Enzymatic Oxidation

Microencapsulated Chloroperoxidase as a Recyclable Catalyst for the Enantioselective Oxidation of Sulfides with Hydrogen Peroxide**

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Microencapsulation of enzymes is a technique that was developed about ten years ago to increase their stability, their ease of recovery, and facilitate their use as catalysts in organic synthesis.[1] Chloroperoxidase (CPO, EC 1.11.1.10) from *Caldariomyces fumago* is a rather versatile heme enzyme that has been extensively studied in recent years.[2,3] Among the wide range of biotransformations carried out by CPO, enantioselective oxidations of olefins and sulfides proved to be very useful for the preparation of important intermediates in organic synthesis.[4] Major drawbacks to the large-scale use of CPO are its high cost, instability at moderate temperatures, and deactivation when using high concentrations of the oxidant hydrogen peroxide. To date, attempts to incorporate CPO into a solid matrix have met with only moderate success, but the enzyme has been adsorbed on mesoporous solids such as MCM-48, SBA-15 and SBA 16,[5] and on microporous solids such as talc.[6] However, adsorption on preformed materials implies there is also a possible desorption process and, in fact, the enzyme was rapidly released into solution during subsequent use of such materials as catalysts. More recently,[7] CPO has been covalently bound to silica gel, thus ensuring a better stability towards leaching, but the catalytic activity of the material has so far not been reported.

Herein we report our successful attempt to apply a different strategy, that is, to build a microporous silica gel cage around CPO and its subsequent use as an enantioselective catalyst in the oxidation of sulfides to sulfoxides.

Tetramethylorthosilicate (TMOS) was prehydrolyzed by addition of a diluted ammonia solution (pH 10) and sonicated for 15 minutes. Either 183 or 3360 CPO units buffered at pH 3.75 were then added and the mixtures stirred for three hours at room temperature until gelation was attained. This yielded two different samples containing 13 units/gram of gel (sample **A**) and 425 units/gram of gel (sample **B**) respectively. The gels were thoroughly washed with acetate buffer

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(pH 3.75) and aged, to complete reticulation, for 2 weeks at 4 °C while covered with buffer (3 mL). This was removed daily and changed with a fresh amount. The xerogels obtained after this time were stored under acetate buffer and used for catalytic studies. The buffer solution aliquots recovered during the preparation were analyzed for their CPO content. The amount of enzyme units lost was calculated using the Morris–Hager assay.[8] As shown in Figure 1, there is a slow

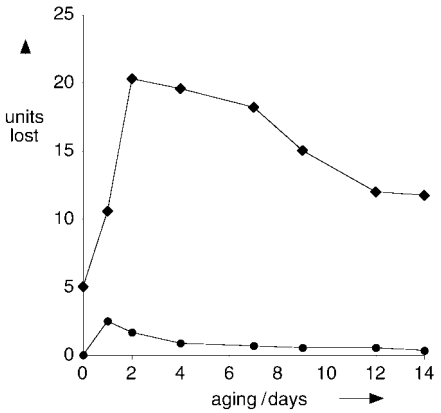


Figure 1. Units of CPO lost during the aging. ●: sample **A**, ◆: sample **B**.

release of CPO from the encapsulated samples that shows a maximum after one–two days and becomes practically negligible with respect to the original amount introduced after two weeks (sample **A**: 1/183 total units introduced; sample **B**: 12/3360 total units introduced). After aging the gels for two weeks the total loss of CPO was $<5\%$ with respect to the original amount and new CPO concentrations are: **A** 12.5 units/gram of gel, **B** 410 units/gram of gel.

Appropriate amounts of xerogels **A** and **B** were dried in vacuo at room temperature and their N_2 adsorption isotherms were analyzed. These indicated that both samples were $>90\%$ microporous (pore diameter <2 nm) with BET surface areas of 700–750 $m^2 g^{-1}$. Microporosity is an essential prerequisite to ensure that CPO remains encapsulated within the silica matrix. However, the presence of a small amount of mesopores could easily explain the loss of approximately 4% of the initially present units during the aging time.

Catalytic reactions using encapsulated CPO (0.3 g) were initially carried out in the oxidation of methylphenylsulfide (MPS) with 0.1M H_2O_2 in 0.1M acetate buffer (pH 3.75) at 15 °C in the absence of solvent. The procedure by which H_2O_2 is added to the system is very important, since it is known that H_2O_2 deactivates CPO.[2,3,4c,5a,6,9] The oxidant solution was added in different aliquots, so that the concentration of the oxidant in the reaction medium never exceeded 10% of the amount of substrate initially present. Some blank tests were also carried out to check the effect of the spontaneous oxidation of MPS in the absence of catalyst and the possible effect of the silica matrix. To this end a pure silica gel sample was prepared using the procedure described above without addition of CPO. The curves obtained are shown in Figure 2, together with the result obtained with free CPO. As expected

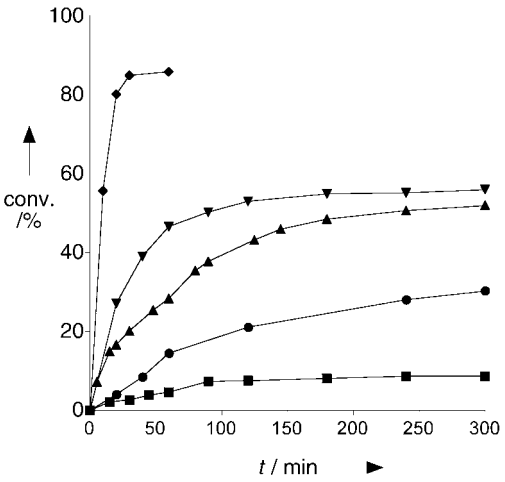


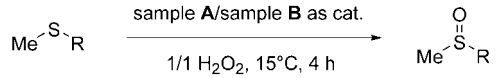
Figure 2. Plots of the conversion of MPS versus time for different catalysts. ■: spontaneous reaction, ●: silica gel, ▲: sample **A**, ◆: sample **B**, ▼: free CPO.

B (123 CPO units in 0.3 g catalyst) is much more active than **A** (3.7 CPO units in 0.3 g catalyst). A small amount of spontaneous oxidation of MPS to sulfoxide is evident as well as some catalysis by the silica gel. The latter reactions are clearly non-enantioselective and hence they will influence the possible overall enantioselectivity observed with **A** as catalyst, while with **B** their effect will be practically negligible.

Figure 2 shows also that 3.7 units of free CPO display a higher conversion rate than the same amount encapsulated in silica (**A**), thus indicating that diffusion through the microporous gel structure may be a limitation. Significantly, however, the maximum conversion attained is the same in both cases.

Samples **A** and **B** were tested also in the oxidation of a series of methylsulfides and a summary of their catalytic properties is reported in Table 1. Formation of sulfones was never observed. Conversions in the range 40–85% and with *ee* $>99\%$ were achieved in all cases with **B** as the catalyst, while the lower conversions and *ee* values observed with **A**

Table 1: Oxidation of different sulfides to sulfoxides with hydrogen peroxide in the presence of encapsulated CPO.[a]



R = Ph, 4-MeC_6H_4, 4-ClC_6H_4, C_5NH_4

Entry	R	Catalyst sample	*t* [min]	Conversion [%][b]	*ee* [%][b]
1	Ph	**A**	240	52	87
2	4-MeC_6H_4	**A**	240	48	76
3	4-ClC_6H_4	**A**	240	40	73
4	[c]	**A**	240	49	92
5	Ph	**B**	30	85	>99
6	4-MeC_6H_4	**B**	30	62	>99
7	4-ClC_6H_4	**B**	30	57	>99
8	[c]	**B**	30	40	>99

[a] Reaction conditions: catalyst 0.3 g; sulfide 12.5 μmol; H_2O_2 12.5 μmol; $T=15$ °C. [b] Determined by GC. [c] 2-pyridyl.

can be attributed to the blank reactions reported in Figure 2, but also to CPO deactivation after the indicated reaction time. In fact, only a slow formation of sulfoxide and a decrease in the *ee* value of the product are observed if the reaction is run for a longer time. For example, if the reaction described in entry 1 is performed for 1440 min the conversion increases to 81 % while the overall *ee* value decreases to 56 %, thus indicating that all the new sulfoxide produced after 240 min is racemic and what is observed is just a dilution effect on the *ee* value of the sulfoxide already present after 240 min. In agreement with this view is the observation that **A** is almost inactive if recycled for a second run (about 15 % methylphenylsulfoxide, 7–8 % *ee*).

The possible recycling of **B** was tested in the oxidation of MPS. Interestingly, this catalyst could be recycled up to four times with only moderate loss of activity, but retaining its enantioselectivity (Table 2). Recycling of the solution adding fresh amounts of sulfide and hydrogen peroxide resulted in no activity, thereby demonstrating that the reaction is truly heterogeneous and that the amount of CPO realeased from the matrix is negligible.

Table 2: Oxidation of methylphenylsulfide with hydrogen peroxide using sample **B** as the catalyst: Effect of catalyst recycling.[a]

Recycle	*t* [min]	Conversion [%][b]	*ee* [%][b]
fresh	30	85	>99
1st	35	82	>99
2nd	50	78	>99
3rd	40	80	>99

[a] Reaction conditions: catalyst 0.3 g; sulfide 12.5 μmol; H_2O_2 12.5 μmol; $T = 15\,^{\circ}C$. [b] Determined by GC.

In conclusion, the protocol reported in this work seems a reliable method for encapsulating CPO and ensure a sufficient stability in the presence of hydrogen peroxide for carrying out the oxidation of a number of organic sulfides with high activity and enantioselectivity. Oxidation reactions are performed using only substrate and hydrogen peroxide with no solvents, in the presence of a catalyst that can be easily separated and recycled. These characteristics taken together make the process described here particularly attractive also for the "greening" of these oxidation reactions.

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