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Chemo-promiscuity of alcohol dehydrogenases: reduction of phenylacetaldoxime to the alcohol

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ABSTRACT

The reduction of phenylacetaldoxime was catalysed by alcohol dehydrogenases in the presence of NAD(P)H yielding finally the primary alcohol via the imine and aldehyde intermediates. This suggests that the hydride of the cofactor NAD(P)H is transferred to the N-atom of the oxime moiety and not to the carbon atom, as usual stated. This reaction represents the first example of a catalytic chemo-promiscuity of alcohol dehydrogenases.

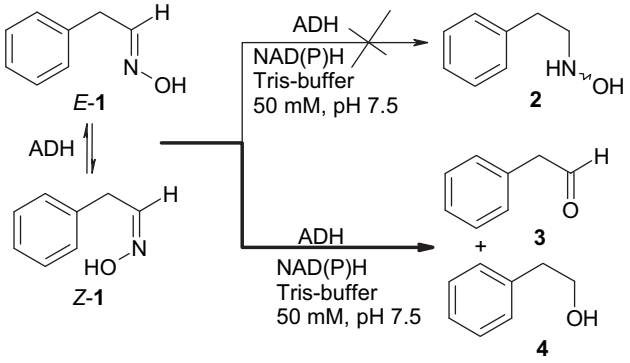
1. Introduction

Alcohol dehydrogenases (ADHs) are enzymes, which are responsible to transform ketone/aldehydes to the corresponding alcohols and vice versa at the expense of a nicotinamide cofactor that acts as hydride donor and acceptor, respectively.[1] Baker's yeast, which is known to possess many ADHs,[2] has been reported to transform oximes to carbonyl compounds or alcohols;[3] however, contradicting reports indicated that Baker's yeast catalysed the reduction of oximes to afford the corresponding hydroxylamines and amines.[4] Since amines are valuable intermediates and products,[5] we envisioned that ADHs might act as suitable catalysts to reduce the C=N double bond of oximes yielding hydroxylamines, which could easily be transformed to the corresponding amines.[6] Overall, this would represent a first example of a promiscuous activity[7] of ADHs.

2. Results and discussion

2.1. Testing ADHs

Using phenylacetaldoxime **1** (*Z*/*E*=95:5) as a test substrate the desired reduction to hydroxylamine **2** was investigated employing various ADH preparations and NAD(P)H recycling systems (Scheme 1, Table 1). Unfortunately, no formation of hydroxylamine or amine could be detected. The control experiment showed that the substrate was stable under the reaction conditions employed. Employing the ADH from *Rhodococcus erythropolis* (RE-ADH) no transformation of **1** was detected at all. The other five ADHs showed *E*/*Z*-isomerisation [e.g., ADH from *Paracoccus pantotrophus* DSM 11072 (PpADH)].[8] Unexpectedly four ADH preparations (HLADH, ADH-A, RasADH, LK-ADH) led to the formation of alcohol **4**, whereby in the case of LK-ADH and RasADH phenylacetaldehyde **3** was detected, too. For instance ADH-A led to the formation of 16% of alcohol **4** within 24 h and 38% within 48 h.



Scheme 1. Biotransformation of oxime **1** employing alcohol dehydrogenases.

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Table 1
Transformation of aldoxime **1** (*Z*/*E*=95:5) by various ADH preparations[a]

Entry	ADH	NAD(P)H recycling	*Z*-**1**[k] (%)	*E*-**1**[k] (%)	Aldehyde **3**[k] (%)	Alcohol **4**[k] (%)
1	—[b]	—	95	5	<0.1	<0.1
2	RE-ADH[c]	2-PrOH	95	5	<0.1	<0.1
3	PpADH[d,e]	2-PrOH	58	42	<0.1	<0.1
4	HLADH[f]	Ethanol	84	15	<0.1	1
5	ADH-A[e,g]	2-PrOH	49 (28)[l]	35 (34)[l]	<0.1	16 (38)[l]
6	RasADH[e,h]	GDH[j]	47	46	1	6[m]
7	LK-ADH[i]	GDH[j]	73	24	1	2

[a] Reaction conditions: Tris/HCl buffer (pH 7.5, 50 mM), 1 mM NAD(P)H, 6 g/L substrate, ADH preparation and cofactor recycling systems, 30 °C, 24 h.
[b] Control in the absence of enzyme.
[c] RE-ADH: ADH from *Rhodococcus erythropolis*, NADH.
[d] PpADH: ADH from *Paracoccus pantotrophus* DSM 11072, NADH, see Ref. 8.
[e] Overexpressed in *E. coli*, used as lyophilised *E. coli* powder.
[f] HLADH: horse liver alcohol dehydrogenase, NADH.
[g] ADH-A: ADH from *Rhodococcus ruber* DSM 44541, NADH, see Ref. 9.
[h] RasADH: ADH from *Ralstonia* sp. DSM 6428, NADPH, see Ref. 10.
[i] LK-ADH: ADH from *Lactobacillus kefir*, NADPH.
[j] Glucose dehydrogenase employing glucose.
[k] Measured by GC–MS.
[l] 48 h reaction time.
[m] In the presence of 5% v/v DMSO; without DMSO: 3% alcohol **4**.

2.2. Isomerisation

These unexpected biocatalytic activities—namely oxime isomerisation as well as reduction of the oxime to the alcohol—prompted us to investigate further this system. The chemical mechanism for (acid- and base-catalysed) oxime isomerisation has been elucidated in literature.[11] In general, oximes are stable at neutral pH towards isomerisation and hydrolysis[12] as confirmed in our blank experiment. In order to elucidate whether the observed *E*/*Z*-isomerisation of **1** was a genuine catalytic activity of the ADHs (except RE-ADH) or mediated in a non-specific fashion, a set of well-known protein preparations were tested (Table 2). The results showed that isomerisation of oxime **1** was not specific for alcohol dehydrogenases, since every preparation tested led to isomerisation. Therefore we did not investigate this unspecific reaction in more detail.

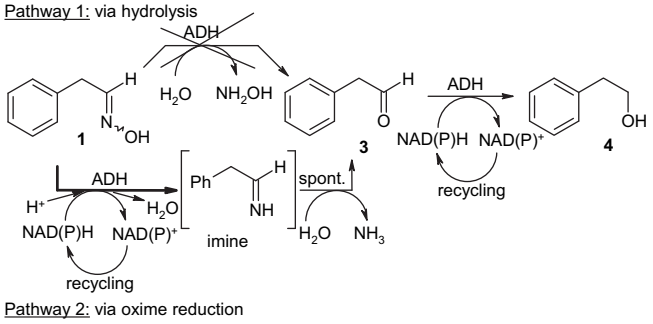
Table 2
E/*Z*-isomerisation of aldoxime **1** (*Z*/*E*=95:5) catalysed by protein preparations[a]

Entry	Protein preparation	*Z*-**1**[c] (%)	*E*-**1**[c] (%)
1	—[b]	95	5
2	Lipase *Aspergillus niger*	90	10
3	Esterase BS3 *Bacillus stearothermophilus*	90	10
4	Bovine serum albumine	76	24
5	CAL-B *Candida antarctica* lipase B	51	49
6	*E. coli*, lyophilised cells	55	45

[a] Reaction conditions: Tris/HCl buffer (pH 7.5, 50 mM), 1 mM NAD(P)H, 6 g/L substrate, crude enzyme preparations, 30 °C, 24 h.
[b] Control: sample without enzymes.
[c] Measured by GC–MS.

2.3. Transformation of oxime to alcohol

Therefore we turned our attention to the ADH-catalysed transformation of the oxime to the alcohol. Alcohol **4** can only be obtained here by reduction of the corresponding aldehyde **3** (Scheme 2), which is also indicated by the detected aldehyde **3** in selected experiments (Table 1, entries 6 and 7). In separate experiments it was proven that the suspected ADH preparations are indeed able to reduce aldehyde **3** to the alcohol **4** in the presence of an NAD(P)H recycling system. As a consequence aldehyde **3** is the obvious precursor for the formation of the alcohol **4** as depicted in Scheme 2. The next question concerns the formation of aldehyde **3**. Aldehyde **3** might be obtained by hydrolysis from aldoxime **1** as generally assumed in literature (Scheme 2, pathway 1).[3b,e,13]



Scheme 2. Pathways for transformation of aldoxime **1** to alcohol **4**.

Hydrolysis might be an unspecific spontaneous reaction, however since the blank experiments (Table 1, entry 1) as well as all other (hydrolytic) enzyme preparations (Table 2) did not lead to hydrolysis of aldoxime **1** to afford the corresponding aldehyde, formation of aldehyde **3** can only be attributed to the activity of the ADHs. In case the ADHs are indeed catalysing this transformation of the oxime via hydrolysis, it can be expected that no reducing equivalents are required: in a test experiment aldoxime **1** was incubated in the presence of various ADHs (ADH-A, PpADH and RasADH) in the absence of NAD(P)H recycling. To our surprise no hydrolysis occurred, thus no aldehyde was formed (neither alcohol, only isomerisation was observed) even if $NAD(P)^+$ was present. As a consequence it was concluded that the ADH in combination with the reduced cofactor might be responsible for the observed transformation. Additional tests showed that NAD(P)H itself is not able to reduce aldoxime **1** spontaneously in the absence of ADH, therefore both ADH and NAD(P)H were required to transform the aldoxime **1** to the aldehyde **3** by *reduction*. The only feasible reduced intermediate one can think of is an imine intermediate, which then spontaneously hydrolyses to yield the aldehyde (Scheme 2, pathway 2).

Attempts to trap the imine intermediate by reduction to the amine employing $NaBH_3CN$, Raney nickel or H_2/Pd/C were not successful; however this was not surprising since additional chemical reduction experiments employing phenylacetaldehyde in the presence of huge excess of ammonia showed that the possible imine intermediate is much too unstable to be trapped at all in water; thus not a trace of amine could be detected when testing the chemical reduction of a possible imine in a mixture of phenylacetaldehyde and ammonia in aqueous solution. This is supported by literature, since no preparative metal-catalysed reductive amination of comparable aldehydes/ketones with ammonia in water at reasonable conditions can be found. Anyway, since aldehyde **3** is an intermediate and since the oxime is reduced (and not hydrolysed), the imine is the only possible unstable intermediate to be expected. Related reductive transformations of oximes to imines have previously been achieved by chemical methods[14] and have been observed by a reconstituted liver microsomal system of a pig liver CYP2D enzyme (NADH-benzamidoxime reductase).[15] Therefore, we propose a reduction pathway via the imine for the ADH-catalysed transformation of oxime **1**. Related observations were reported recently for flavo-proteins for the reduction of aliphatic nitro-compounds leading finally to aldehydes and not to amines or hydroxylamines.[16]

The sequential reduction of the aldehyde to the alcohol was much faster than the aldoxime reduction, since only traces of the aldehyde could be detected in selected cases (LK-ADH and