Process Biochemistry 50 (2015) 1788–1792

Contents lists available at ScienceDirect

Process Biochemistry

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

Short communication

Baker's yeast-mediated asymmetric reduction of ethyl 3-oxobutanoate in deep eutectic solvents

Marina Cvjetko Bubalo[a], Marcelina Mazur[b], Kristina Radošević[a], Ivana Radojčić Redovniković[a,*]

[a] Faculty of Food Technology and Biotechnology, University of Zagreb, Pierotijeva 6, 10000 Zagreb, Croatia
[b] Department of Chemistry, Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland

ARTICLE INFO

Article history:
Received 18 June 2015
Received in revised form 23 July 2015
Accepted 24 July 2015
Available online 26 July 2015

Keywords:
Bioreduction
Deep eutectic solvents
Ethyl 3-hydroxybutanoate
Ethyl 3-oxobutanoate
Saccharomyces cerevisiae

ABSTRACT

Cholinium-based deep eutectic solvents (DES) were evaluated as solvents for yeast-mediated reduction of ethyl 3-oxobutanoate to ethyl 3-hydroxybutanoate. The type of hydrogen bond donor and the amount of water present in DES considerably influenced the reaction yield. At water content of 50% (w/w), sugar and alcohol containing DES were the most appropriate solvents for the given reduction, with yields similar to those obtained in phosphate buffer (<93.0%), while in DES based on acids or amide poor yields were observed regardless water content (<49.3%). The results suggest that pH value of reactiom medium and cell viability are the curtail variables influencing bioreduction yield. Furthermore, by changing the water content in DES and/or DES itself it is possibly to alter enantioselectivity. Overall, the results suggest that sugar containing DES possess good biocompatibility with yeast cells and can be applied as green reaction medium for yeast-mediated bioreduction.

1. Introduction

Baker's yeast, *Saccharomyces cerevisiae*, is an ideal biocatalyst for industrial application since it is cheap, readily available and well-studied microorganism, also non-pathogenic (GRAS status) and easy to cultivate in non-sterile environment [1]. This microorganism has a high catalytic capacity as a redox biocatalyst and is especially important in enantioselective reductions of carbonyl group to generate the stereogenic center. In the contrary to the use of pure redox enzyme, no cofactors addition or system for cofactors regeneration is required. Among prochiral substrates, 3-oxo esters are of special interest since asymmetric reduction of the latter gives corresponding 3-hydroxy esters, chiral products that are used as building blocks of fine chemicals, including pharmaceuticals, flavours, and fragrances [2]. The ethyl 3-oxobutanoate is often the model compound for investigation of the microbial reduction process. In the case of stereoselective reduction of carbonyl group the (*S*)-ethyl 3-hydroxybutanoate or the corresponding *R*-enantiomer can be obtained as a product. First compound has a great meaning as a key chiral intermediate for the synthesis of lavandulol, sulcatol and prenophorin, while *R*-enantiomer is versatile intermediate in the synthesis of β-lactamase inhibitors or ±decarestrictine [3,4].

Biocatalytic reactions are traditionally carried out in various solvents to bring reactants and catalysts together to deliver mass, heat, and momentum. However, solvents are accountable for a large part of the waste and pollution generated by chemical processes [5]. Therefore, the choice of a reaction solvent not only depends on its chemical, and physical properties, but also on its environmental impact (e.g. ecotoxicity and biodegradability), sustainability (possibility of recycle and reuse) and process safety (e.g. flammability and volatility). Over the past decade various green alternative solvents have been proposed among which super- and subcritical fluids, fluorinated solvents, ionic liquids, glycerol-derived solvents and deep eutectic solvents stand out as the most promising ones [6]. Deep eutectic solvents (DES) are the youngest class among these solvents, but their green properties such as non-volatility, non-flammability and stability, together with low ecological footprint owning to natural raw material used for their preparation, makes them almost ideal solvents for organic synthesis. By definition, DES are mixtures of nontoxic quaternary ammonium salts (e.g. choline chloride) and a naturally-derived uncharged hydrogen bond donor (e.g. amines, sugars, alcohols and carboxylic acids) with melting point much lower than starting materials. These solvents are also easily prepared by totally green procedures (solvent-free, atom economy 100%, cheap components) [7].

* Corresponding author.
E-mail address: iradojci@pbf.hr (I. Radojčić Redovniković).

http://dx.doi.org/10.1016/j.procbio.2015.07.015
1359-5113/

Table 1
List of the DES used in this study.

Combination	Abbreviation	Components ratio
Choline chloride:glycerol	ChCl:Gly	1:2[a]
Choline chloride:ethylene glycol	ChCl:EG	1:2[a]
Choline chloride:oxalic acid	ChCl:OA	1:1[a]
Choline chloride:malic acid	ChCl:Ma	1:1[a]
Choline chloride:glucose	ChCl:Glc	2:1[a]
Choline chloride:fructose	ChCl:Fru	3:2[b]
Choline chloride:xylose	ChCl:Xyl	2:1 [a]
Choline chloride:urea	ChCl:U	1:2[a]

[a] Molar ratio.
[b] Weight ratio.

Since DES are relatively new solvents, the literature regarding their potential in biocatalysis is scarce, especially for systems that use whole cells as catalyst [8]. To explore the possibility of DES implementation into baker's yeast enantioselective reductions of prochiral compounds, the reduction of ethyl 3-oxobutanoate to ethyl 3-hydroxybutanoate in various cholinium-based DES was herein studied.

2. Methods

2.1. Biological and chemical materials

Ethyl 3-oxobutanoate, racemic ethyl3-hydroxybutyrate and all chemicals for DES syntheses (choline chloride, glucose, fructose, xylose, glycerol, ethylene glycol, oxalic acid, malic acid and urea) and analytics were purchased from Sigma–Aldrich, Germany (purity of ≥99%), and used without further purification. Organic solvents used were of analytical grade and supplied from Merck, Germany. Lyophilised baker's yeast was purchased from KVASAC D.O.O., Croatia.

2.2. Synthesis of DES

Choline chloride (ChCl) and hydrogen bond donor (HBD) were dried in the vacuum concentrator (Savant SPD131DDA SpeedVac Concentrator, Thermo scientific, USA) at 60 °C for 24 h before use. The ChCl and HBD in certain molar ratio (Table 1) were directly weighed and the mixture was stirred in the sealed flask at 80 °C for 2–6 h until a homogeneous transparent colourless liquid was formed. The water content of the synthesised DES was determined by Karl Fischer titration (Metrohm, 787KF Titrino, Switzerland) at 20 °C, and was found to be <1% (w/w). The solutions of DES in water were prepared from the starting solvent by adding the right amount of water in certain weight ratio. Additionally, the pH values of DES aqueous solutions (DES containing 10-90% of water, w/w), were measured by digital pH meter (Mettler Toledo, Switzerland).

2.3. Bioreduction reaction

Lyophilised baker's yeast was firstly washed with potassium phosphate buffer (100 mM, pH 7.4) and cells were centrifuged at 5000 *g* for 15 min. To 0.6 g of wet yeast 2 mL of appropriate solvent (buffer or DES) was added. Finally, substrate ethyl 3-oxobutanoate (20 μL) was added and the mixture was stirred at room temperature at 200 rpm. After 24 h the reaction was stopped by centrifuging the mixture and supernatant was extracted with diethyl ether (3 × 2 mL). The organic phases were collected, and diethyl ether was evaporated under reduced pressure, yielding crude products. All experiment was repeated at least three times.

2.4. Analytical methods

To determine reaction yield, crude products was resuspended with 1 mL dichloromethane and analysed by a gas chromatograph equipped with a Rxi_5Si/MS column (30 m × 0.25 mm i.d. × 0.25 mm) with electrospray ionization mass spectrometry (Shimadzu, Japan). Helium was used as a carrier gas at a flow rate of 155.4 mL min^{-1}. The temperature of the oven at the injection was 60 °C and was kept constant during whole analysis time (6 min). Injector and detector temperatures were set at 220 °C. The ionization of the samples was achieved at 70 eV using the SCAN mode (ion source temperature 200 °C; interface temperature 250 °C). Quantification of data was done by the calibration with standard samples.

Enantiomeric excess of ethyl 3-hydroxybutyrate was determined as follows.The crude products were dissolved in 2 mL of dry diethyl ether and 0.2 mL of dry pyridine. The reaction was stirred at room temperature on magnetic stirrer and 0.1 mL of propionyl chloride was slowly added. Then the reaction was continued at room temperature for 12 h. When the substrate reacted completely, the mixture was acidified by 2 mL of 0.1 M HCl and the products were extracted with diethyl ether (3 × 2 mL). Combined organic layers were washed with saturated $NaHCO_3$, brine (until neutral) and dried over anhydrous $MgSO_4$. Chiral gas chromatography analysis was performed on Agilent Technologies 6890N instrument equipped with autosampler and FID detector. The enantiomeric excesses were determined by using Varian CP Chirasil-DEX CB column (25 m × 0.25 mm × 0.25 μm) with following temperature programs: injector (250 °C), detector (250 °C), column temperature: 50 °C, 30 min, 50–80 °C (0.5 °C min^{-1}), 80–200 °C (20 °C min^{-1}), 200 °C (9 min).

2.5. Viability of yeast within DES

Impact of DES on yeast viability was estimated by setting-up similar experiment as for bioreduction reaction (inoculation of 0.6 g of wet yeast in 2 mL of buffer or DES containing 50% of water (w/w) without addition of substrate). Yeast viability was determined 3 and 24 h after inoculation. Briefly, the cell suspension was mixed with an equal volume of methylene blue and incubated for 5 min at room temperature. Blue-coloured cells were visualised and counted as dead cells [9]. Up to 500 cells were counted for each sample in individual experiment, and every experiment was repeated at least three times.

3. Results and discussion

Several cholinium-based deep eutectic solvents (DES) containing sugar, alcohol, organic acid or amide as hydrogen bond donor (HBD) (Table 1) were assayed for bioreduction of ethyl 3-oxobutanoate to ethyl 3-hydroxybutyratewith baker's yeast *S. cerevisiae*. The reaction was chosen as a bench reaction for yeast-mediated preparation of β-hydroxy esters, valuable chiral synthons for preparation of industrially important chemicals [1]. Bearing in mind that enzymes need a certain amount of water for their activity [8], DES containing at least 10% of water (10–50%, w/w) were initially prepared as a media for the proposed reaction. Addition of water also allows coping with the noticeable mass transfer problem caused by the relatively high viscosity of DES. A reaction was also conducted in phosphate buffer as reference solvent. Yields obtained in different solvents are summarized in Fig. 1. As can be observed, the type of HBD present in DES dramatically influenced the reaction yield. For the aqueous solutions of DES (50% of water, w/w) containing sugars (glucose, fructose, and xylose) or glycerol as the HBD the highest reactions yield, similar to those obtained in phosphate