A Biocatalytic Aza-Achmatowicz Reaction

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Supporting Information

ABSTRACT: A catalytic, enzyme-initiated (aza-) Achmatowicz reaction is presented. The involvement of a robust vanadium-dependent peroxidase from Curvularia inaequalis allows the simple use of H2O2 and catalytic amounts of bromide.

KEYWORDS: Achmatowicz reaction, biocatalysis, hypohalogenites, oxidation, peroxidase

The Achmatowicz reaction over the years has demonstrated its usefulness in the conversion of furan rings into heterocyclic scaffolds containing multiple functional handles for further synthetic transformations. Key in the Achmatowicz process is an oxidative activation of the furan ring, giving rise to a reactive dicarbonyl intermediate, which cyclizes to give the corresponding pyranone (X = O) or piperidinone (X = N-EWG) structure (Scheme 1).4

Despite frequent application of the Achmatowicz protocol in the synthesis of pharmaceutically relevant building blocks and natural products, the oxidative rearrangement is usually conducted using stoichiometric amounts of an oxidative reagent such as m-CPBA, and catalytic methods are scarce.

Recently, however, Deska and co-workers reported an enzymatic version of the Achmatowicz reaction using the well-known chloroperoxidase from Caldariomyces fumago (CfCPO).9 One of the major challenges using CfCPO (as with any heme-dependent enzyme) is its poor resistance against the oxidant H2O2.10–13 Essentially, this renders any in situ H2O2 generation redundant and allows for simple and clean (yielding H2O as the sole byproduct) reaction schemes.

Furthermore, most in situ H2O2 generation methods yield additional byproducts such as gluconic acid that not only negatively influences the atom economy of the overall reaction but also may complicate the reaction scheme. In addition, the chemoenzymatic process seems restricted to Achmatowicz reactions, while the correspondingaza-Achmatowicz products are synthetically equally relevant. Therefore, we decided to follow up on the seminal contribution by Deska using the vanadium-dependent peroxidase from Curvularia inaequalis [CiVCPO (Scheme 2)].15–20

The major advantage of CiVCPO over CfCPO lies in the prosthetic group used (vanadate as compared to heme iron) and the resulting high robustness of the peroxidase in the presence of H2O2.16 Essentially, this renders any in situ H2O2 generation redundant and allows for simple and clean (yielding H2O as the sole byproduct) reaction schemes.

Pleasingly, already in initial aza-Achmatowicz experiments under arbitrarily chosen reaction conditions, full conversion of 1a was observed within 24 h. It is worth noting here that in the absence of either of the catalysts (CiVCPO or Br−) or the oxidant (H2O2), no significant conversion was observed.

Encouraged by these promising results, we set out to determine the parameters influencing the efficiency of the chemoenzymatic aza-Achmatowicz reaction. Acidic pH values appeared to be necessary to achieve full conversion (Figure 1).
This observation is in line with the previously determined preference of CiVCPO for slightly acidic pH values.\textsuperscript{15–20}

To ensure the high catalytic efficiency of CiVCPO while minimizing the acidolytic degradation of the reagents, we performed all subsequent reactions at pH 5.

Next we turned our attention to the influence of the catalysts. Obviously, reducing the concentration of the biocatalyst (CiVCPO) is desirable, but substoichiometric amounts of bromide would avoid possible inhibitory effects on the enzyme.\textsuperscript{21,22} Pleasingly, both catalyst concentrations could be reduced very significantly without impairing the apparent activity of the overall chemoenzymatic aza-Achmatowicz reaction (Figures 2 and 3).

Reducing the biocatalyst concentration from 0.1 to 0.01 μM resulted in a somewhat reduced reaction rate (Figure 2), indicating that below 0.05 μM the enzymatic oxidation of Br\textsuperscript{−} becomes overall rate-limiting.

As shown in Figure 3, a decrease in the bromide concentration from 10 mM to 50 μM (1 mol %) had no apparent influence on the conversion of 1a into 2a. The corresponding Achmatowicz reaction of alcohol 1d, however, exhibited a very distinct dependency on the bromide concentration applied (Figure 3, dark blue bars). Within the time frame of these experiments, full conversion of the starting material (1d) into product (2d) was observed only in the presence of (super)stoichiometric amounts of KBr. One possible explanation for this observation may be a low reactivity of 1d with OBr\textsuperscript{−}. The accumulating OBr\textsuperscript{−} reacts with hydrogen peroxide, giving rise to the formation of singlet oxygen,\textsuperscript{16} therefore necessitating higher in situ concentrations of the latter. Nevertheless, catalytic amounts of bromide were feasible.

Encouraged especially by the high efficiency of the chemoenzymatic aza-Achmatowicz reaction in the presence of low bromide concentrations, we also evaluated seawater as a reaction medium and source of bromide (Figure 4). The overall rate of the chemoenzymatic aza-Achmatowicz reaction fell significantly behind the rate in defined buffers. Instead of being complete within maximally 30 min, full conversion was achieved only within approximately 2.5 h (Figure 4).

Most probably, the reduced reaction rate can be attributed to the huge molar surplus of chloride (∼550 mM) over bromide (<1 mM) present in seawater, leading to a predominant

\textsuperscript{a}In situ-formed, freely diffusable hypohalogenites (OBr\textsuperscript{−} and OCl\textsuperscript{−}) presumably account for the (aza-)Achmatowicz conversion of starting materials 1a–d. Abbreviations: Boc, tert-butyloxycarbonyl; Cbz, carboxybenzyl.

Figure 1. Influence of pH on the chemoenzymatic aza-Achmatowicz reaction: blue for c(1a) and red for c(2a). General conditions: solvent, ethanol with 100 mM universal B&K (Britton–Robinson) buffer [1:1 (v/v)]; c(1) = 5 mM substrate; c(KBr) = 10 mM; c(H\textsubscript{2}O\textsubscript{2}) = 10 mM; c(CiVCPO) = 0.1 μM (52 units mL\textsuperscript{−1}); T = 30 °C; t = 24 h.

Figure 2. Influence of c(CiVCPO) on the chemoenzymatic aza-Achmatowicz reaction: blue for c(1a) and red for c(2a). General conditions: solvent, ethanol with 0.1 M citrate at pH 5 [1:1 (v/v)]; c(1) = 5 mM; c(H\textsubscript{2}O\textsubscript{2}) = 10 mM; c(KBr) = 0.05 μM; T = 30 °C; t = 24 h.

Figure 3. Influence of c(KBr) on the chemoenzymatic Achmatowicz (dark blue) and aza-Achmatowicz (light blue) reactions. General conditions: solvent, ethanol with 100 mM citrate buffer at pH 5 [1:1 (v/v)]; c(1a or 1d) = 5 mM substrate; c(H\textsubscript{2}O\textsubscript{2}) = 10 mM; c(CiVCPO) = 0.1 μM (52 units mL\textsuperscript{−1}); T = 30 °C; t = 24 h.

Figure 2. Influence of c(CiVCPO) on the chemoenzymatic aza-Achmatowicz reaction: blue for c(1a) and red for c(2a). General conditions: solvent, ethanol with 0.1 M citrate at pH 5 [1:1 (v/v)]; c(1) = 5 mM; c(H\textsubscript{2}O\textsubscript{2}) = 10 mM; c(KBr) = 0.05 μM; T = 30 °C; t = 24 h.
formation of hypochlorite over hypobromite. Indeed, control
experiments in defined buffers and KCl as halogen resulted in
only 46% conversion after 24 h under otherwise identical
conditions. Alternatively, substrate inhibition of GVCPO by
cloride may also contribute to the reduced overall activity
observed in seawater.21 Further experiments clarifying the
chemical reactivities are currently ongoing in our laboratories.
Nevertheless, the experiments mentioned above suggest that
simple (and cheap) seawater may serve as the reaction medium
and source of catalysts for chemoenzymatic aza-Achmatowicz
reactions.

Admittedly, the characterization experiments reported above
are not suitable for preparative-scale application of the
proposed biocatalytic (aza-)Achmatowicz reaction. Therefore,
we set out to perform reactions at more practical reagent
concentrations [100 mM, approximately 25 g L⁻¹] and source of catalysts for chemoenzymatic aza-Achmatowicz
reactions.

Next to broadening the scope of the proposed chemoenzymatic
protocols, we will particularly focus on mechanistic studies and
frequencies have been observed, making it a promising catalyst.

In summary, we have presented a chemoenzymatic
alternative to the established stoichiometric (aza-)Achmatowicz
procedures. GVCPO is an efficient catalyst for generating
hypohalogenites in situ under mild reaction conditions from
catalytic amounts of halogenides. Because of its high robustness
and catalytic activity, excellent turnover numbers and
frequencies have been observed, making it a promising catalyst.
Next to broadening the scope of the proposed chemoenzymatic
protocol, we will particularly focus on mechanistic studies and
investigation of the stereochemochemical outcome.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the
ACS Publications website at DOI: 10.1021/acscatal.6b01636.

Control experiments detailing experimental information,
including full characterization of the products (PDF)
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Notes
The authors declare no competing financial interest.

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