfoxides **2b** and **2c** are higher than those previously obtained in tandem glucose oxidase/CiP oxidation.[4] Moreover, the enantiomeric excesses stay constant during the reaction (entry 8) and no further oxidation to the sulfone was observed. CiP can also be replaced by microperoxidase-11 (entry 13), but the reaction leads to racemic sulfoxide.[19]

The reaction can be easily scaled up and carried out on a multigram scale without decrease of yield or enantiomeric excess (entry 14). The immobilised DAO could be reused several times without significant loss of activity.[20] The total turnover number of CiP in this system and for oxidation of thioanisole is superior to 500 (versus 100–200 for the same reaction catalysed by the same enzyme fed directly with hydroperoxide[12,13]).

Table 1. Asymmetric oxidation of arylmethyl sulfides **1a-c** catalysed by oxidase/CiP systems

Entry	Sulphide	Oxidase substrate (mmol)	Oxidase (mg)	Peroxidase (ml)	Temp. (°C)	Time (h)	Conversion (%) (yield[d])	Ee (%)
1	**1a**	D-Glucose, 2.00	GOD, 4	–	Rt	6	43	0
2	**1a**	D-Glucose, 2.00	GOD, 4	1	Rt	6	90	72
3	**1a**	DL-Ala, 5.60	TvDAO, 125	–	Rt	6	2	0
4	**1a**	DL-Ala, 5.60	PKDAO, 23	1	Rt	6	60	77
5	**1a**	D-Phe, 2.80	TvDAO, 125	1	Rt	6	48	56
6	**1a**	Gly, 2.00	TvDAO, 125	1	40	24	40	76
7	**1a**	D-Ala, 2.80	TvDAO, 125	1	Rt	6	89	75
8	**1a**	DL-Ala, 5.60	TvDAO, 125	1	Rt	2	38	75
						6	79 (70)	76
9	**1a**	DL-Ala, 2.80	TvDAO, 125	1	Rt	10	93 (85)	79
10	**1a**	DL-Ala, 2.80	TvDAO, 125	1	40	6	97	73
11	**1b**	DL-Ala, 2.80	TvDAO, 125	1	Rt	8	82	96
12	**1c**	DL-Ala, 5.60	TvDAO, 125	1	40	6	88 (76)	97
13[a]	**1a**	DL-Ala, 0.28	TvDAO, 3	0.5 mg	Rt	10	56	0
14[b]	**1a**	DL-Ala, 42.00	TvDAO, 1875	15	40	10	93 (86)	75[c]

Reaction conditions: sulfide **1** (1 mmol), water (20 mL), pH 8.5

[a] Microperoxidase-11 (Sigma), water (2 mL), sulfide **1a** (0.1 mmol).

[b] Sulfide **1a** (15 mmol); water (200 mL).

[c] $[\alpha]=-110$ ($c=3$, EtOH; lit.:[18] $[\alpha]=+146$ for the (*R*)-enantiomer)

[d] Yield of pure isolated product.

3. Experimental

3.1. Typical procedure

The sulphide (1 mmol) and immobilised TvDAO (125 mg) were suspended in a mixture of aqueous DL-alanine solution (20 mL, 2.8 mmol) and CiP (1 mL, previously dialysed against 0.5 M NaCl). The pH of the mixture was adjusted to 8.5 and the mixture was mechanically stirred either at rt or 40°C until completion of the reaction (Table 1). The formed sulfoxide was then extracted with ethyl acetate, and purified if necessary on a short silica gel column. The enantiomeric excess was determined by HPLC on a Chiracel OD-H column (*iso*-hexane/propan-2-ol 95:5). The degree of conversion and chemical purity of the sulfoxides was determined by GC and NMR.

4. Conclusions

The devised TvDAO/CiP system mediates the preparative oxidation of sulphides by oxygen, leading to the corresponding sulfoxides in high yield and with good enantiomeric excess. Immobilised oxidase and its low cost substrates ensure a 'cheap and green' synthetic entry to chiral sulfoxides. Product isolation is easy and background non-selective oxidation, often observed with slow reacting peroxidase, is suppressed by self-controlled hydrogen peroxide production.

Acknowledgements

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20. Immobilised commercial TvDAO loses 30% activity after the first washing with water, probably as an indication of the presence of some non-immobilised enzyme adsorbed on the support. No further loss of activity of recycled TvDAO was observed even after three runs.