Visible light-driven and chloroperoxidase-catalyzed oxygenation reactions†

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Robust peroxidase-catalyzed enantiospecific oxyfunctionalizations can be achieved by simple light-driven *in situ* generation of hydrogen peroxide.

Chloroperoxidase from *Caldariomyces fumago* (CPO, E.C. 1.11.1.10) is a versatile oxidation and oxyfunctionalization catalyst.[1] It catalyzes a broad range of synthetically useful transformations such as oxidation of alcohols,[2] hydroxylation of allylic, propargylic and benzylic C–H bonds,[3] C=C double bond epoxidations,[4] halohydroxylations,[5] and heteroatom oxygenation reactions.[6] Many of these proceed with high to exclusive regio-, chemo-, and enantioselectivity. Unlike the related P450 monooxygenases,[7] CPO does not rely on prohibitively expensive nicotinamide cofactors and molecular oxygen to regenerate its catalytically active oxyferryl heme active site.[1b] Instead, CPO utilizes simple hydrogen peroxide, which makes CPO an attractive catalyst for preparative organic chemistry.

Its high synthetic potential however is impaired by its rather poor operational stability, particularly towards H_2O_2.[8] The exact inactivation mechanism is still under debate but it is clear that oxidative degradation of the heme prosthetic group plays a major role.[9] Portionwise addition of H_2O_2[10,11] to maintain the H_2O_2 concentration at acceptable levels significantly increased the total turnover number (TTN) of CPO over stoichiometric use of H_2O_2 or less reactive organic peroxides.[3a,12] But the heterogeneous nature of the external addition results in 'hot spots' comprising locally high H_2O_2 concentrations and fast CPO inactivation.[6b] This may be circumvented by generating H_2O_2 in homogeneous phase (*in situ*) by reduction of molecular oxygen. Chemical,[13] electrochemical,[6c,14] or enzymatic methods have been reported.[6b,15,16] But each approach comprises specific disadvantages such as need for specialized equipment or a second, costly enzyme. Furthermore, sufficiently high TTNs have not been achieved yet for CPO. Consequently, the quest for a simple, easily applicable, and robust *in situ* H_2O_2-generation method continues.

Here, we report on a novel, light-driven approach for the *in situ* generation of H_2O_2 to promote CPO-catalyzed oxidation–oxyfunctionalization reactions. We make use of (1) facile reduction of visible light-excited isoalloxazines (*e.g.* flavine adenine dinucleotide FAD, -mononucleotide FMN, or riboflavin Rf) by simple and cheap sacrificial electron donors such as ethylenediaminetetraacetate (EDTA), (2) high reactivity of reduced flavins ($FADH_2$, $FMNH_2$, RfH_2) with O_2 yielding H_2O_2 (ESI†).[17] We hypothesized that the reaction sequence outlined in Scheme 1 might be useful to promote CPO-catalysis.

As a starting point for our light-driven *in situ* H_2O_2 generation system we (arbitrarily) chose FMN as photocatalyst and EDTA as sacrificial electron donor. An ordinary slide projector equipped with a 250 W bulb (Philips 7748 XHP, see ESI† for experimental setup) served as light-source. Illumination of an anaerobic solution of FMN (0.1 mM in potassium phosphate buffer (KP_i) pH 5.1) in the presence of EDTA resulted in fast (<1 minute) decolourization of the solution indicating full reduction of FMN. The characteristic yellow colour of oxidized FMN returned upon aeration accompanied by the formation of H_2O_2. This sequence could be repeated several times. From these experiments we estimated a catalytic performance for the flavin photocatalyst (TF) of approximately 1.6 turnovers per minute.

Next we combined the photocatalytic H_2O_2 generation with CPO to perform a range of typical CPO-oxidation and -oxyfunctionalization reactions (Table 1).

In all cases, the photoenzymatic approach turned out to be superior to the stoichiometric addition of H_2O_2 which we attribute to an increased stability of CPO (*vide infra*). Performing these experiments either in the absence of EDTA, FMN, or in darkness yielded no conversion. Also in the absence of CPO, no conversion was observed with the exception of thioanisole where trace amounts of racemic sulfoxide were found. The enantioselectivity of the CPO-catalyzed sulfoxidation reaction was not impaired (Table 1).

The system is not confined to FMN as photocatalyst: substituting FMN by FAD or Rf under otherwise identical conditions influenced neither rate nor the stereochemical outcome of the sulfoxidation reaction (data not shown).

Encouraged by these results, we further evaluated the possibility to control the H_2O_2 formation rate *via* the photocatalyst concentration (Fig. 1). The overall rate correlated with the flavin concentration applied. Thus, the sulfoxide formation rate increased from 2.3 mM h^{-1} to 11.8 mM h^{-1} with increase of [FMN] from 10 μM to 250 μM. In terms of TF(CPO) this corresponds to an increase from 9.8 min^{-1} to 123 min^{-1}. Furthermore, significant accumulation of H_2O_2 was not observed. This indicates that the photocatalytic generation of H_2O_2 was overall rate-limiting and H_2O_2 was quickly consumed by CPO-catalyzed sulfoxidation. The very low *in situ* H_2O_2 concentration also explains the significantly

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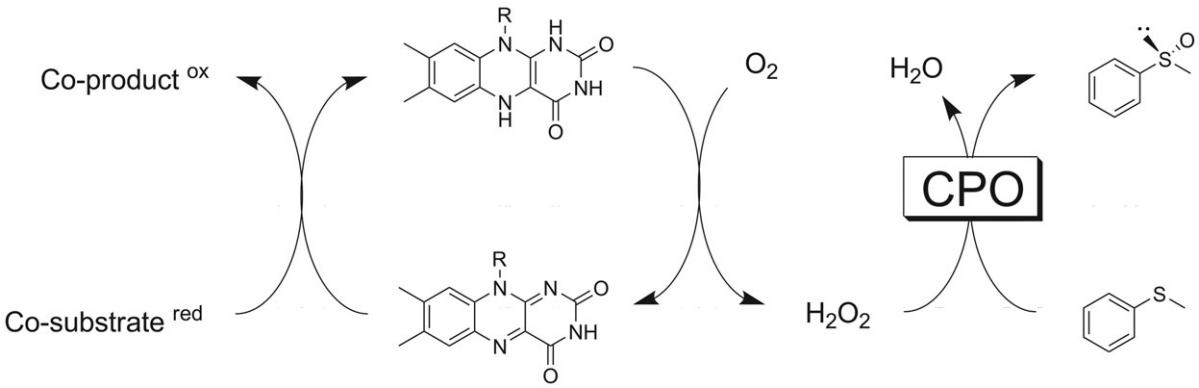

Scheme 1 Light-driven *in situ* H_2O_2 generation to promote CPO-catalyzed sulfoxidation reactions. Co-substrates used in this study comprise EDTA and formate (see ESI†).

Table 1 Typical CPO-catalyzed oxidation–oxyfunctionalization reactions driven by the proposed photochemical *in situ* H_2O_2 generation approach compared to the stoichiometric use of H_2O_2[a]

		Conversion (%)/TN (CPO)	
Substrate	Product	H_2O_2	FMN/$h\nu$/O_2
		30/6720	100/22 400
		26/830	40/1270
		60/1910	96/3050

[a] Conditions: [thioanisole] = 8 mM, [CPO] = 0.357 μM, [H_2O_2] = 8 mM or [EDTA] = 8 mM, [FMN] = 80 μM, $h\nu$, both methods yielded (*R*)-sulfoxide in optical purity >99% ee; [indole] = [furfuryl alcohol] = 25 mM, [CPO] = 7.85 μM, [H_2O_2] = 25 mM or [EDTA] = 25 mM, [FMN] = 250 μM, $h\nu$; no over oxidation products such as sulfone or furfurylic acid were observed. TN = turnover number = mol(product) × mol(CPO)$^{-1}$.

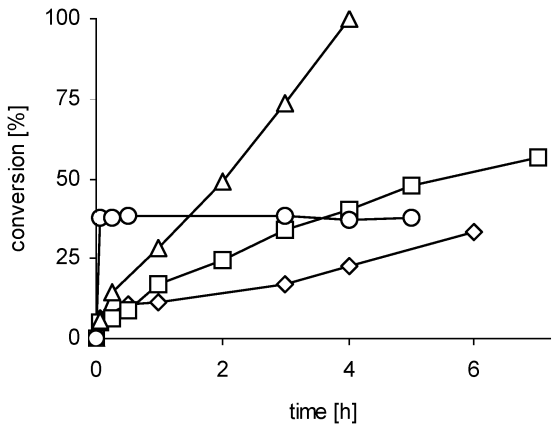

Fig. 1 Influence of FMN concentration on the light-driven CPO-catalyzed oxidation of thioanisole. Conditions: 25 mL ^{t}BuOH–50 mM phosphate buffer pH = 5.1 (25/75), *T* = 25 °C, [thioanisole] = 50 mM, [CPO] = 3.93 μM, oxidant: [H_2O_2] = 50 mM (○) or [EDTA] = 50 mM, [FMN] = 10 μM (◇), 50 μM (□), 250 μM (△). In all samples, the enantiomeric purity of the sulfoxide was greater than 98% ee.

increased operational stability of CPO: while with stoichiometric H_2O_2, CPO was fully inactivated within maximally 3 minutes, stable production of (enantiopure) sulfoxide continued for at least 7 h in case of the photocatalytic *in situ* formation of H_2O_2 corresponding to an at least 100-fold improved operational stability of CPO. Thus, we were able to demonstrate that the H_2O_2 supply rate can easily be controlled *via* the photocatalyst concentration.

Envisaging an environmentally benign photoenzymatic reaction, EDTA may not be the sacrificial electron donor of choice. Especially the generation of formaldehyde and ethylene diamine as waste products is not desirable from an environmental and toxicological point-of-view.[18] Other sacrificial electron donors such as methionine, adrenaline, or nicotine[17a] are also not attractive as again significant amounts of waste are generated. Therefore, we evaluated the suitability of formate as simple and cheap sacrificial electron donor, yielding CO_2 as the sole by-product. In fact, EDTA could be substituted by formate as sacrificial electron donor to promote CPO-catalyzed sulfoxidation and full conversion was observed (ESI†). Interestingly, here the enantioselectivity was somewhat lower (78% ee, *R*) than using EDTA as the sacrificial electron donor (>99% ee, *R*). This reduction of enantioselectivity was even more pronounced if H_2O_2 was added stoichiometrically (ESI†). Similar effects have been reported recently for the CPO-catalyzed epoxidation of limonene[4d] and can be rationalized by assuming binding of formate to the heme-iron as suggested by crystallographic data.[19] Thus, presence of formate might impair precise positioning of thioanisole within the CPO active site.

Overall, we have demonstrated that the proposed light-driven approach for *in situ* generation of H_2O_2 is practicable to promote CPO-catalyzed oxygenation reactions. The H_2O_2 generation rate can be easily controlled *via* the photocatalyst concentration. Further characterization will result in an optimized ratio of photo- and biocatalyst enabling high reaction rates while minimizing H_2O_2-related inactivation of the enzyme. Thus, we have turned a previously undesired side reaction[17c,d] into a simple, robust, and easily applicable novel method for peroxidase-catalyzed oxidation and oxyfunctionalization reactions. Compared to established approaches (Table 2), one major advantage of the photocatalytic generation of

Table 2 CPO-catalyzed sulfoxidation of thioanisole using different H_2O_2 generation/dosage methods

H_2O_2 generation method	TTN		Yield (%)	ee (%)	Remark/requirements
	CPO	Co-catalyst			
Stoichiometric H_2O_2[a]	4900	—	30	>99	—
Sensor-controlled[2b]	148 000	—	n.d.	n.d.	Additional equipment
Glucose oxidase/glucose/O_2[1b,6b]	250 000	n.d. (glucose oxidase)	79	99	2nd enzyme
Pd/H_2/O_2[13]	6500	12.8 (Pd/C)	11–60	9–36	Autoclave/detonation gas
Cathode/O_2[6c]	58 900	—	76	93	Electrochemical cell
Flavin/EDTA/$h\nu$/O_2[a]	22 400	1250 (FMN)	100	<99	Visible light
Flavin/$NaHCO_2$/$h\nu$/O_2[a]	22 000	>1000 (FMN)	100	78	Visible light

n.d. = not determined.[a] This study.

H_2O_2 lies in its simplicity. No specialized equipment or catalysts are required, all reactions were performed at ambient pressure and temperature using a readily available light-source (also sunlight) and commercially available catalysts. Furthermore, already under non-optimized conditions turnover numbers of more than 1.000 and 22.000 have been achieved for the photocatalyst and CPO, respectively. Current work ongoing in our laboratory comprises full characterization and optimization of the reaction setup and evaluation of further simple sacrificial electron donors *e.g.* phosphite. Thus, we are convinced to eventually obtain a simple, compatible, and environmentally benign reaction setup. Furthermore, preliminary results suggest a general applicability to heme enzyme-catalyzed oxidation reactions such as horseradish peroxidase-catalyzed oxidative C–C coupling reactions and cytochrome C-catalyzed oxygenations. Also this approach might be used for simplified P450 oxygenation reactions *via* the hydrogen peroxide shunt pathway[20] and thereby become useful *e.g.* for screening and drug metabolite synthesis.

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