Halofunctionalization of alkenes by vanadium chloroperoxidase from Curvularia inaequalis†

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The vanadium-dependent chloroperoxidase from Curvularia inaequalis is a stable and efficient biocatalyst for the hydroxyhalogenation of a broad range of alkenes into halohydrins. Up to 120 000 TON with 69 s⁻¹ TOF were observed for the biocatalyst. A bioenzymatic cascade to yield epoxides as reaction products is presented.

Vic-Halohydrins are valuable building blocks e.g. in natural product synthesis.1 Two functional groups endow the organic chemist with handles for further transformations such as conversion into epoxides and other functionalities.2 To attain vic-halohydrins, halohydroxylation of olefins appears to be the most direct approach next to ring-opening of epoxides by nucleophilic attack.3

Treatment of olefins with elementary halogens in water provides halohydrins. This approach, however, is hampered by the high reactivity of the reagents leading to side products and the corrosive reaction conditions.4 Also the formation of significant inorganic wastes renders this approach questionable from an environmental point of view. Therefore, N-halo compounds such as N-halosuccinimide represent the most commonly used reagents, or sodium periodate, despite the fact of low atom efficiency causing large amounts of waste products.5 More recently, transition metal catalysts (including W and V) have received increasing interest for the conversion of alkenes to halohydrins using H₂O₂ and inorganic halide as reagents.6 In addition, ozone has been successfully applied as oxidant using NH₄Cl as halogen source.7

Finally, biocatalysis also in principle enables the conversion of alkenes to halohydrins using so-called haloperoxidases (E.C. 1.11.1.8, 1.11.1.10 and 1.11.1.18). These enzymes generate reactive hypohalites from the corresponding halides and hydrogen peroxide. This has been reported for example using the heme-dependent chloroperoxidase from Caldariomyces fumago (CfuCPO).8 However, the poor stability of heme-dependent haloperoxidases against H₂O₂ limits the broad applicability of this enzyme class and make control over the in situ concentration of H₂O₂ inevitable.9 In fact, the catalytic performance (in terms of turnover numbers, TON) reported for CfuCPO is too low to be of preparative value (generally a few thousand turnovers can be estimated from the publications).8c,10 Vanadium-dependent haloperoxidases are principally more robust against H₂O₂ and therefore maybe more practical catalysts.11

The vanadium chloroperoxidase from Curvularia inaequalis (CvCPO) for example excels by its superb stability against H₂O₂ as well as demanding environmental conditions such as cosolvents and temperature.12 It was successfully applied for the catalytic halogenation of phenols13 as well as to mediate the (Aza-) Achmatowicz reaction.14

Therefore, we became interested in evaluating CvCPO as catalyst for the halogenation of alkenes striving for more robust reactions than reported for the heme-dependent halogenases (Scheme 1).

In a first set of experiments we investigated the halohydroxylolation of styrene. As starting point we chose the optimised reaction conditions determined previously.13,14 Full conversion was achieved after 4 hours with high selectivity towards vic-bromohydrin (>90%) with only trace amount of side products (epoxide, ketone derivatives) detectable (Table 1, entry 2). The regio chemistry of the hydroxybromination reaction exclusively followed Markovnikov’s rule. In accordance with the suspected chemo-enzymatic reaction mechanism, no enantioselectivity was observed. When the enzyme was omitted from the reaction, no product formation was observed (Table 1, entry 3), which confirms the proposed chemoenzymatic cascade. Likewise, no conversion was observed in the absence of H₂O₂ or KBr (data not shown).

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When using ethanol a cosolvent, also very significant amounts (13%) of [2-bromo-1-ethoxyethyl]benzene were obtained (Table 1, entry 8). We attribute this to the competition of water and ethanol as nucleophiles.

Adding the oxidant (H₂O₂) portion-wise proved to be more efficient than a single addition at the beginning of the reaction (Table 1, entries 2 vs. 9). This can be attributed to the well-known formation of singlet oxygen (¹O₂) from hypohalites and H₂O₂, which in our case represents an undesired side reaction consuming H₂O₂.¹⁵

Varying the pH value of the reaction medium had the most significant effect on the reaction (Fig. S1, ESI†). Consistent with previous observations, highest CVCPO activity was observed at slightly acidic to neutral conditions. Even in the absence of buffer, essentially the same results were observed as using BR (Britton–Robinson) buffer (Table 1, entries 4 and 5). Raising or lowering the pH value lead to dramatic activity decreases. Under acidic reaction conditions the bromohydrin was the predominant sole product of the reaction. The relative epoxide yield increased with increasing pH. This is consistent with the well-known base-catalysed ring-closure of haloaldrenes.

To obtain further insights into the kinetics of the hydroxybromination reaction, we followed the time course of the chemoenzymatic conversion of (water soluble) sodium styrene-4-sulfonate 1g (Fig. 1). Following a very short lag-phase, the product accumulation proceeded linearly until approx. 75% conversion and then considerably slowed down. After 5–6 h, full conversion was obtained. It is worth emphasising the excellent catalytic performance of the biocatalyst here: Over 6.5 h CVCPO performed 400,000 catalytic turnovers corresponding to an average turnover frequency of more than 15 s⁻¹ (over 6.5 h). Within the first 2 h, this value was even higher (69 s⁻¹). Raising the concentration of 1g from 40 mM to 160 mM (while concomitantly increasing the concentration of KBr as well as the buffer strength to avoid pH shifts, vide infra) resulted in 80% of the desired product 2g. Hence CVCPO performed at least 1,280,000 catalytic cycles. These values underline the robustness and efficiency of the biocatalyst.

Applying the optimised reaction conditions (Table 1, entry 9) we advanced to evaluate the product scope of the chemo-enzymatic hydroxyhalogenation reaction. As shown in Fig. 2 both aromatic and aliphatic alkenes were converted into the corresponding haloaldrenes. Styrene was transformed into the corresponding bromohydrin 2a and chlorohydrin 2b with 81% and 77% yield, respectively. α- and β-methylstyrene were converted in satisfactory yields. The (E)/(Z) configuration of the latter influenced the product conformation leading to the anti-bromohydrin

![Scheme 1 Chemoenzymatic oxidation of alkenes using the vanadium chloroperoxidase from Curvularia inaequalis (CVCP1) as hypohalite generation catalyst. Here, the most popular chemo-enzymatic mechanism is shown where the enzyme forms the hypohalite in situ followed by spontaneous reaction of the latter with an alkene. It should, however, be mentioned that also some indications for a direct enzymatic halohydroxylation within the enzyme active site exist.¹¹](Image)

![Table 1 Characterisation of the reaction conditions for the chemoenzymatic bromohydroxylation of styrene](Table)

![Fig. 1 Time course of the chemoenzymatic oxidation of alkene 1g (●) to 2g (■). General conditions: aqueous medium (D₂O, 0.1 M citrate pH 5), c(1g) = 40 mM, c(KBr) = 160 mM, c(H₂O₂) = 170 mM, c(CVCPO) = 100 mM, T = 25 °C. The concentration of substrate and product was determined by ¹H NMR (see ESI†).](Image)
Circumvented by a two-step procedure wherein the halohydrin formation is performed first at acidic pH followed by a base treatment to pH 10 with NaOH. This method proved to be successful for most products with the exception of the halohydrin obtained from oleic acid (2k). Here, intensive foaming (of the carboxylate) interfered with product isolation, which could be avoided by substituting NaOH with trimethylamine. Also, it is worth mentioning here that the previously mentioned 10(9)-bromo-9(10)-ethoxycadecanoic acid was also retained here (approx. 30% yield).

Even though this two-step procedure was efficient for the production of epoxides, we envision a more elegant (and less wasteful) approach. For this, we evaluated a bienzymatic cascade reaction comprising CVCPO-initiated halohydrin formation followed by halohydrin dehalogenase (HHe)-catalysed ring-closure (Scheme 2). Such a cascade would, in principle, also allow for catalytic use of the halides. For this, we recombiantly expressed the HHe from Agrobacterium radiobacter. Particularly, its poor enantioselectivity appeared attractive in this case as a more enantioselective version would lead to kinetic resolution of the racemic bromohydrin and consequently in decreased yields of the desired epoxide.16

A reaction employing 20 mM styrene 1a in the presence of 5 mM KBr indeed gave 6.6 mM of the desired epoxide and 1.8 mM of the bromohydrin indicating the principal feasibility of the reaction shown in Scheme 2, despite the reduced activity of the biocatalysts at pH 7 (Fig. S2, ESI†).

The present study underlines the synthetic potential of the vanadium-dependent chloroperoxidase from Curvularia inaequalis. Its high robustness and activity enabled efficient halohydroxylation of various alkenes to the corresponding halohydrins on preparative scale. Despite the promising first results, a range of issues will have to be overcome to render this method truly practical. First, efficient in situ H2O2 generation systems17 will be applied to this reaction in order to circumvent the undesired 1O2 formation reaction. Also the envisioned bienzymatic cascade forming epoxides from alkenes (while using catalytic amounts of halides) will need further improvements such as the application of CVCPO mutants with engineered pH optima,18 reaction engineering approaches utilizing spatially separated reaction compartments and immobilized enzymes.19

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Notes and references