Available online at www.sciencedirect.com
ScienceDirect
Enzyme and Microbial Technology 42 (2008) 308–314
ENZYME AND MICROBIAL TECHNOLOGY
www.elsevier.com/locate/emt

# Reduction of aliphatic nitro groups using an obligately anaerobic whole cell biocatalyst

Hassan Korbekandi, Paul Mather, John Gardiner, Gill Stephens *

*Manchester Interdisciplinary Biocentre, University of Manchester, 131 Princess Street, Manchester M1 7DN, UK*

Received 30 May 2007; received in revised form 11 October 2007; accepted 12 October 2007

**Abstract**

*Peptostreptococcus productus* U-1 was grown in a fructose-limited chemostat with caffeate ($D = 0.19\,h^{-1}$), and washed, harvested cells were used to reduce (*E*)-2-nitro-1-phenyl-1-propene **1a** and (*E*)-2-nitro-1-phenyl-1-butene **1b** in a two-liquid phase reaction system. The aminoalkanes **3a** and **3b** were formed in 47% and 7.5% yield, respectively, by reduction of both the aliphatic nitro group and the C–C double bond. Direct reduction of a nitroalkane was also demonstrated using synthetic, racemic **2a**, which was reduced to **3a** in 45% yield. Optimum activity was obtained with **1a** provided at 2.5 mM (0.25 mmol and 0.041 g) at a scale of 100 mL, at pH 7.0. The reaction was complete in 24 h using only 0.66 $g_{dry\ weight\ biocatalyst}\ L^{-1}$, and the rate of aliphatic nitro reduction was up to 154 mmol (25.1 g) $(kg_{dry\ weight}\ h)^{-1}$.


*Keywords:* Aliphatic nitro reduction; Anaerobe; Biotransformation; Nitroalkene reduction; *Peptostreptococcus productus*

## 1. Introduction

Many pharmaceuticals are derived from amine intermediates. However, there are only a relatively small number of chemical routes to homochiral and complex amines with the required selectivity, scalability and environmental acceptability for large-scale manufacturing [1–10]. For this reason, a variety of biocatalytic routes have also been developed. Several biocatalytic kinetic resolutions are available [11–14], but suffer from the disadvantage that costs are high unless the residual starting material can be racemised and recycled. Recently, deracemisations and dynamic kinetic resolutions have been developed to overcome this problem, using amino acid oxidases, lipases or hydantoinases [15–20]. Even so, products are still restricted mainly to amino acids and some primary amines. There have been some real successes in expanding substrate ranges by directed evolution [21,22], but there are still many inaccessible products. A few secondary amines can be produced by biocatalytic desymmetrization, but these are restricted to a very small substrate class (e.g. prochiral propane diols) [23]. Asymmetric routes are also available. Here, the substrate range is mainly limited to α-amino acids [24,25], although the reduction of oximes with Baker's yeast affords secondary amines in modest e.e. [26]. Therefore, it would be extremely useful to develop complementary biocatalytic routes for synthesis of a wider range of amines.

In principle, chemoselective reduction of aliphatic nitro groups should provide a versatile route to numerous chiral and achiral targets. In practice, this is problematic, due to the requirement for forcing conditions employing very strong reducing agents (e.g. lithium aluminium hydride or hydrogenation), which precludes inclusion of various other reduction-sensitive functionality in substrates. As a result, there is considerable interest in developing mild, chemoselective and biocatalytic routes as alternatives [25,27–29]. Reduction of aromatic nitro groups to amine has been demonstrated using a number of whole cells and enzymes [27,28,30–39]. In contrast, biocatalytic reduction of aliphatic nitro groups is extremely rare [40–42]. Furthermore, the existing biocatalysts exhibit very poor rates and yields [40,41] or they have restricted substrate ranges and are unstable [42]. Therefore, better biocatalysts are needed for aliphatic nitro reduction.

We wish to report the discovery of an efficient, aliphatic nitro reduction using whole cells of *Peptostreptococcus productus* U-1 (Fig. 1). This discovery was somewhat serendipitous, since we discovered the reaction whilst screening anaerobic bacteria for asymmetric hydrogenation of nitroalkenes **1**, building on earlier observations that these organisms are excellent biocatalysts for

* Corresponding author. Tel.: +44 161 306 4377; fax: +44 161 306 4399.
*E-mail address:* gill.stephens@manchester.ac.uk (G. Stephens).

0141-0229/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.enzmictec.2007.10.009

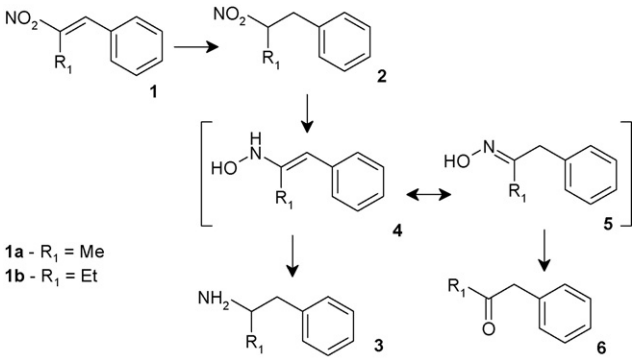


Fig. 1. Proposed reaction scheme for nitroalkene reduction using *P. productus*.

reducing C–C double bonds [43,44]. We included the homoacetogenic anaerobe, *P. productus* U-1 [45] in the screen because it can hydrogenate a range of cinnamic acid derivatives [46,47]. We found that *P. productus* U-1 reduced the C–C double bond as expected, but, most significantly, it also reduced the aliphatic nitro group with excellent efficiency. This afforded chiral aminoalkanes from nitroalkenes in one step.

## 2. Materials and methods

### 2.1. Microorganism and growth

*P. productus* strain U-1 (DSM 3507) was grown in an anaerobic cabinet (Don Whitley Scientific Mark III) at 30 °C. The medium was sterilized and made anaerobic as described previously [48] except that $NaHCO_3$ solution was degassed with oxygen-free $CO_2$. The basal medium (924 mL and pH 7.0) contained: $KH_2PO_4$ (25 mg), $K_2HPO_4$ (400 mg), $NH_4Cl$ (3 g), resazurin (0.1 mg) and yeast extract (Oxoid, 2 g). Agar (10 g) was also added for preparation of stock cultures. After sterilization, the following sterile, anaerobic solutions were added: $MgSO_4{\cdot}7H_2O$ (0.4 g in 20 mL), $NaHCO_3$ (2 g in 20 mL), fructose (2.52 g in 10 mL), selenite-tungstate solution (2 mL), cysteine (250 mg $L^{-1}$ and 2 mL), $Na_2S$ (250 mg $L^{-1}$ and 2 mL) and trace elements (10 mL) and vitamins (10 mL) [49], but with 0.024 g $L^{-1}$ $NiCl_2{\cdot}6H_2O$ and 0.01 g $L^{-1}$ NaOH and without $MgSO_4{\cdot}7H_2O$, and with only 0.01 mg $L^{-1}$ cyanocobalamin, respectively. Selenite-tungstate solution was prepared from $H_2SeO_4$ (250 mg $L^{-1}$ and 2 mL) and $Na_2WO_4{\cdot}2H_2O$ (50 mg $L^{-1}$ and 2 mL).

The growth of *P. productus* in batch cultures is extremely variable, so we grew the organism in fructose-limited chemostat culture [43,50] in the medium described above, but containing 15 g $L^{-1}$ fructose and 1 mM caffeate [48], and without $Na_2S$ or $NaHCO_3$, at pH 7.0. The culture was mixed at 400 rpm and sparged (1.5 mL $min^{-1}$) with $N_2$ (80%) and $CO_2$ (20%). The dilution rate was 0.19 $h^{-1}$. The inoculum (60 mL) had been transferred twice in the same medium with fructose and caffeate since adaptation to caffeate is necessary, and steady state was reached after 20 volume changes.

### 2.2. Cell harvesting and biotransformations

Cells (approximately 350 mL) were harvested and washed [48] using 0.1 M potassium phosphate buffer pH 7.0, and the cells were suspended in 21 mL of buffer to prepare the cell suspension used for biotransformations. Reactions were started by adding an aliquot of the harvested cell suspensions to the reaction mixtures (to final biomass concentrations stated in the text) and stirred using a magnetic stirrer bar at 400 rpm. Reaction mixtures [48] contained 0.1 M potassium phosphate pH 7 and fructose (56 mM) in a volume of 100 mL for reduction of (*E*)-2-nitro-1-phenyl-1-propene **1a** and 12 mL for reduction of (*E*)-2-nitro-1-phenyl-1-butene (**1b**) and 2-nitro-1-phenylpropane (**2a**). The biotransformations were done in a two-liquid phase reaction system by dissolving the substrates in anaerobic tetradecane [48], and the phase ratio was 20% (v/v) in all cases. The initial concentration of nitroalkenes **1a** and **1b** was 2.5 mM across the combined tetradecane and aqueous phases, except for the experiments presented in Table 1, and nitroalkane **2a** was added to 1.1 mM. A constant phase ratio was maintained during the reaction by sampling both the organic and aqueous phase in a volume ratio of 1:4. The toxicity of nitroalkenes was studied by testing for inhibition of caffeate reduction, and caffeate (final concentration 1 mM) was dissolved in water as described above.

### 2.3. Analytical methods

Biomass and caffeate concentrations were determined as described previously [48], and an OD value of 1 was equivalent to a dry weight of 0.21 g $L^{-1}$. Samples from biotransformations were prepared and the tetradecane phase analysed by GCMS as described previously [48]. The aqueous phase was also analysed, since the products were distributed between both phases. The aqueous samples were adjusted to pH 11.0 with KOH (0.1 M), NaCl (0.2 g) was added, the samples centrifuged and the supernatant (3 mL) extracted three times with ethyl acetate (3 mL). The extracts were combined, dried and dissolved in ethyl acetate (0.5 mL), and 0.25 μL aliquots were analysed by GCMS as described above. **1a**, **1b**, **2a**, **2b** and **5b** were partitioned preferentially into the tetradecane phase, whereas **3a**, **5a**, **6a** and **3b** were found only in the aqueous phase. Biotransformations were monitored routinely by HPLC analysis using a Waters 990 HPLC equipped with photodiode array detector. The nitroalkenes and nitroalkanes **1a**, **1b** and **2a** were found in the tetradecane phase, and were analysed by mixing samples of the tetradecane phase with an equal volume of the mobile phase before analysis of a 20 μL sample on a Chiralcel® OJ column (Daicel, 250 mm × 4.6 mm) with hexane/2-propanol (9:1 v/v and 0.5 mL $min^{-1}$) as the mobile phase. Products were detected at 254 nm. Amphetamine **3a** was found in the aqueous phase, and concentrations and enantiopurity were determined after derivatizing samples of the aqueous phase [51] except that 0.1 M $Na_2CO_3/NaHCO_3$ buffer pH 11.0 (6 mL) was added to thawed aqueous samples (0.5 mL), and the mixture was extracted three times with hexane–ethyl acetate (9:1 v/v and 10 mL). The residue was dissolved in hexane/2-propanol (9:1 v/v and 500 μL) and samples (20 μL) were analysed on tandem Chiralcel® OJ and Chiralcel® OB (Daicel, 500 mm × 4.6 mm) columns at 25 °C with hexane/2-propanol (9:1 v/v and 1 mL $min^{-1}$) as the mobile phase. Products were detected

Table 1
Effect of substrate concentration on reduction of 2-nitro-1-phenyl-1-propene

| Substrate concentration (mM) | Substrate amount (mg) | Substrate consumed (mM) | % added substrate consumed | **2a** | | **3a** | |
|---|---|---|---|---|---|---|---|
| | | | | Yield isolated[a] (%) | e.e. (%) | Yield isolated[a] (%) | e.e. (%) |
| 2.4 | 39.1 | 2.09 | 87 | 38 | 2.1 | 47 | 1.2 |
| 5.1 | 83.2 | 2.65 | 52 | 11 | 48.2 | 5 | 19 |
| 8.5 | 139 | 3.49 | 41 | 6 | 19.1 | 0 | NA |
| 12.0 | 196 | 3.84 | 32 | 2 | 16.3 | 0 | NA |

Harvested cells (0.18 $g_{dry\ weight\ biocatalyst}$ $L^{-1}$) were tested for reduction of **1a** at various substrate concentrations at 100 mL scale. Substrate and product concentrations and enantiopurities were determined by HPLC after 14 h. NA, not applicable.

[a] Determined by HPLC.