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Identification of a marine NADPH-dependent aldehyde reductase for chemoselective reduction of aldehydes

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ABSTRACT

A putative aldehyde reductase gene from *Oceanospirillum* sp. MED92 was overexpressed in *Escherichia coli*. The recombinant protein (OsAR) was characterized as a monomeric NADPH-dependent aldehyde reductase. The kinetic parameters K_m and k_{cat} of OsAR were 0.89 ± 0.08 mM and $11.07 \pm 0.99\ s^{-1}$ for benzaldehyde, 0.04 ± 0.01 mM and $6.05 \pm 1.56\ s^{-1}$ for NADPH, respectively. This enzyme exhibited high activity toward a variety of aromatic and aliphatic aldehydes, but no activity toward ketones. As such, it catalyzed the chemoselective reduction of aldehydes in the presence of ketones, as demonstrated by the reduction of 4-acetylbenzaldehyde or the mixture of hexanal and 2-nonanone, showing the application potential of this marine enzyme in such selective reduction of synthetic importance.

1. Introduction

The reduction of carbonyl compounds to alcohols is one of the most important reactions in synthetic organic chemistry. Although many methods are available for the reaction, few are chemoselective for the reduction of aldehydes in the presence of ketones and such methods are still in demand [1–4]. Enzymatic reduction offers an attractive approach because the enzymes usually display a better selectivity and the biocatalytic reaction conditions are considered to be more environmentally benign. However, there are only a few examples where biocatalysts have been exploited to the chemoselective reduction of aldehydes in the presence of ketones and other functional groups [5–7]. On the other hand, the benzylic alcohols are key starting materials in the synthesis of scented substances for cosmetics, fragrances and flavor industry, which in general are produced from the less expensive aldehydes. As such, aldehyde reductases have been searched with aim to develop biocatalytic methods for chemoselective reduction of aldehydes.

The aldo–keto reductases (AKR) are a superfamily of enzymes that includes a number of related NAD(P)H-dependent oxidoreductases, such as aldehyde reductase, aldose reductase. The AKR enzymes share a common $(\alpha/\beta)_8$ structure, and conserved catalytic mechanism, although there is considerable variation in the substrate-binding pocket [8,9]. These enzymes metabolize a diverse range of substrates, including aliphatic and aromatic aldehydes, monosaccharides, steroids, prostaglandins [8,10–12]. A NADPH-dependent human liver aldehyde reductase (HlAR) was extracted from human liver and the enzyme catalyzed the reduction of various aldehydes [13,14]. HlAR was thus used as the template in genome mining to seek for effective aldehyde reductase in AKR superfamily. Since the enzymes originated from the highly diverse marine microorganisms offered a great source of biocatalysts for organic synthesis [15–17], a putative aldehyde reductase (OsAR) from *Oceanospirillum* sp. MED92 [18], which had 41.64%, 35.82%, 15.34% and 13.69% identity with HlAR [14], *Sporobolomyces salmonicolor* aldehyde reductase (SsAR) [19], *Vigna* radiata aldehyde reductase (VrAR) [20] and aflatoxin-metabolizing aldehyde reductase (AfAR) [21], was selected as the target enzyme. Herein we report the biochemical characterization and substrate profile of the recombinant OsAR enzyme. The results showed that OsAR was an aldehyde specific reductase, and catalyzed the chemoselective reduction of aldehydes in the presence of ketones.

2. Materials and methods

2.1. Materials

FastDigest Restriction Enzymes were purchased from Fermentas, St. Leon Roth, Germany. Vector pET21a(+) was purchased

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Table 1
Purification parameters of QsAR.

	Specific activity (U mg^{-1})	Total activity (U)	Purification factor	Yield (%)
Cell-free extract	1.8	200	1	100
Ni column	16	172	8.9	86

from Novagen (Merck, Germany). NADPH was from Codexis (USA); all aldehydes and ketones were purchased from Alfa Aesar or Sigma–Aldrich.

2.2. Cloning, expression and purification of OsAR

The OsAR gene from *Oceanospirillum* sp. MED92 with six His-tag at N-terminal and recognition sites for the restriction enzymes *Nde*I and *Sal*I was synthesized by Shanghai Xuguan Biotechnological Development Co. LTD. The gene product was digested with *Nde*I/*Sal*I, and cloned into the *Nde*I/*Sal*I sites of pET21a (+) (Novagen). After confirmation of the sequence, the plasmid PET-21a-QsAR was transformed into *E. coli* BL21 (DE3).

E. coli BL21 (DE3) cells carrying the recombinant plasmid were cultivated in 5 ml LB medium containing ampicillin (100 μg/ml) overnight at 37 °C. The culture was inoculated into 1 l LB medium containing ampicillin (100 μg/ml) and grown at 37 °C. The culture was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG) with a final concentration of 1 mM when OD_{600} reached 1.2, and then allowed to grow for additional 12 h at 20 °C. After centrifugation at 6000 × *g* for 15 min at 4 °C, the bacterial pellet was washed with phosphate buffer (50 mM, pH 7.0), and resuspended in a phosphate buffer (20 mM pH 7.4) containing 0.5 M of NaCl and 40 mM of imidazole.

The cells were lysed by sonication and the supernatant was collected by centrifugation at 10,000 × *g* for 30 min at 4 °C. Protein purification was performed at 23 °C using an AKTA purifier 10 system with UNICORN 5 software (GE Healthcare). The OsAR was purified using affinity chromatography with a HisTrapTM FF crude column (GE, USA). The column was pre-equilibrated with 50 ml equilibrium buffer (20 mM phosphate buffer, 0.5 M NaCl, 40 mM imidazole, pH 7.4). The sample (30 ml) was loaded with a flow rate of 1.0 ml/min. After washing with 50 ml of the equilibrium buffer, the bounded target protein was eluted with elution buffer (20 mM PB, 0.5 M NaCl, 0.5 M imidazole, pH 7.4). The target protein fraction was desalted through dialyzed against 20 mM phosphate buffer (pH 6.5). The protein was collected and stored at −20 °C. Protein concentration was measured by Bradford method [22].

2.3. Molecular weight determination

The molecular mass of OsAR was determined by gel filtration chromatography using a Superdex 200 10/300 GL column and the low molecular weight gel filtration calibration kit (GE). The sodium phosphate buffer (50 mM, pH 7.2) containing 150 mM NaCl was used as the eluent. The flow rate was 0.4 ml/min and the absorbance at 280 nm was monitored.

2.4. Activity assay

Enzyme activity was determined in a 0.2 ml of sodium phosphate buffer (0.1 M, pH 6.5) containing substrate (2 mM), NADPH (0.3 mM), and 20 μL (20 μg/ml) of the purified enzyme OsAR. The substrate was added as solution in dimethyl sulfoxide (DMSO), which did not exceed 1% of the total volume [23]. The reaction was initiated by the addition of the enzyme, and monitored for 1–3 min with SPECTRAMAX M2e (MD, USA) at 30 °C. The activity was determined by measuring NADPH oxidation from the decrease

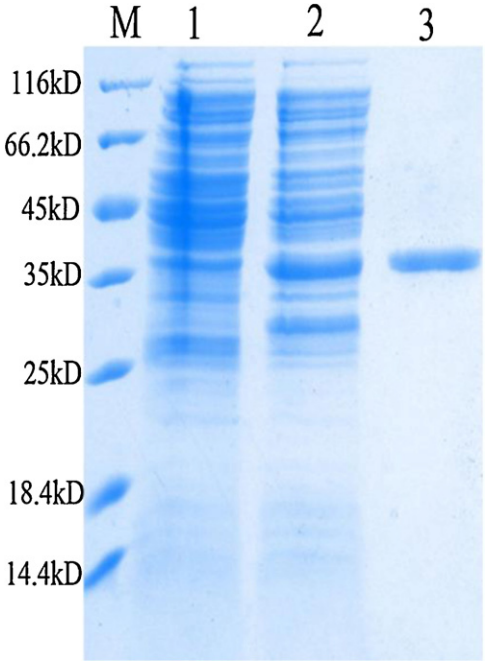

Fig. 1. SDS-PAGE analysis of OsAR. M: protein marker. (1) Control with empty vector. (2) Cell-free extract. (3) Purified QsAR.

in absorbance at 340 nm using a molar absorption coefficient of 6.22 mM^{-1} cm^{-1}. One unit of enzyme activity was defined as the amount of enzyme catalyzing the conversion of 1 μmol NADPH per minute.

2.5. Effects of pH, temperature and metal ions on enzyme activity

The pH dependence of OsAR activity was studied using the following buffer system (100 mM): sodium acetate (pH 5.0–6.0), sodium phosphate (pH 6.0–8.0), and Tris–HCl (pH 8.0–9.0). For determining pH stability, the enzyme was pre-incubated in buffers

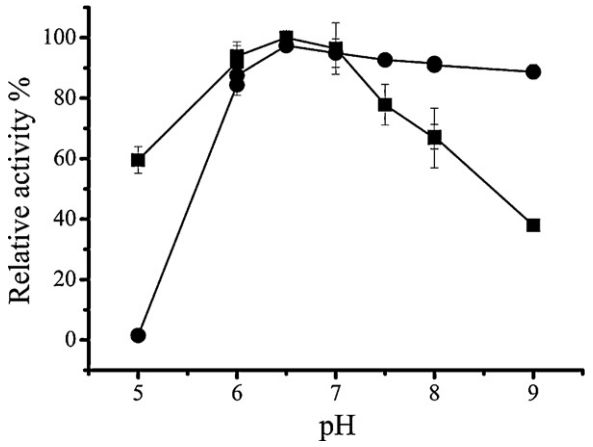

Fig. 2. Effects of pH on the enzyme activity (square) and stability (circle). The enzyme activity (16 U/mg) measured in sodium phosphate buffer (100 mM, pH 6.5) was defined as 100%.