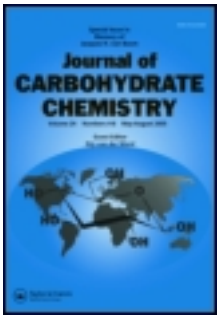

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Chloroperoxidase-Catalyzed Oxidation of 5-Hydroxymethylfurfural

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CHLOROPEROXIDASE-CATALYZED OXIDATION OF 5-HYDROXYMETHYLFURFURAL

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ABSTRACT

Chloroperoxidase (CPO) catalyzes the oxidation of 5-hydroxymethylfurfural (HMF) with hydrogen peroxide as the oxidant. The reaction proceeds with 60-74% selectivity to furan-2,5-dicarboxaldehyde (FDC). The main byproduct is 5-hydroxymethyl-2-furancarboxylic acid (HFCA); a minor amount of 5-formylfuran-2-carboxylic acid (FFCA) was also detected. When $H_2{}^{18}O_2$ was used a virtually quantitative incorporation of ^{18}O was observed in the HFCA product, whereas no ^{18}O was incorporated from $H_2{}^{18}O$. Hence, the CPO-catalyzed oxidation of aldehydes to acids proceeds with direct oxygen transfer from the iron-oxo complex of CPO. Controlling the H_2O_2-addition with a H_2O_2-stat facilitated the reaction procedure and a conversion of 87% of HMF was reached within 21 min.

INTRODUCTION

Chloroperoxidase (CPO, E.C. 1.11.1.10) from *Caldariomyces fumago* is a heme peroxidase, containing iron(III)protoporphyrin(IX) as the prosthetic group. It is an extracellular enzyme that can readily be isolated in synthetically useful quantities.[1] CPO catalyzes a variety of oxidations with H_2O_2, via the formation of a formally oxoiron(V)porphyrin intermediate, without the need for expensive cofactors. Since the oxidant is H_2O_2 no undesirable inorganic waste is generated in these processes. Examples

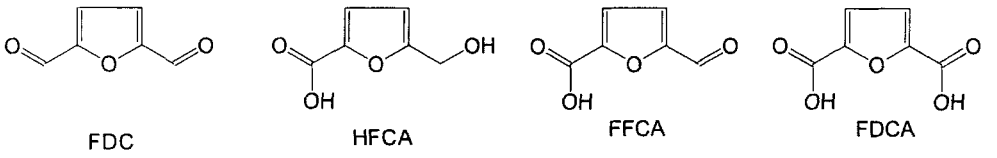

Figure 1. Possible oxidation products of HMF.

include, in addition to the natural reaction of oxidative halogenation,[2] enantioselective sulfoxidations,[3] epoxidations[4] and benzylic hydroxylations,[5] and oxidation of indoles to 2-oxindoles.[6] CPO has been reported[7] to catalyze the selective oxidation of primary alcohols. This prompted us to study the use of CPO as a catalyst for the selective oxidation of 5-hydroxymethylfurfural (HMF).

The oxidation of HMF yields products of potential interest as industrial monomers.[8] Possible oxidation products of HMF are furan-2,5-dicarboxaldehyde (FDC), 5-hydroxymethylfuran-2-carboxylic acid (HFCA), 5-formylfuran-2-carboxylic acid (FFCA) and furan-2,5-dicarboxylic acid (FDCA) (Figure 1).

Although there are several catalytic methods available for obtaining FDCA or FFCA in good yield,[9] reports on selective oxidation of HMF to FDC are rare. Oxidation of HMF to FDC occurs selectively with stoichiometric amounts of manganese dioxide.[10] However, large amounts of manganese salts are produced which is an obvious disadvantage. A mixture of products, including FDC is obtained when platinum is used as catalyst and oxygen as the oxidant.[9a] Oxidation of HMF in the presence of 2,2,6,6-tetramethylpiperidinyloxy derivatives (Tempo), oxygen and copper salts, yields FDC in 55% yield.[11]

RESULTS AND DISCUSSION

The CPO-catalyzed oxidation of HMF proceeded smoothly to a nearly complete conversion of HMF. In view of the known sensitivity of CPO for H_2O_2 the oxidant was added over 150 min at a low constant rate of 0.5 equiv/h. As expected, oxidation of the alcohol moiety took place with the formation of FDC. Besides this reaction, however,

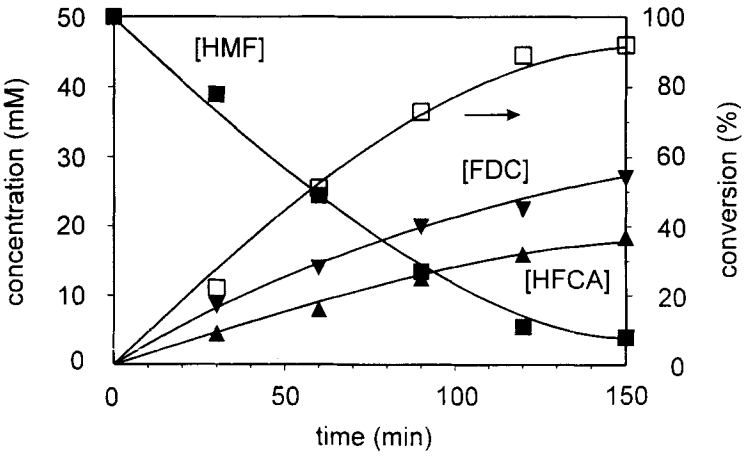

Figure 2. Oxidation of HMF, 50 mM HMF in 25 mL pH 5, 200 μL CPO, 1 equiv H_2O_2/2 h, □ conversion of HMF, ■ [HMF], ▼ [FDC], ▲ [HFCA].

oxidation of the aldehyde function in HMF, yielding HFCA, also took place. This was unexpected, because the CPO-catalyzed oxidation of alcohols by H_2O_2 was reported to proceed selectively to the aldehydes without any detection of over-oxidation to the acid for the alcohols tested, as, for example, furfuryl alcohol.[7b] We repeated the oxidation of furfuryl alcohol and confirmed a virtually 100% selectivity to aldehyde at a conversion of furfuryl alcohol of 92%. For HMF, however, the maximum selectivity to FDC was 74%, depending on the reaction conditions used, and HFCA was obtained as a major by-product in 25-40% yield (Figure 2 and Table 1). A minor amount of FFCA (<5% under the conditions used) was also detected.

Optimum activity for oxidation of HMF was obtained at pH 5. Under the conditions used, 92% conversion of HMF to oxidized products was obtained within 2.5 h with a turnover number (= mole HMF oxidized/mole CPO used) of 62×10^3. Recently we have shown that *tert*-butyl alcohol as a cosolvent stabilizes CPO in the oxidation of indole.[12] Hence, we also performed the oxidation of HMF in *tert*-butyl alcohol/ aqueous buffer (3:7, v/v, citrate buffer 0.1 M pH 5). However, the conversion dropped from 89% to 66% (Table 1) thereby also decreasing the turnover number and the selectivity did not improve.

Table 1. Conversion and selectivity of HMF oxidation.[a]

pH	conversion 120 min. (%)	selectivity to FDC (%)
3	25	74
4	59	68
5	89	59
6	83	60
5, argon	88	60
5, *tert*-butyl alcohol[b]	66	63

a. 50 mM HMF/25 mL, 0.1 M buffer, 200 μL CPO, 1 equiv H_2O_2/2 h, reaction time: 2.5 h.
b. *tert*-butyl alcohol/aqueous buffer 30:70 (v/v).

Table 2. Oxidation of HMF, FDC and HFCA with CPO and H_2O_2.[a]

Starting Material	Conversion (%)	Products
HMF	92	FDC, HFCA, FFCA
HFCA	68	FFCA
FDC	22	FFCA

a. 50 mM starting material/25 mL, 0.1 M buffer pH 5, 200 μL CPO, 1 equiv H_2O_2/2 h, reaction time: 2.5 h.

In order to assess the role of CPO in the production of HFCA and FFCA a number of blank experiments without enzyme were carried out. No reaction was observed with either HMF, HFCA or FDC as the reactant. However, all these reactants were oxidized by CPO and hydrogen peroxide (Table 2).

Clearly, the formation of HFCA, FDC and FFCA is due to CPO-catalyzed oxidation of HMF with H_2O_2. Based on these results the following reaction scheme can be presented for the oxidation of HMF (Figure 3).

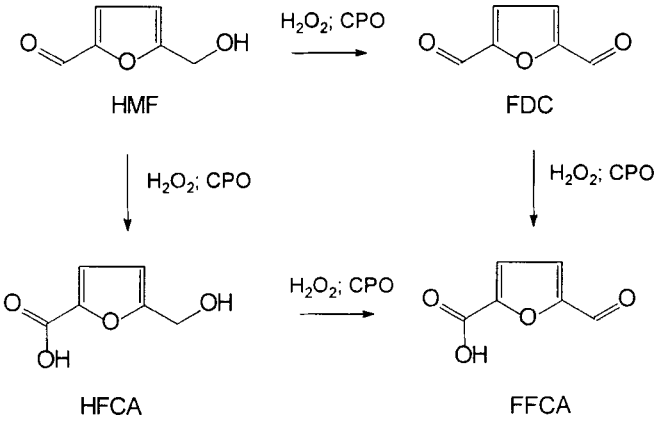

Figure 3. Reaction scheme for CPO catalyzed HMF oxidation.

The observed formation of HFCA is, to the best of our knowledge, the first evidence for CPO-catalyzed oxidation of aldehydes to acids. An indication for the CPO-catalyzed oxidation of aldehydes to acids was previously obtained in benzylic hydroxylation, in which a large amount of benzoic acid was detected as a by-product.[5b]

To investigate the mechanism of oxygen transfer in aldehyde oxidation labelling experiments were carried out. Reactions with labelled $H_2{}^{18}O_2$ yielded virtually complete incorporation of ^{18}O into HFCA, whereas no labelled oxygen from H_2O (^{16}O:^{17}O:^{18}O= 50.4:25.7:23.9) was incorporated into FDC. Exclusion of oxygen had no effect on selectivity or conversion for the oxidation of HMF (Table 1). From these results we conclude that the CPO-catalyzed oxidation of aldehydes to acids proceeds via direct oxygen transfer from the iron(V)oxo species to the aldehyde, analogous to the corresponding sulfoxidations,[13] epoxidations,[14] oxidation of arylamines[15] and indole oxidation to oxindole.[6b]

The oxygen transfer from the iron(V)oxo intermediate to the aldehyde could involve concerted insertion or two discrete one-electron transfers. In the latter case oxygen rebound from the oxoiron(IV) intermediate would result in the observed incorporation of ^{18}O from $H_2{}^{18}O_2$. Hence we propose that in the oxidation of HMF the initial step involves competing hydrogen abstraction, by the iron(V)oxo intermediate of CPO, from the alcohol and aldehyde moieties (Figure 4).

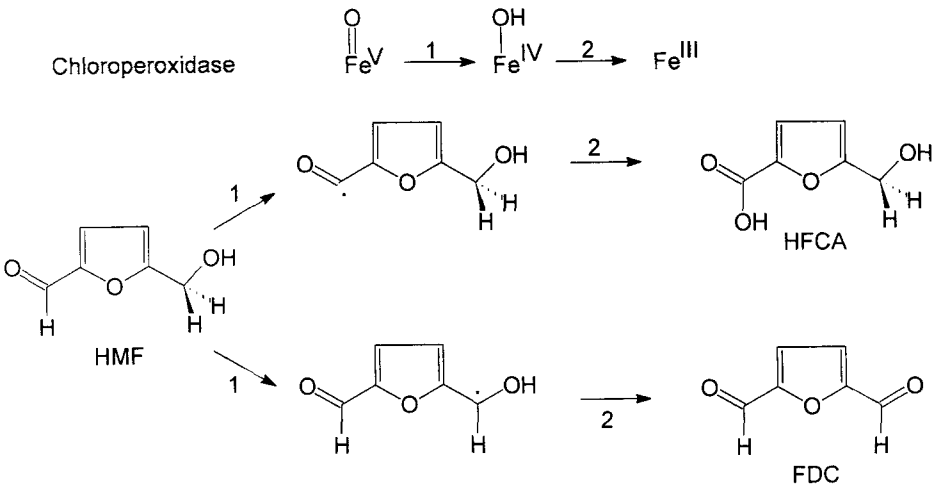

Figure 4. Proposed mechanism for HMF oxidation.

Furfuryl alcohol oxidation catalyzed by CPO shows a selectivity of virtually 100% to aldehyde at a conversion of 92%. Thus, the oxidation of HMF involves more than just a competitive oxidation of alcohol and aldehyde as otherwise the oxidation of furfuraldehyde to 2-furoic acid should have competed with the furfuryl alcohol oxidation. Hammett parameters indicate that the hydroxymethyl substituent does not exert an electron-donating effect on aromatic systems (σ_m= 0.08 and σ_p= 0.08), thus the positive effect of this group on the reactivity of the aldehyde group for oxidation presumably is negligible. Hence, the observed oxidation of the aldehyde moiety of HMF is not due to a higher intrinsic activity of the aldehyde group of HMF compared to 2-furfural, caused by the 2-hydroxymethyl substituent. Possibly the hydroxyl moiety in HMF is involved in binding the reactant to the enzyme thus directing the aldehyde group into a favourable position for reaction with the oxoiron intermediate.

The oxidation of 2,5-bis(hydroxymethyl)furan (BHF) yielded, in contrast to the oxidation of furfuryl alcohol, not only the products from alcohol oxidation to aldehyde (HMF and FDC) but also HFCA, comparable to the HMF oxidation (Figure 5). As expected, about the same selectivity to FDC was obtained as for HMF oxidation (67% *vs.* 63%, both at 60% conversion of HMF).

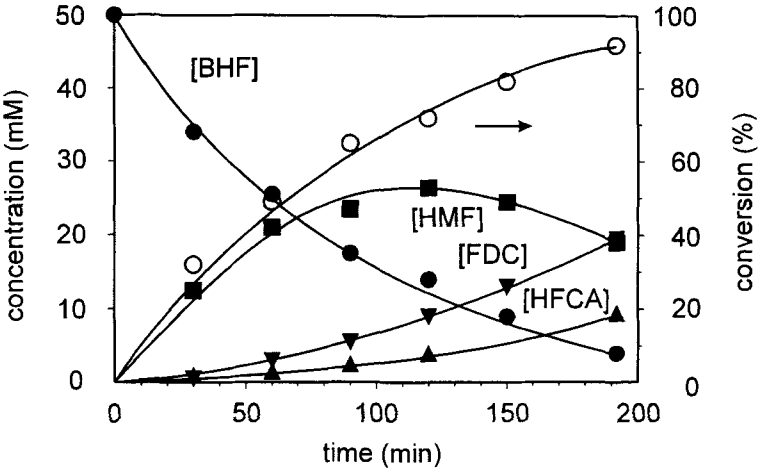

Figure 5. Oxidation of 2,5-bis(hydroxymethyl)furan (BHF), 50 mM BHF in 25 mL pH 5, 200 μL CPO, 1 equiv H_2O_2/2 h, ○ conversion of BHF, ● [BHF], ■ [HMF], ▼ [FDC], ▲ [HFCA].

Use of a H_2O_2-stat

CPO is deactivated even by low concentrations of H_2O_2. Therefore, the H_2O_2 should preferably be added rate limiting to the reaction mixture. In the reactions described above, this was done by slow addition of H_2O_2 to the reaction mixture (1 equiv/2 h). We have recently demonstrated that a hydrogen peroxide-stat is very useful for controlling CPO-catalyzed oxidation reactions.[12] The hydrogen peroxide-stat measures the concentration of H_2O_2 in solution and keeps it at a constant low level (30 μM). This set-up results in a more efficient use of CPO and it also facilitates the detection of the end point of the reaction. Its advantages in the oxidation of HMF became immediately apparent because the reaction proceeded very rapidly; after 12 min a conversion of 77% was already reached and after 21 min a final conversion of 87% was obtained (Figure 6), this in contrast to slow addition of H_2O_2 in which a conversion of 89% was reached after 2 h. Thus the oxidation of HMF is another example of the practical utility of a hydrogen peroxide-stat for controlling reactions using CPO as the catalyst. The selectivity to FDC was comparable to the selectivity obtained with slow addition of H_2O_2 (66% *vs.* 59%).

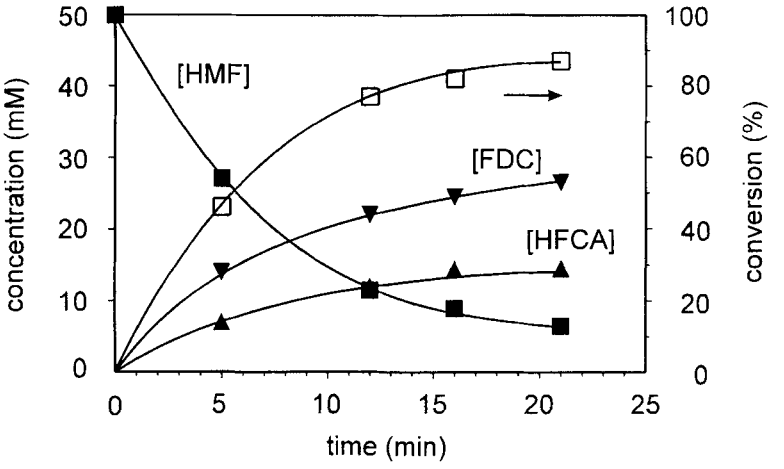

Figure 6. Oxidation of HMF using a hydrogen peroxide-stat, 50 mM HMF in 25 mL pH 5, 200 μL CPO, $[H_2O_2]$ = 30 μM, □ conversion of HMF, ■ [HMF], ▼ [FDC], ▲ [HFCA].

CONCLUSIONS

The oxidation of HMF by CPO and hydrogen peroxide proceeds with a maximum selectivity of 74% to FDC. Virtually quantitative conversion of HMF can be obtained. HFCA is the major by-product of the HMF oxidation and is formed by CPO catalyzed oxidation of the aldehyde moiety of HMF. The latter involves direct oxygen transfer from the iron(V)oxo intermediate to the aldehyde. As the oxidation of furfuryl alcohol does proceed selectively to the aldehyde, in contrast to HMF oxidation, it can be concluded that the oxidation of HMF involves more than competitive oxidation of aldehydes and alcohols. CPO provides an environment to HMF in which oxidation of the aldehyde moiety becomes more favourable than in furfuraldehyde, possibly by interaction of the hydroxyl moiety of HMF with amino acids in the vicinity of the active site of CPO. A hydrogen peroxide-stat facilitates the reaction procedure for CPO-catalyzed oxidation of HMF and leads to lower reaction times.

EXPERIMENTAL

Materials and Analytical Methods

Chloroperoxidase from *Caldariomyces fumago* was isolated and purified as described in literature.[1a] The enzyme preparation (93 μM) contained 8000 U/mL according to the method of Morris *et al.*[16] Hydrogen peroxide 35% was obtained from Merck. 5-Hydroxymethylfurfural was obtained from Südzucker. Furfuryl alcohol and 2,5-bis(hydroxymethyl)furan were purchased from Aldrich Chemical Company. *tert*-Butyl alcohol was obtained from Baker. The hydrogen peroxide-stat, Dulcometer Perox 20/21, was obtained from Prominent Dosiertechnik, Heidelberg, Germany. The measurement of the hydrogen peroxide was based on amperometric measurements at a platinum electrode which is operated as a potentiostat. Two point calibrations were made before each reaction. A Metrohm Dosimat 655 was used for addition of hydrogen peroxide (1.66 M).

HPLC-analysis: Reaction samples were analyzed on a Phenomenex Rezex Organic Acids column, 60 °C, eluent 0.01 M trifluoroacetic acid at 0.6 mL/min. Detection was performed on a Shodex RI SE-51 refractive index detector and on a Shimadzu SPD-6A UV detector (220 nm). Products were identified by comparing the HPLC-retention times of the products to authentic samples and by mass spectroscopy, for which the HPLC column was coupled to a VG70-SE spectrometer operating in plasma spray mode.

General Oxidation Procedure

At room temperature 1.25 mmol of HMF was dissolved in 25 mL buffer (0.1 M citrate buffer pH 5). Subsequently, 200 μL CPO (8×10^3 U/mL, A_{400} = 8.5) was added to the reaction mixture, followed by 5 min. of stirring. The reaction was started by continuous addition of 1.66 M H_2O_2 in buffer (0.1 M citrate, pH 5). In total 1.25 equiv of H_2O_2 was added in 150 min. In the case of the hydrogen peroxide-stat controlled reactions the $[H_2O_2]$ was set at 30 μM. The reactions were monitored by removing aliquots which were analyzed on HPLC. Citric acid was used as internal standard. Oxidations at pH 3 and pH 6 were performed in phosphate buffer (0.1 M) and oxidations at pH 4 were performed in acetate buffer (0.1 M). Blank experiments were carried out under the same conditions as above in which CPO was discarded.

Labelling Experiments

$H_2{}^{18}O_2$: To 1 mL of a 50 mM solution of HMF in 0.1 M formate buffer pH 5 which contained 25 μL CPO was added $H_2{}^{18}O_2$ (2.7%) in 8 portions of 8 μL in 35 min. H_2O (^{16}O:^{17}O:^{18}O = 50.4:25.7:23.9): 1 mg HMF was dissolved in 200 μL labelled H_2O. Subsequently 0.5 mg of ammonium formate and 5 μL CPO were added to the reaction mixture. H_2O_2 (2.7%) was added in 8 portions of 2 μL in 35 min. The reaction products of the labelling experiments were analyzed by HPLC-MS as described under materials and analytical methods.

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