Protein Expression and Purification 92 (2013) 208–213

Contents lists available at ScienceDirect

Protein Expression and Purification

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

Expression, purification, and characterization of formaldehyde dehydrogenase from *Pseudomonas aeruginosa*

Wangluo Zhang [a,b,1], Shuai Chen [a,1], Yuanping Liao [a], Dingli Wang [a], Jianfeng Ding [a], Yingming Wang [a], Xiaoyuan Ran [a], Daru Lu [b,*], Huaxing Zhu [a,*]

[a] *R&D Department, Novoprotein Scientific Inc (Shanghai), Room 202, Building 2, 720 Cailun Road, Shanghai 201203, China*
[b] *State Key Laboratory of Genetic Engineering and MOE, Key Laboratory of Contemporary Anthropology, Institute of Genetics, School of Life Sciences, Fudan University, 220 Handan Road, Shanghai 200433, China*

ARTICLE INFO

Article history:
Received 19 August 2013
and in revised form 26 September 2013
Available online 11 October 2013

Keywords:
Pseudomonas aeruginosa
Formaldehyde dehydrogenase
Molecular chaperone
Coexpression
Protein purification
Characterization

ABSTRACT

As a member of zinc-containing medium-chain alcohol dehydrogenase family, formaldehyde dehydrogenase (FDH) can oxidize toxic formaldehyde to less active formate with NAD^+ as a cofactor and exists in both prokaryotes and eukaryotes. Most FDHs are well known to be glutathione-dependent in the catalysis of formaldehyde oxidation, but the enzyme from *Pseudomonas putida* is an exception, which is independent of glutathione. To identify novel glutathione-independent FDHs from other bacterial strains and facilitate the corresponding structural and enzymatic studies, high-level soluble expression and efficient purification of these enzymes need to be achieved. Here, we present molecular cloning, expression, and purification of the FDH from *Pseudomonas aeruginosa*, which is a Gram-negative pathogenic bacterium causing opportunistic human infection. The FDH of *P. aeruginosa* shows high sequence identity (87.97%) with that of *P. putida*. Our results indicated that coexpression with molecular chaperones GroES, GroEL, and Tig has significantly attenuated inclusion body formation and improved the solubility of the recombinant FDH in *Escherichia coli* cells. A purification protocol including three chromatographic steps was also established to isolate the recombinant FDH to homogeneity with a yield of ~3.2 mg from 1 L of cell culture. The recombinant *P. aeruginosa* FDH was properly folded and biologically functional, as demonstrated by the mass spectrometric, crystallographic, and enzymatic characterizations of the purified proteins.

Introduction

Formaldehyde is a potent toxic and carcinogenic compound because of its reactivity to DNA. Both prokaryotic and eukaryotic organisms have developed cellular systems to counteract formaldehyde from environmental and endogenous resources [1–3]. Most of these systems are mediated by NAD(P)(H)-dependent oxidoreductases (dehydrogenases/reductases) [4,5] to initially oxidize formaldehyde to less active formate, which is further oxidized to carbon dioxide or incorporated into the one carbon pool via normal metabolic pathways [6]. The NAD(P)(H)-dependent oxidoreductases have been categorized into three groups: the short-, medium-, and long-chain families [7–9].

In the last three decades, the medium-chain family (MDR)[2] has been well studied in terms of both structural and functional properties in a number of species [10–13]. An example is formaldehyde dehydrogenase (FDH) that catalyzes the oxidation of formaldehyde with NAD^+ as an electron acceptor and exists in a wide variety of organisms from *Escherichia coli* to humans [4,5,14]. The FDH belongs to the zinc-containing medium-chain alcohol dehydrogenase (ADH) family, which is a subgroup of the MDR enzymes. Most FDHs are glutathione-dependent during the catalysis of formaldehyde oxidation, and the reaction product is S-formylglutathione instead of free formate [15–18]. The FDH from *Pseudomonas putida* is, so far, the only identified member of the ADH family that can catalyze irreversible oxidation of formaldehyde without glutathione [14,19]. The crystal structure of this enzyme has revealed a tetramer as the active form,

* Corresponding authors. Fax: +86 50798028 813 (H. Zhu).
E-mail addresses: drlu@fudan.edu.cn (D. Lu), zhuhuaxing@novoprotein.com.cn (H. Zhu).
[1] These authors contributed equally to this work.

[2] *Abbreviations used:* ADH, alcohol dehydrogenase; MDR, family the medium-chain family; FDH, formaldehyde dehydrogenase; FDM, formaldehyde dismutase; IPT, Gisopropyl-d-thiogalactoside; SEC, size-exclusion chromatography; BSA, bovine serum albumin; MALDI-TOF, matrix-assisted laser desorption ionization time-of-flight; MAP, methionine aminopeptidase.

1046-5928/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pep.2013.09.017

with each monomer comprising catalytic and coenzyme-binding domains, together with two bound zinc ions [20]. All this information suggests that it is very interesting to identify novel glutathione-independent FDHs from different bacterial strains.

In this study, we focus on the FDH from *Pseudomonas aeruginosa*, which is a versatile Gram-negative bacterium that exists in various environments. *P. aeruginosa* is a major pathogen that causes opportunistic human infections because of its intrinsic resistance to antibiotics and disinfectants [21,22]. To obtain enough amounts of soluble recombinant *P. aeruginosa* FDH proteins for structural and enzymatic studies, efficient expression and purification methods need to be established. The overproduction of recombinant proteins in *E. coli* has been known to cause formation of the insoluble aggregate-inclusion bodies. To overcome this bottleneck, some novel approaches have been developed, for example, coexpression of the recombinant proteins with molecular chaperones [23–26]. As a ubiquitous class of proteins that are essential for proper folding of other proteins during translation *in vivo*, molecular chaperones could increase the proportion of recombinant proteins in their native folded states. For instance, coexpression of the *groES/L* genes encoding chaperones GroES and GroEL in *E. coli* has significantly improved the solubility of *P. putida* formaldehyde dismutase (FDM) [27]. Here, we present the studies describing the effects of coexpression with chaperones GroES, GroEL, and Tig on the solubilization of recombinant *P. aeruginosa* FDH in *E. coli* BL21(DE3)/pG-TF2 cells. Protein purification, as well as the mass spectrometric, crystallographic, and enzymatic characterizations, of the recombinant FDH is also reported.

Materials and methods

Chemicals, enzymes, and the vector pCold II

The restriction enzymes NdeI, HindIII, the plasmid pG-TF2 and the expression vector pCold II were purchased from Takara. Novo-Rec seamless cloning kit and molecular-mass protein standards were from Novoprotein (Shanghai, China). The bacterial strains DH5a and BL21(DE3) were purchased from Tiangen. The chaperone coexpression strain BL21(DE3)/pG-TF2 was obtained by transformation of the plasmid pG-TF2 into *E. coli* BL21(DE3) cell. The Ni^{2+} Sepharose Fast Flow chelating resin was purchased from GE Healthcare. All other chemicals were from Sigma in analytical grade.

Cloning of P. aeruginosa FDH gene in pCold II

The FDH gene of *P. aeruginosa* (strain LESB58) was synthesized based on the authentic protein sequence (residues 1-399, UniProt accession number: **B7V5W2**) by Genscript (Nanjing, China). The DNA sequence was optimized to adapt the codon usage of the expression host using JCat [28]. An N-terminal His-tag (MNHKVHHHHHH) was also introduced into the FDH protein. The expression vector pCold II was linearized by NdeI and HindIII restriction sites, and the synthesized FDH gene was subcloned into the vector by recombinant seamless cloning with the following primers: 5′-CATCATCATCATCATCATATGTCTGGTAACCGTGGTGTTG-3′ (forward); 5′-CTAGACTGCAGGTCGACAAGCTTTTAAGCAGCACGGAACAGGTTG-3′ (reverse). The resulting plasmid pCold II-FDH was verified by DNA sequencing and transformed to *E. coli* BL21(DE3) or BL21(DE3)/pG-TF2 for gene expression.

Expression of P. aeruginosa FDH in E. coli

E. coli BL21(DE3)/pG-TF2 cells transformed with the plasmid pCold II-FDH were grown in LB medium containing ampicillin (100 μg ml^{-1}) and chloramphenicol (34 μg ml^{-1}) at 37 °C to an OD_{600} of 0.6–0.7; 5 ng ml^{-1} tetracycline was then introduced to produce chaperones GroEL, GroES, and Tig for 30 min. The His-tagged FDH protein was then produced at 16 °C for 16 h after adding 0.1 mM isopropyl β-D-thiogalactoside (IPTG) to the cell culture. The cells were finally harvested by centrifugation at 4000*g*, 4 °C for 30 min, and the pellets were frozen at −80 °C.

Expression of the *P. aeruginosa* FDH gene in *E. coli* BL21(DE3) cells was performed under similar experimental conditions without adding tetracycline to the cell culture.

Purification of P. aeruginosa FDH

The FDH protein of *P. aeruginosa* was purified from *E. coli* BL21(DE3)/pG-TF2 cells as follows. Freeze–thawed cell pellets were resuspended in lysis buffer (20 mM Tris–HCl pH 8.0, 150 mM NaCl, and 20 mM imidazole), followed by sonication on ice. The crude lysate was centrifuged at 12,000*g* for 60 min, and the supernatant was filtered through a 0.45-μm filter followed by loading onto a 2-ml column of pre-equilibrated Ni^{2+} Sepharose Fast Flow chelating resin. The column was washed extensively with lysis buffer and eluted with an imidazole gradient (50–250 mM). The eluted samples were then dialyzed against buffer A (20 mM Tris–HCl pH 8.0, 1 mM DTT), and the dialyzed samples were loaded onto a 10-ml Mono Q High Performance column (GE Healthcare) pre-equilibrated with buffer A. The column was washed and then eluted with a NaCl gradient (50–500 mM) in buffer A. Peak fractions containing the FDH were pooled and further purified using size-exclusion chromatography (SEC) with a HiLoad 26/60 Superdex 200 column (GE Healthcare) in buffer B (10 mM Tris–HCl pH 8.0, 1 mM DTT).

The multimeric state of the *P. aeruginosa* FDH was also analyzed by comparing its elution profile with those of molecular-mass protein standards. The homogeneity of the purified FDH was analyzed with SDS–PAGE, and the protein concentration was measured using Bradford assay, with bovine serum albumin (BSA) as the standard.

Mass spectrometry analysis and crystallization of P. aeruginosa FDH

The highly purified protein of *P. aeruginosa* FDH (0.2 mg ml^{-1}, 10 mM Tris–HCl pH 8.0, 1 mM DTT) was dialyzed against distilled water, and the molecular mass of the protein (1 μl) was analyzed using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) with a 4800 Plus MALDI TOF/TOF™ Analyzer (AB SCIEX). The mass spectrometer is equipped with 4000 Series Explorer V3.5 software (AB SCIEX) for data acquisition and spectra handling.

The crystals of *P. aeruginosa* FDH were obtained using sitting-drop vapor-diffusion method with the setup of 2-μl drops consisting of 1 μl of purified protein (15 mg ml^{-1}, 10 mM Tris–HCl pH 8.0, 1 mM DTT) and 1 μl of reservoir solution. Optimum plate-like crystals grew at 18 °C in 0.1 M Bis–Tris pH 5.5, 1.8 M ammonium sulfate as the reservoir solution.

Enzymatic activity assay

$$\text{Formaldehyde} + NAD^+ + H_2O \xrightarrow{FDH} \text{Formate} + NADH + H^+ \quad (1)$$

As indicated by (Eq. (1)), the enzymatic activity of the *P. aeruginosa* FDH was assayed by measuring the formation of NADH in terms of the increase of absorbance at 340 nm [14]. About 0.1-ml aliquot of the purified enzyme (50 μg ml^{-1}) was incubated with or without glutathione (3 mM), followed by adding 1 ml of cofactor NAD^+ (1 mM) and substrate formaldehyde (1 mM) in 50 mM potassium phosphate buffer, pH 7.5, at 37 °C in a quartz cuvette with