Disulfide-bridge formation through solvent-free oxidation of thiol amino acids catalysed by peroxidase or hemin on mineral supports

Eryka Guibé-Jampel [a] **and Michel Therisod** *[b]

[a] *UMR 8615 Réactivité et Synthèses Sélectives and* [b] *Lab. Chimie Bioorganique et Bioinorganique, I.C.M.O. - Bat. 410-420, Université Paris-Sud, F-91405 Orsay cedex. E-mail: therisod@icmo.u-psud.fr; eriguibe@icmo.u-psud.fr*

Received (in Cambridge, UK) 31st August 1999, Accepted 1st October 1999

Air oxidation of hydrophilic thiols to disulfides was performed in neutral conditions on mineral supports activated by hemin or peroxidase.

Oxidation of cysteine and related compounds has been extensively studied. Despite its importance in biochemical processes, the controlled formation of disulfide bridges remains a significant challenge.[1] We have recently reported that aromatic thiols dispersed on a weakly basic support could be easily and selectively oxidised by air in solvent-free conditions.[2] Likewise, DL-cysteine dispersed on wet Hyflo Super Cel (HSC) is rapidly converted in 30 min into a mixture of racemic and *meso*-cystine by heating in air at 75 °C (Table 1, entry 1). However, several compounds like cysteine hydrochloride, cysteine methyl ester hydrochloride, *N*-acetylcysteine and glutathione remained unchanged under these conditions. Thus, in order to perform smooth oxidation of these thiols in neutral conditions, we have foreseen the use of a support activated with an efficient catalyst. Transition metal salts or complexes have been employed for the catalytic or stoichiometric oxidation of thiols into disulfides.[3] Among them, hemin (ferriprotoporphyrin(IX) chloride) was reported to catalyse at a 0.2% molar ratio the oxidation by oxygen of alkylthiols in alkaline aqueous solution.[4] Horseradish peroxidase (HRP), one of the most common hemeproteins, is known to catalyse the oxidation of cysteine and dithiothreitol;[5] it has recently been used to prepare aryl and alkyl disulfides under similar conditions to hemin catalysis.[6] Consequently, we studied the performance of a weakly basic mineral support (Hyflo Super Cel®, Fluka: HSC), activated by hemin or peroxidase (EC 1.11.1.7, Novo SP 676) to catalyse the oxidation by air of amino acid-thiols, as exemplified below for *N*-acetylcysteine (Fig. 1).

Freeze-dried peroxidase and the substrate were co-dispersed on HSC; the coated support was hydrated by a small amount of water, and shaken until a loose powder was obtained. Alternatively, the enzyme was dispersed on the support, and the powder was impregnated by a methanolic solution of the substrate, afterwards the mixture was dried. *N*-Acetylcysteine thus gave 85% of *N*-acetylcystine at 75 °C after 2 hours if impregnated by methanol, after 45 min if dispersed on hydrated HSC, and less than 5% of disulfide after 2 hours at 75 °C if dispersed on dry HSC (Fig. 2).

Hemin-activated HSC was prepared by impregnation with a pyridine solution of hemin and was then dried. The substrate was then added to the coated support, and the mixture was hydrated with a small amount of water. Under these solvent-free optimised conditions, complete oxidation of cysteine is achieved in 15 min at 75 °C (Table 1); cysteine hydrochloride is

Table 1 Oxidation of thiols to symmetrical disulfides

Entry	Thiol (100 mg)	Catalyst [a] (3×10^{-3} mol equiv.)	Temperature/ °C	Time/h	Conversion (%) [b]
1	DL-Cysteine	—	75	0.5	>98
2	DL-Cysteine	Hemin	75	0.25	96
3	DL-Cysteine	Peroxidase	75	0.5	100
4	L-Cysteine·HCl	—	75	3	<1.5
5	L-Cysteine·HCl	Hemin	75	3	85
6	L-Cysteine·HCl	Peroxidase	75	3	90
7	*N*-Ac-L-Cysteine	—	75	0.5	0
8	*N*-Ac-L-Cysteine	FeCl3	75	0.5	2.5
9	*N*-Ac-L-Cysteine	Hemin	20	18	80
10	*N*-Ac-L-Cysteine	Hemin	75	0.5	85
11	*N*-Ac-L-Cysteine	Peroxidase	20	18	80
12	*N*-Ac-L-Cysteine	Peroxidase	75	0.5	73
13	L-CysteineOMe·HCl	—	20	24	0
14	L-CysteineOMe·HCl	Hemin	20	5	77
15	L-CysteineOMe·HCl	Peroxidase	20	5	95
16	Glutathione	—	75	3	<3
17	Glutathione	Hemin	20	28	84
18	Glutathione	Hemin	45	5	>92
19	Glutathione	Peroxidase	20	28	87
20	Glutathione	Peroxidase	45	5	>98
21	Penicillamine	Hemin	6	72	>95
22	Penicillamine	Peroxidase	6	72	>95
23	Penicillamine	—	75	0.2	30
24	Penicillamine	Hemin	75	0.2	35
25	Penicillamine	Peroxidase	75	0.2	60

[a] The hemin content of peroxidase was determined spectrophotometrically as 1.5 mg of hemin per 100 mg of peroxidase. [b] Determined by NMR. [c] 40% decomposition.

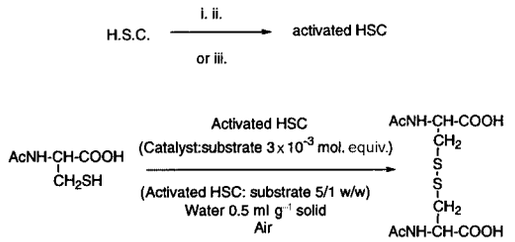

Fig. 1 Preparation of activated HSC and catalysed oxidation of *N*-acetylcysteine (2 h, 80 °C). i: hemin (pyridine), 1.5 mg g^{-1} HSC; ii: drying; iii: freeze-dried peroxidase, 100 mg 500 mg^{-1} HSC.

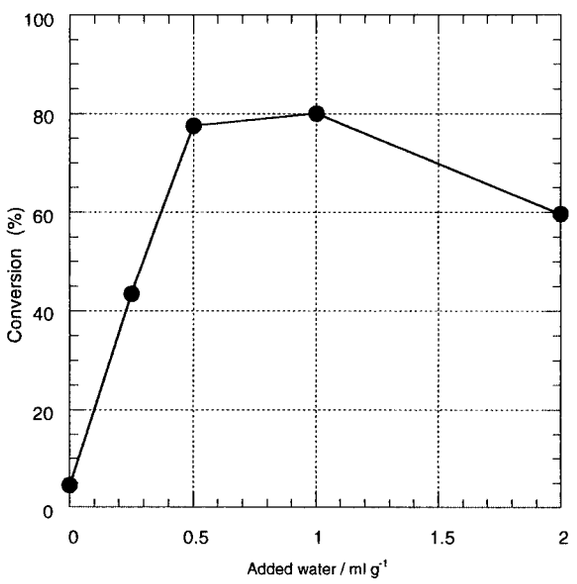

Fig. 2 Dependence of the oxidation of *N*-acetylcysteine catalysed by hemin-activated HSC on the amount of added water (2 h, 80 °C).

oxidised within 3 hours, *N*-acetylcysteine within 60 min at 75 °C or 24 h at 20 °C. Cysteine methyl ester hydrochloride, which decomposes rapidly in alkaline solutions, can be quantitatively oxidised under neutral conditions at 20 °C.†

We have tested the method on the peptide model, reduced glutathione. Like *N*-acetylcysteine, glutathione is not oxidised in the absence of a catalyst. On heating or after a long storage in air on HSC, some decomposition (up to 40%) occurs. On hemin or peroxidase activated HSC, clean and complete oxidation can be achieved at 20 or 45 °C. The rate of oxidation of *N*-acetylcysteine or glutathione is similar if catalysed by hemin or peroxidase. Nevertheless, an effect of the protein environment can be observed for the oxidation of tertiary thiols like penicylamine (dimethylcysteine) (Fig. 3). Neither overoxidation nor other side reactions could be observed with any substrates under study. All products were quantitatively recovered as described below in the Experimental section.

To summarise, disulfide bridges can be successfully formed by oxidation of amino acid-thiols catalysed by hemin or peroxidase immobilised on a weakly basic mineral support without solvent. This environmentally friendly method offers considerable synthetic advantages in terms of yield, selectivity and simplicity of reaction and work-up procedures.

Experimental

Preparation of catalysts

Peroxidase: Novo SP 676 is a freeze-dried enzyme from Novo Nordisk; specific activity: 2675 KPODU/g [one PODU converts 1 μmol of hydrogen peroxide per min in a system where 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) is oxidised]. Peroxidase-activated support was prepared by mechanical dispersion of the enzyme (200 mg g^{-1}) on HSC with a vortex mixer. Hemin-activated support was prepared as follows: 32.6 mg of hemin (Fluka) dissolved in 10 ml of pyridine were added to 10 g of HSC, and the mixture was dried on a rotatory evaporator (pyridine was not retained on HSC under these conditions). The activated supports remains active for months if stored in the refrigerator (peroxidase) or at room temperature (hemin).

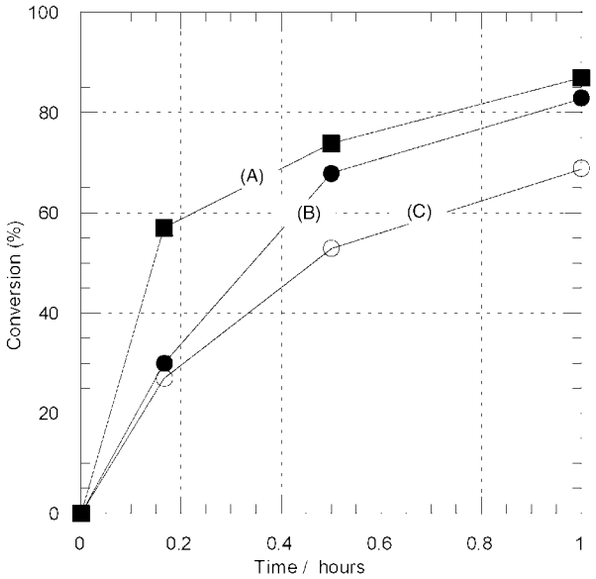

Fig. 3 Oxidation of DL-penicillamine dispersed on hydrated HSC (75 °C) activated by: (A): peroxidase; (B): hemin; (C): no catalyst.

Typical procedure for an oxidation reaction

Reaction mixtures were prepared by mechanical dispersion of the thiol (200 mg g^{-1}) on the activated support. Water (0.5 ml g^{-1}) was added and the mixture was carefully mixed until an homogeneous (hydrated) loose solid was obtained. This solid was left at room temperature or heated in an open vessel for the indicated time. Products were eluted from the support either with diluted hydrochloric acid (cystine, oxidised glutathione, penicylamine) or methanol (*N*-acetylcystine, cystine methyl ester). The solution was decolorised by addition of activated carbon, filtered and evaporated.

Acknowledgements

We are grateful to Novo Nordisk for the kind supply of peroxidase.

Notes and references

† Hemin on Celite, neutral and basic alumina or silica gel instead of HSC can be used under similar conditions.

1 I. Annis, B. Hargittai and G. Barany, *Methods Enzymol.*, 1997, **289**, 198; T. Shi and D. L. Rabenstein, *J. Org. Chem.*, 1999, **64**, 4590.
2 L. Sainte-Marie, E. Guibé-Jampel and M. Therisod, *Tetrahedron Lett.*, 1998, **39**, 9661.
3 D. J. Procter, *J. Chem. Soc.*, *Perkin Trans. 1*, 1999, 641; S. Uemura, in *Comprehensive Organic Synthesis*, ed. B. M. Trost and I. Fleming, Pergamon Press, New York, 1991, vol. 7, p. 757; P. C. B. Page, R. D. Wilkes and D. Reynolds, in *Comprehensive Organic Functional Group Transformations*, ed. A. R. Katritzky, O. Meth-Cohn, C. W. Rees and S. V. Ley, Pergamon, 1995, vol. 2, p. 210; H. Firouzabadi, M. Abbassi and B. Karimi, *Synth. Commun.*, 1999, **29**, 2527.
4 C. F. Cullis, J. D. Hopton, C. J. Swan and D. L. Trimm, *J. Appl. Chem.*, 1968, **18**, 335.
5 J. Olsen, L. Davis and M. F. Tsan, *Biochim. Biophys. Acta*, 1976, **445**, 324; L. S. Harman, C. Mottley and R. P. Mason, *J. Biol. Chem.*, 1984, **259**, 5606.
6 M. Sridhar, S. Kumara Vadivel and U. T. Bhalerao, *Synth. Commun.*, 1998, **28**, 1499.

Communication 9/07027C