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Adzuki bean: A new resource of biocatalyst for asymmetric reduction of aromatic ketones with high stereoselectivity and substrate tolerance

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ABSTRACT

A new resource of biocatalyst for asymmetric reduction of aromatic ketones has been discovered for the first time from a common plant seed, adzuki bean, i.e. *Phaseolus angularis* (Willd.) W.F. Wight. The study investigated the best methods to prepare the biocatalyst and its ability to reduce ketones. Our results indicated that the biocatalyst from adzuki bean could reduce various aromatic ketones at relatively high concentrations (*e.g.* 100 mM), exhibiting excellent stereoselectivity (>98% e.e.). In addition, it was found that NADPH acts as the reducing cofactor, which can be regenerated by the crude enzyme system itself using glucose as an auxiliary substrate.

1. Introduction

Chirality is one of the most important symbols of nature and living organisms (Wu and Xiao, 2007). Recently, single enantiomers are widely used in pharmaceuticals, flavour, agricultural chemicals and speciality materials. The active "–OH" at chiral center of chiral alcohols makes them key intermediates for synthesis of optically active products. Biocatalysts can be used in both simple and complex transformations without the need for tedious steps. Such high selectivity also affords efficient reactions with few by-products, thereby making them an environmentally benign alternative to conventional chemical catalysts. Among all the methodologies of biocatalysis to obtain chiral alcohols, asymmetric bioreduction of prochiral ketones is preferred because it can give 100% theoretical yield. The use of biocatalysis for industrial synthesis of chemicals increases rapidly (Nakamura et al., 1996; Schmid et al., 2001; Soni et al., 2007; Yazbeck et al., 2004).

Plant cell cultures represent a unique class of potential biocatalysts for the transformations of synthetically important foreign substrates (Baskar et al., 2004; Kumaraswamy and Ramesh, 2003; Nagaoka, 2004; Utsukihara et al., 2006; Yadav et al., 2001). Compared to other biocatalysts, plants have many merits, e.g. they do not make microbe contaminations, may be edible and are therefore more suitable for being used in food or pharmaceuticals industry. Bioreductions mediated by growing or immobilized plant cell cultures have been known for several decades (Blanchard and Van de Weghe, 2006; Ishihara et al., 2003). But there are still many problems for the use of plants as well as other microbial catalysts in actual production, such as low substrate tolerance and expensive cofactor regeneration etc. In addition, the plant cell cultures are difficult to perform cloning and protein engineering, so often have to make due with wildtype. This work aimed to address these issues by using a newly discovered biocatalyst from adzuki bean or *Phaseolus angularis* (Willd.) W.F. Wight, which is an annual vine widely grown throughout East Asia and the Himalayas for its small (approximately 5 mm) bean.

2. Methods

2.1. Typical procedure for preparing the acetone powder of crude enzyme

Adzuki beans were purchased from the local supermarket at ca. 0.6 US$ for 1 kg. Using a coffee bean grinder to grind adzuki beans to small particulates, and then these particulates were soaked in water (pH 6–7, the mass ratio of particulate to water was 1:5) for 5–6 h at room temperature with gentle agitation. The supernatant after soaking was centrifuged with 8000*g* for 20 min and then filtrated using four layers of gauzes. The supernatant after filtration was slowly mixed with chilled acetone (−20 °C, v:v = 1:1) on a magnetic stirrer. The suspension was centrifuged again at 8000*g* for 20 min, and the precipitation was collected and stored in 4 °C

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for acetone to volatilize. A dry crude enzyme powder could also be obtained by lyophilization and stored at 4 °C.

2.2. General procedure for performing the bioreduction

Fifty micrograms of the crude enzyme powder were suspended in 0.5 mL of potassium phosphate buffer solution (PBS, pH 7.0, 100 mM) in an Eppendorf tube (1.5 mL). Different substrates at a certain concentration (10, 50 or 100 mM) were added into the system with a cosolvent of ethanol or DMSO (5%). Reactions were carried out in Eppendorf shaker (1,100 rpm) at 30 °C for certain periods (1–72 h).

Centrifugation and extraction were used to stop reactions. Two times volume of ethyl acetate (EtOAc) were mixed adequately with reaction solutions, and then the mixtures were centrifuged at 12000*g* for 5 min. The organic layer was dried over anhydrous sodium sulfate overnight before GC analysis.

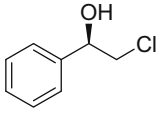
The substrate conversion and enantiomeric excess (e.e.) of product were detected by chiral GC (SUPCOL DEX β-120) analysis, and the absolute configuration was identified by comparing the GC retention times with those of standard samples.

2.3. Bioreductions at mini-preparation scale

To a suspension of about 1 g powder of the crude enzyme in 10 mL of PBS (100 mM, pH 7.0), was added 2-chloro-1-phenylethanone (**1a**, 0.155 g, 1.0 mmol) or 2-bromo-1-phenylethanone (**3a**, 0.199 g, 1.0 mmol) dissolved in 0.5 mL cosolvent of ethanol. A double amount (2 g) of the crude enzyme powder was used for reducing phenylethanone (**2a**, 0.120 g, 1.0 mmol), 1-(4′-nitrophenyl)ethanone (**4a**, 0.165 g, 1.0 mmol) and 1-(pyridin-4′-yl)ethanone (**5a**, 0.121 g, 1.0 mmol). The reaction mixtures were incubated in an orbital shaker (160 rpm) at 30 °C for a certain period necessary to obtain an appropriate conversion. After centrifugation at 8000*g* for 10 min, the supernatant was saturated with NaCl and then extracted with EtOAc (10 ml × 3). The yield and e.e. of products were determined by GC analysis. The organic solution was dried over anhydrous Na_2SO_4 and evaporated under vacuum. The resultant crude product was purified by silica gel column chromatography (eluent: petroleum ether/ethyl acetate, 2:1–10:1, v/v), followed by evaporation under vacuum. The structure of product was confirmed by ^{1}H NMR analysis. The absolute configuration was determined by comparing the specific rotation with the literature values or by comparing the retention times on chiral GC with those of authentic samples.

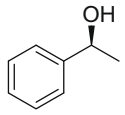
2.4. Spectral and rotation data for the biosynthesized compounds

(*R*)-2-Chloro-1-phenylethanol (1b):



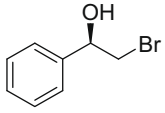
^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 2.73 (d, J = 3.3 Hz, 1 H, O*H*), 3.64 (dd, J = 11.4, 9.0 Hz, 1H, CH_2), 3.74 (dd, J = 11.4, 3.6 Hz, 1H, CH_2), 4.90 (dt, J = 9.0, 3.3 Hz, 1H, C*H*), 7.25–7.39 (m, 5H, Ar*H*); α_D^{21} −58.3 (c 1.36, $CHCl_3$) {lit. (Ema et al., 2001) α_D^{24} −20.8 (c 1.89, $CHCl_3$), 88% ee, *R*}; 99.3% ee; GC analysis: Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm; oven temperature 150 °C, injector temperature 280 °C, detector temperature 350 °C; carrier gas, nitrogen (0.1 MPa); retention times: t_R (*R*) 15.2 min, t_R (*S*) 14.6 min.

(*S*)-Phenylethanol (2b):



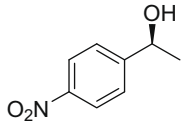
^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.49 (d, J = 6.3 Hz, 3H, CH_3), 1.96 (s, 1H, O*H*), 4.89 (q, J = 6.5 Hz, 1H, C*H*), 7.25–7.38 (m, 5H, Ar*H*); α_D^{21} −58.6 (c 1.00, $CHCl_3$) {lit.(Nagaoka, 2004) α_D^{25} −55.1 (c 1.63, $CHCl_3$), *S*}; 98.6% ee; GC analysis: Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm; oven temperature 130 °C, injector temperature 280 °C, detector temperature 350 °C; carrier gas, nitrogen (0.1 MPa); retention times: t_R (*R*) 9.0 min, t_R (*S*) 9.4 min.

(*R*)-2-Bromo-1-phenylethanol (3b):



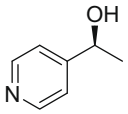
^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 2.69 (d, J = 3.0 Hz, 1H, O*H*), 3.54 (dd, J = 10.2, 8.7 Hz, 1H, CH_2), 3.64 (dd, J = 10.2, 3.0 Hz, 1H, CH_2), 4.93 (dt, J = 9.3, 3.0 Hz, 1H, C*H*), 7.33–7.39 (m, 5H, Ar*H*); α_D^{21} −41.5 (c 1.00, $CHCl_3$) {lit. (Gilmore et al., 2004) $[\alpha]_D$ −30.9 (c 1.00, $CHCl_3$), 79% ee, *R*}; 99.6% ee; GC analysis: Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm; oven temperature 150 °C, injector temperature 280 °C, detector temperature 350 °C; carrier gas, nitrogen (0.1 MPa); retention times: t_R (*R*) 23.7 min, t_R (*S*) 22.9 min.

(*S*)-1-(4′-Nitrophenyl)ethanol (4b):



^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.52 (d, J = 6.3 Hz, 3H, CH_3), 5.03 (q, J = 6.3 Hz, 1H, C*H*), 7.56 (d, J = 8.7 Hz, 2H, Ar*H*), 8.21 (d, J = 8.7 Hz, 2H, Ar*H*); α_D^{22} −27.5 (c 1.00, C_2H_5OH) {lit.(Homann et al., 2004) α_D^{25} −29.7 (c 2.59, C_2H_5OH), *S*}; >99.9% ee; GC analysis: Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm; oven temperature 190 °C, inject temperature 280 °C, detector temperature 350 °C; carrier gas: nitrogen (0.1 MPa); retention times: t_R (*R*) 14.4 min, t_R (*S*) 14.8 min.

(*S*)-1-(Pyridin-4′-yl)ethanol (5b):



^{1}H NMR (300 MHz, $CDCl_3$, TMS) δ 1.47 (d, J = 6.6 Hz, 3H, CH_3), 4.89 (q, J = 6.6 Hz, 1H, C*H*), 7.30 (d, J = 4.2 Hz, 2H, Ar*H*), 8.40 (d, J = 4.2 Hz, 2H, Ar*H*); α_D^{22} −44.8 (c 1.32, C_2H_5OH) {lit.(Ohkuma et al., 2000) α_D^{26} −54.9 (c 1.02, C_2H_5OH), 99.8% ee, *S*}; >99.9% ee; GC analysis: Supelco β-DEX 120 column, 30 m × 0.25 mm × 0.25 μm; oven temperature 140 °C, injector temperature 280 °C, detector temperature 350 °C; carrier gas, nitrogen (0.1 MPa); retention times: t_R (*R*) 15.2 min, t_R (*S*) 15.8 min.