Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Molecular Catalysis B: Enzymatic 41 (2006) 141–145
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Galactose oxidase and alcohol oxidase: Scope and limitations for the enzymatic synthesis of aldehydes

Arjan Siebum [a,*], Arjan van Wijk [a], Rob Schoevaart [b], Tom Kieboom [a]

[a] *Leiden Institute of Chemistry, Gorlaeus Laboratories, Leiden University, P.O.Box 9502, 2300 RA Leiden, The Netherlands*
[b] *CLEA Technologies BV, Julianalaan 136, 2628 BL, Delft, The Netherlands*

Received 17 February 2006; received in revised form 31 March 2006; accepted 5 April 2006
Available online 7 July 2006

Abstract

The utility of both galactose oxidase and alcohol oxidase for alcohol-to-aldehyde oxidation has been investigated, from a synthetic point of view. The speed of reaction and degree of conversion has been measured for 29 different primary alcohols. The two oxidative enzymes show complementary synthetic use, i.e. galactose oxidase for galactose-derived polyols and alcohol oxidase for aliphatic mono- and diols. Alcohol oxidase has been successfully used in combination with the aldolase DERA in a two-step, one-pot reaction cascade.

Keywords: Reaction cascades; Alcohol oxidase; Galactose oxidase; Biocatalysis; Synthesis

1. Introduction

The current trend in chemistry towards 'green' reactions emphasizes the inherent advantages of catalytic methods. Biocatalytic methods, especially, promise a host of improvements compared to standard organic conversions [1]. Their capacity for highly (stereo-)selective transformations under mild, aqueous conditions, without the need for stoichiometric addition of heavy metal reagents or protection/deprotection schemes is practically unequalled within organic synthesis. With this in mind, we approached the selective conversion of several highly functionalized alcohols into the corresponding aldehydes as this oxidation reaction cannot be easily achieved by clean chemical means. In addition, the aldehydes obtained by enzymatic oxidation are useful as acceptor in subsequent enzymatic aldol reactions in a cascade mode without intermediate recovery steps.

Galactose oxidase (*D. dendroides*, E.C. 1.1.3.9.) and alcohol oxidase (*P. pastoris*, E.C. 1.1.3.13) were the biocatalysts of choice. Both enzymes are oxidases that do not require cofactors in the course of their catalytic cycles, but use molecular oxygen as an electron acceptor in the oxidation of alcohols to aldehydes (Fig. 1). The fact that they do not need stoichiometric addition of a cofactor like NADPH (or the presence of a second enzyme system to regenerate a catalytic amount of cofactor) makes oxidases more suitable for biocatalytic oxidations than the cofactor-dependent dehydrogenases.

Galactose oxidase (GO) is a copper-containing, free-radical catalyst that was first described in 1959 by Cooper et al. [2]. This publication triggered a steady stream of further publications detailing investigations into the enzyme's mechanism [3,4], its structure [5,6] and its range of possible substrates [7,8]. From the outset it was reported that the enzyme could transform a range of primary alcohols into the corresponding aldehydes, though at widely varying velocities.

Alcohol oxidase (AO) is a flavin-dependent, alcohol oxidizing protein originating in methylotropic yeasts and part of the GMC-oxidoreductase superfamily [9]. AO, like GO oxidizes a range of (aliphatic) primary alcohols to the corresponding aldehyde with concomitant production of H_2O_2. AO was earlier reported to have a range of unbranched aliphatic alcohols as its substrate [10–12].

Despite all the work carried out on the GO and AO systems, however, a clear picture on the limits of these systems' reactivities has failed to emerge and some ambiguities concerning the scope of GO especially remain [13]. Therefore, we have investigated the preparative scope and limitations of these enzymes on the basis of 30 different alcohol substrates.

* Corresponding author. Tel. +31 71 5275034; fax: +31 71 5274488.
E-mail address: A.Siebum@chem.leidenuniv.nl (A. Siebum).

1381-1177/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2006.04.003

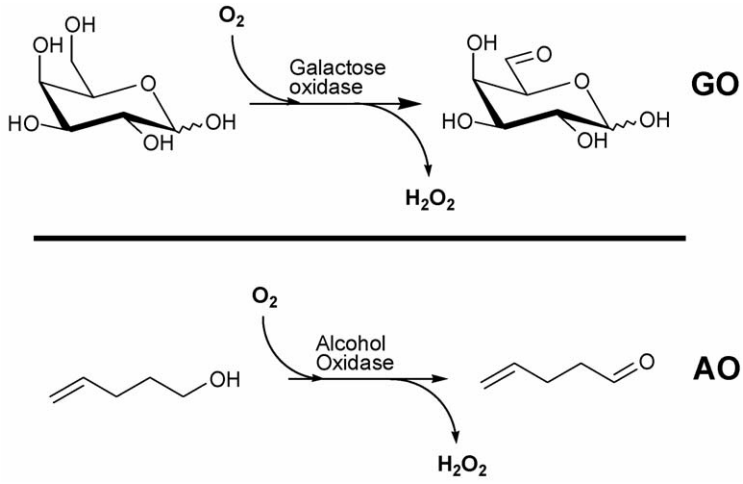

Fig. 1. The galactose oxidase (GO) and alcohol oxidase (AO) catalytic systems.

2. Experimental

^{1}H NMR spectra were recorded with a Jeol FX-200, a Bruker DPX-300 or a Bruker DPX 400 spectrometer, using methanol (CH_3OH: $\delta = 3.3$ ppm) or water (H_2O: $\delta = 4.8$ ppm) as an internal standard. ^{13}C noise-decoupled NMR spectra were recorded with a Jeol FX-200 at 50.1 MHz a Bruker DPX-300 spectrometer at 75.5 MHz and a Bruker DPX-400 at 100.7 MHz, using $CDCl_3$ ($\delta = 77$ ppm), CH_3OH ($\delta = 49$ ppm) or TSP (3-(trimethylsilyl)tetradeuteropropionic acid sodium salt, $\delta = 0$ ppm) as internal standard. All spectra were recorded in 10% D_2O in H_2O, except where noted otherwise. UV–vis-measurements were performed on a Perkin-Elmer Lambda 25 at 425 nm and at a temperature of 21 °C. Spots on thin-layer chromatography were detected with phosphomolybdic acid or $KMnO_4$-spraying. Enantiomeric excesses were determined by ^{1}H NMR using a chiral additive (*R*-(−)-1-phenyl-2,2,2-trifluoroethanol). All commercially available chemicals were purchased from Sigma–Aldrich, Acros or Fluka. All chemicals were used without further purification, unless stated otherwise. Galactose oxidase was a gift from Hercules (Barneveld, The Netherlands) and had the same specificity as the enzyme bought from Sigma. All other enzymes were bought from Sigma or Fluka.

2.1. General synthetic procedure

To 5 mL of a 200 mM solution of the substrate dissolved in 50 mM phosphate buffer 2 mg of catalase (Bovine liver, E.C. 1.11.1.6, 2440 units/mg) was added, followed by 30 units oxidase. The reaction mixture was brought under an oxygen atmosphere using a balloon and gently shaken on a rotatory shaker for 24 h. The conversion was determined by ^{1}H NMR.

2.1.1. GO

To 5 mL of a 200 mM solution of methyl α-D-galacto-pyranoside dissolved in 50 mM phosphate buffer (pH 7), 2 mg of catalase was added followed by 30 units galactose oxidase. The reaction mixture was brought under an oxygen atmosphere using a balloon and gently shaken on a rotatory shaker for 24 h (21 °C, 1 atm O_2). The conversion as determined by integration of the H1/H6 and C1/C6 signals of starting material and product using ^{1}H and ^{13}C NMR proved to be quantitative. Methyl α-D-galacto-hexodialdo-1,5-pyranoside could be isolated as an off-white powder in 95% yield by recrystallizing from 2-propanol/petroleum ether 40–60.

^{13}C NMR (75 MHz, 10% D_2O in H_2O): $\delta = 103.93$, 88.14 (C6, hydrate form), 76.85, 72.79, 70.69, 68.35, 57.20 (OCH_3) ppm.

2.1.2. AO

To 5 mL of a 200 mM solution of 4-penten-1-ol dissolved in 50 mM phosphate buffer (pH 7.4), 10 mg of catalase CLEA [14] (Cross-Linked Enzyme Aggregate) was added followed by 30 units alcohol oxidase. To the reaction mixture 5 mL of toluene was added and the two-phased reaction mixture was brought under an oxygen atmosphere using a balloon and gently shaken on a rotatory shaker for 24 h (21 °C, 1 atm O_2). The conversion was determined by GC, ^{1}H and ^{13}C NMR to be quantitative. The product could be isolated by separation of the two layers and extraction of the water layer with 5 mL diethyl ether. The combined organic layers were dried with $MgSO_4$ and evaporated in vacuo to half the volume, giving a 200 mM solution of 4-pentenal in toluene.

^{1}H NMR: (300 MHz, $CDCl_3$): $\delta = 9.69$ (1 H, t, H-1), 5.73 (1 H, m, H-4), 4.95 (2 H, m, H-5), 2.44 (2 H, m, H-2), 2.31 (2 H, m, H-3) ppm.

2.2. Reaction velocities

A hydrogen peroxide coupled assay similar to the one described in literature, was applied at pH 7 and 21 °C [7]. To a saturated solution of toluidine in 50 mM phosphate buffer (5 mg in 10 mL) was added 10 μL chloroperoxidase (*C. fumago*) obtained from Fluka (22429 units per mL). Subsequently 1 mL of the assay solution and 500 μL of a 150 mM substrate solution in 50 mM phosphate buffer were mixed in a cuvet and 1 unit of GO/AO was added, after which the mixture was rapidly homog-