Molecular Catalysis 443 (2017) 92–100

Contents lists available at ScienceDirect

Molecular Catalysis

journal homepage: www.elsevier.com/locate/mcat

MCAT

Research Paper

Enzymatic halogenation and oxidation using an alcohol oxidase-vanadium chloroperoxidase cascade

Andrada But[a], Aster van Noord[a], Francesca Poletto[a], Johan P.M. Sanders[a,c], Maurice C.R. Franssen[b,*], Elinor L. Scott[a,*]

[a] Chair of Biobased Chemistry and Technology, Wageningen University, P.O. Box 17, 6700AA Wageningen, The Netherlands
[b] Laboratory of Organic Chemistry, Wageningen University, P.O. Box 8026, 6700EG Wageningen, The Netherlands
[c] Wageningen Food & Biobased Research, P.O. Box 17, 6700AA Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 15 May 2017
Received in revised form
11 September 2017
Accepted 13 September 2017

Keywords:
Alcohol oxidase
Vanadium chloroperoxidase
Enzymatic cascade
Biobased nitrile
Enzymatic oxidation

ABSTRACT

The chemo-enzymatic cascade which combines alcohol oxidase from *Hansenula polymorpha* (AOX_{Hp}) with vanadium chloroperoxidase (VCPO), for the production of biobased nitriles from amino acids was investigated. In the first reaction H_2O_2 (and acetaldehyde) are generated from ethanol and oxygen by AOX_{Hp}. H_2O_2 is subsequently used in the second reaction by VCPO to produce HOBr *in situ*. HOBr is required for the non-enzymatic oxidative decarboxylation of glutamic acid (Glu) to 3-cyanopropanoic acid (CPA), an intermediate in the production of biobased acrylonitrile. It was found that during the one pot conversion of Glu to CPA by AOX_{Hp}-VCPO cascade, AOX_{Hp} was deactivated by HOBr. To avoid deactivation, the two enzymes were separated in two fed-batch reactors. The deactivation of AOX_{Hp} by HOBr appeared to depend on the substrate: an easily halogenated compound like monochlorodimedone (MCD) was significantly converted in one pot by the cascade reaction of AOX_{Hp} and VCPO, while conversion of Glu did not occur under those conditions. Apparently, MCD scavenges HOBr before it can inactivate AOX_{Hp}, while Glu reacts slower, leading to detrimental concentrations of HOBr. Enzymatically generated H_2O_2 was used in a cascade reaction involving halogenation steps to enable the co-production of biobased nitriles and acetaldehyde.

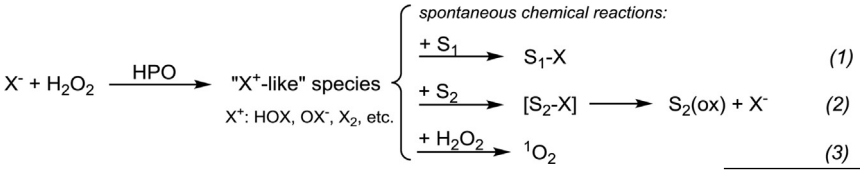
1. Introduction

Halogenation and oxidation reactions are key steps in the production of both commodity and fine chemicals. Halogen atoms are inserted because of their unique physicochemical and biological properties, in commodities such as vinyl chloride [1] but also in medicines like diclofenac [2]. The reagents involved are often molecular halogens: toxic, corrosive and reactive compounds that are produced in energy-intensive processes [3]. Oxidation reactions in bulk chemistry are usually carried out using molecular oxygen and a solid catalyst, but in fine chemical industry hypervalent heavy metal oxides are used, which is undesirable due to toxicity and low atom efficiency [4]. Environmental concerns are stimulating more sustainable production of chemicals [5]. There is an increasing amount of research dedicated to develop greener halogenation and oxidation processes. For example, the *in situ* generation of halogenating species, such as X^+ (where X: Cl, Br) is desired [6]. In addition to the typical halogenation reaction, X^+ species can also be used as oxidizing reagents where a substrate is oxidised via an intermediate halogenation step.

The *in situ* production of X^+ species (X^+: HOX, X_2, etc.) by catalysis using haloperoxidases (HPO) has received a lot of attention due to the mild reaction conditions such as aqueous solutions at ambient temperature [6–8]. HPO use H_2O_2 in acidic conditions to oxidise halides to the corresponding hypohalous acid (HOX). In the presence of an organic halogen acceptor (S_1), HOX will react to give a halogenated product (S_1-X) according to Eq. (1) [9]. When an unstable intermediate (S_2-X) is formed, hydrolysis and/or further oxidation to a more stable product will occur as shown in Eq. (2).

* Corresponding authors.
E-mail addresses: maurice.franssen@wur.nl (M.C.R. Franssen), elinor.scott@wur.nl (E.L. Scott).

http://dx.doi.org/10.1016/j.mcat.2017.09.014



The most investigated HPO is the Fe-heme containing chloroperoxidase from *C. fumago* (CPO) [10–14]. Despite the broad application of CPO [15], excess of H_2O_2 in the reaction mixture causes deactivation of the haloperoxidase by oxidation of its heme moiety [10]. A solution to overcome deactivation by H_2O_2, is to replace Fe-heme HPO with the more robust vanadium containing haloperoxidase (VHPO) which has been successfully used for halogenation reactions like the conversion of alkenes into halohydrins [16], the halogenation of phenols [17,18] and oxidation reactions like the aza-Achmatowicz [7] or the oxidative decarboxylation reaction of amino acids [8]. It was shown that VCPO has superior resistance against H_2O_2 [19]. However, with an excess of H_2O_2, HOBr may react with H_2O_2 to form singlet oxygen [19] (Eq. (3)) which may lead to undesired oxidation products and enzyme inactivation [16]. Gradual addition of H_2O_2 could be an option to alleviate this problem [8,10]. As well as this, the production of H_2O_2 itself is not sustainable due to energy use and safety issues[20]. Another option would be the *in situ* production of H_2O_2 by the use of oxidases. These enzymes can activate molecular oxygen for the production of H_2O_2 together with the oxidation of a substrate (Eq. (4)).

$$\text{Substrate} + O_2 \rightarrow \text{Substrate}_{ox} + H_2O_2 \quad (4)$$

The majority of processes for the enzymatic generation of H_2O_2 use glucose oxidases (GOX) [12]. GOXs are well characterised enzymes [21] which have flavin adenine dinucleotide (FAD) as a cofactor. To produce one equivalent of H_2O_2, GOX converts equimolar amounts of glucose to gluconic acid which results in the acidification of the reaction mixture which requires pH control and results in salt by-products. In addition, the atom efficiency of this system is poor.

Other FAD-containing oxidases could lead to the same performance as GOX for the *in situ* production of H_2O_2 but without the drawbacks related to gluconic acid. For example, alcohol oxidases (AOX) [22] convert short chain primary alcohols into (highly) volatile aldehydes. The substrate affinity of AOXs decreases with the length of the carbon chain of the alcohol, therefore, AOXs have the highest activity with methanol and ethanol as substrates [23]. Formaldehyde is the oxidation product of methanol, while ethanol is converted to acetaldehyde; as formaldehyde is more toxic compared to acetaldehyde [24], ethanol is the preferred substrate. It was shown that acetaldehyde can be easily separated by pervaporation [25] or air stripping [26]. Deactivation of AOX by its own product H_2O_2 is possible due to oxidation of the –SH residues of AOX [27]. However, this can be prevented by *in situ* product removal techniques such as cascade reactions. Deactivation of AOX was prevented in a cascade reaction where H_2O_2 was immediately consumed by the next reaction with a catalase [27] or peroxidase [28]. Replacing GOX by AOX and glucose with ethanol for the *in situ* generation of H_2O_2 would avoid complex downstream processing. Furthermore, the cascade reaction of AOX with an HPO which will use H_2O_2 for the production of X^+ species would give access to more sustainable reactions involving halogenation reagents.

Examples for the cascade reactions of oxidases with HPO are known. The majority of these reactions use HPO for its so-called classical peroxidase activity. Some HPO, in addition to the haloperoxidase activity, catalyse a typical peroxidase reaction where a substrate is oxidised using H_2O_2 in the absence of halides [15]. For example, the cascade reaction of GOX with an HPO in the absence of halides was shown to be successful for the oxidation of thioanisole or indole [12,13]. However, only a few examples are known for the cascade reaction of oxidases with haloperoxidases for the production of HOX and the use of it as a halogenating agent (Eq. (4) + (1)). GOX was coupled to CPO in presence of Cl^- for microbial disinfection purposes of surfaces [29] and for chlorination reactions of model substrates such as monochlorodimedone [30] or barbituric acid [31]. Recently, the cascade GOX-CPO was also used in presence of Br^- for the bromination of allenes [32]. An industrial example for the combination of an AOX using methanol with a HPO for a chlorination reaction was briefly mentioned for propylene chlorohydrin generation from propene for the Cetus process [33]. However, to the best of our knowledge no details are given about the reaction conditions or the results obtained.

The combination of AOX with HPO for the production of HOX and the use of it in a further chemical reaction as oxidizing agent (Eq. (4) +)2)) has not yet been investigated. Therefore, to have more sustainable oxidation reactions *via* halogenation more insight is required for the combination of oxidases with haloperoxidases in a chemo-enzymatic cascade reaction.

A recent publication reported the use of vanadium chloroperoxidase from *C. inaequalis* (VCPO) for the oxidative decarboxylation of amino acids to nitriles. A reaction that occurs *via* brominated amines. When biomass-derived amino acids are used as a feedstock, a biobased alternative for the production of nitriles is obtained [8]. It was shown that glutamic acid (Glu) can give access to biobased acrylonitrile, an important building block in the polymer industry, via the intermediate 3-cyanopropanoic acid (CPA) [34]. To produce CPA from Glu, two equivalents of an oxidised bromine species such as HOBr are required. For this, VCPO generates HOBr by oxidizing Br^- with H_2O_2. Subsequently HOBr brominates Glu to give N,N-dibrominated Glu which is further converted to CPA, the corresponding nitrile.

This paper describes the reaction configuration required to perform the chemo-enzymatic cascade that combines an AOX with VCPO for the production of nitriles from amino acids. The required HOBr is generated *in situ* by the VCPO-mediated oxidation of Br^- with H_2O_2. The necessary H_2O_2 is generated *in situ* as well by an AOX which activates molecular oxygen. To do this AOX converts ethanol to acetaldehyde, a volatile product which can be readily removed (Scheme 1).

2. Experimental

2.1. Materials

L-glutamic acid (98.5% pure), NaBr (99% pure), H_2O_2 (35 wt-%), citric acid, Na_3VO_4 (99.98% pure), 2 2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (98% pure) and Trizma base were purchased from Sigma-Aldrich. HPLC grade ethanol, methanol were received from Actu-All chemicals. –3-Cyanopropanoic acid (95.9% pure) was provided by Interchim and monochlorodimedone by BioResourse Products. Oxygen was used as received from Linde-gas.

2.2. Enzymes

Vanadium chloroperoxidase was expressed in *E. coli* cells containing the VCPO plasmid using a protocol described elsewhere