Journal of Photochemistry and Photobiology A: Chemistry 313 (2015) 154–162

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

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

Expression systems for soluble metal-dependent formate dehydrogenase

Masaki Ihara[a,b,*], Yusuke Kawano[c], Yusuke Fujiwara[a], Tetsuya Kodo[a], Manami Mizuguchi[a], Yusuke Mochiduki[a], Kai Kodoh[a], Ayako Okabe[a], Izumi Matsuno[a]

[a] Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Nagano 399-4511, Japan
[b] JST, PRESTO, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[c] Graduate School of Biological Sciences, Nara Institute of Science and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192, Japan

ARTICLE INFO

Article history:
Received 20 March 2015
Received in revised form 23 June 2015
Accepted 24 June 2015
Available online 29 June 2015

Keywords:
Formate dehydrogenase
Recombinant protein
Expression system

ABSTRACT

Molybdenum or tungsten-dependent formate dehydrogenases (FDH) can reduce carbon dioxide (CO_2) to formate under ordinary conditions, and therefore, are considered promising catalysts for CO_2 fixation. However, to our knowledge, no study on the modification of metal-dependent FDHs has been published, likely because of a lack of convenient expression systems for the recombinant enzymes. We attempted to establish an methodology for the preparation and modification of soluble oxygen-tolerant metal-dependent FDHs, for the following three strategies: (1) *Escherichia coli* FDH is converted from a membrane bound protein into a soluble protein by deleting the C-terminal membrane-anchor of small subunit (FdoH) and the whole membrane subunit (FdoI) and expressed homologously in *E. coli*; (2) originally soluble FDHs from *Desulfovibrio* are expressed heterologously in *E. coli*; and (3) *Desulfovibrio* FDHs are genetically engineered by the homologous gene recombination method and expressed homologously in *Desulfovibrio*. We successfully established the expression systems for (1) and (3), and succeeded in purification of the soluble FDHs with a tag by using affinity columns.

1. Introduction

Formate dehydrogenase (FDH) is an enzyme that can reduce carbon dioxide (CO_2) to formate. Two classes of FDHs have been discovered thus far: metal-independent nicotinamide adenine dinucleotide (NADH)-based FDHs [1,2] and metal-dependent molybdenum (Mo)-based or tungsten (W)-based FDHs [3–5]. We had earlier created several NADPH-dependent type FDHs and combined them with photosystem I (PSI), ferredoxin, and ferredoxin-$NADP^+$-reductase to establish an artificial light-driven carbon dioxide reduction system (Fig. 1A). The system successfully produced formate in vitro and in vivo [6]. However, the reduction of carbon dioxide by NADPH ($CO_2 + NADPH \rightarrow HCOO^- + NADP^+$) is thermodynamically unfavorable. To create a more efficient system, it is necessary to use a metal-dependent FDH that can directly accept electrons from a low potential electron donor, such as PSI (Fig. 1B).

Metal-dependent FDHs consist of a catalytic subunit and electron transfer subunits. The catalytic subunit possesses a Mo or W coordinated by a selenocysteine (SeCys) residue, two molybdopterin guanine dinucleotides (MGDs), and [4Fe-4S] clusters [7–10]. *Escherichia coli* has three metal-dependent FDHs; formate dehydrogenase H (EcFDH-H), formate dehydrogenase N (EcFDH-N) and formate dehydrogenase O (EcFDH-O). EcFDH-H is a part of the multifunctional membrane-bound protein, the formate hydrogenlyase complex, responsible for the anaerobic oxidation of formate to CO_2 and molecular hydrogen. It can be isolated as a soluble monomeric enzyme [7,11–13]. EcFDH-N is a component of an anaerobic respiratory chain, formate–nitrate oxidoreductase, which is also a membrane-bound protein complex and catalyzes oxidation of formate coupled to nitrate respiration [14]. It was purified and crystallized as a trimeric enzyme containing a catalytic subunit (FdnG), an electron transfer subunit (FdnH), and a transmembrane subunit with type-b heme (FdnI) (Fig. 2A). EcFDH-O, an isoenzyme of EcFDH-N, is a membrane-bound enzyme composed of three subunits, FdoG, FdoH, and FdoI, which exhibit high sequence similarity to the corresponding subunits of EcFDH-N. The expression of EcFDH-O is induced under micro-aerobic and aerobic conditions. It is proposed that a physiological role of EcFDH-O is to ensure rapid adaptation during a sudden shift

* Corresponding author at: Faculty of Agriculture, Shinshu University, 8304 Minamiminowa, Nagano 399-4511, Japan. Fax: +81 265 77 0508.
E-mail address: m_ihara@shinshu-u.ac.jp (M. Ihara).

http://dx.doi.org/10.1016/j.jphotochem.2015.06.028
1010-6030/

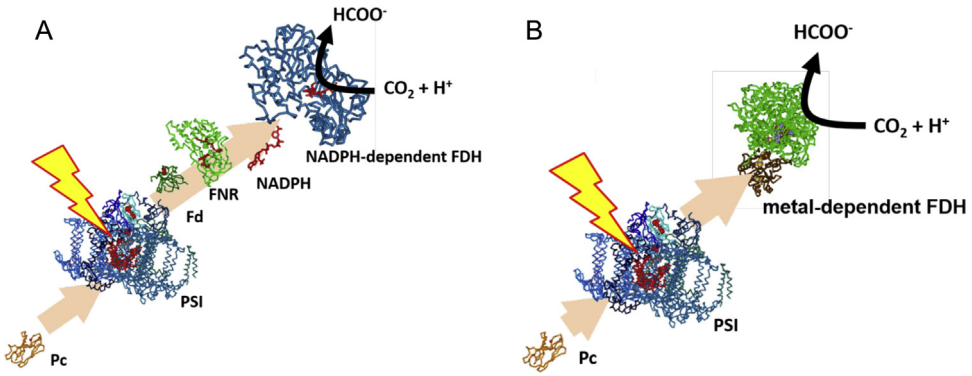

Fig. 1. (A) The artificial light-driven carbon dioxide reduction system using NADPH-dependent FDH and photo-electron relay system consisting of plastocyanin (Pc), PSI, ferredoxin (Fd), ferredoxin-$NADP^+$ reductase (FNR) and NADP(H). (B) An example of direct light-driven carbon dioxide reduction using metal-dependent FDH and PSI.

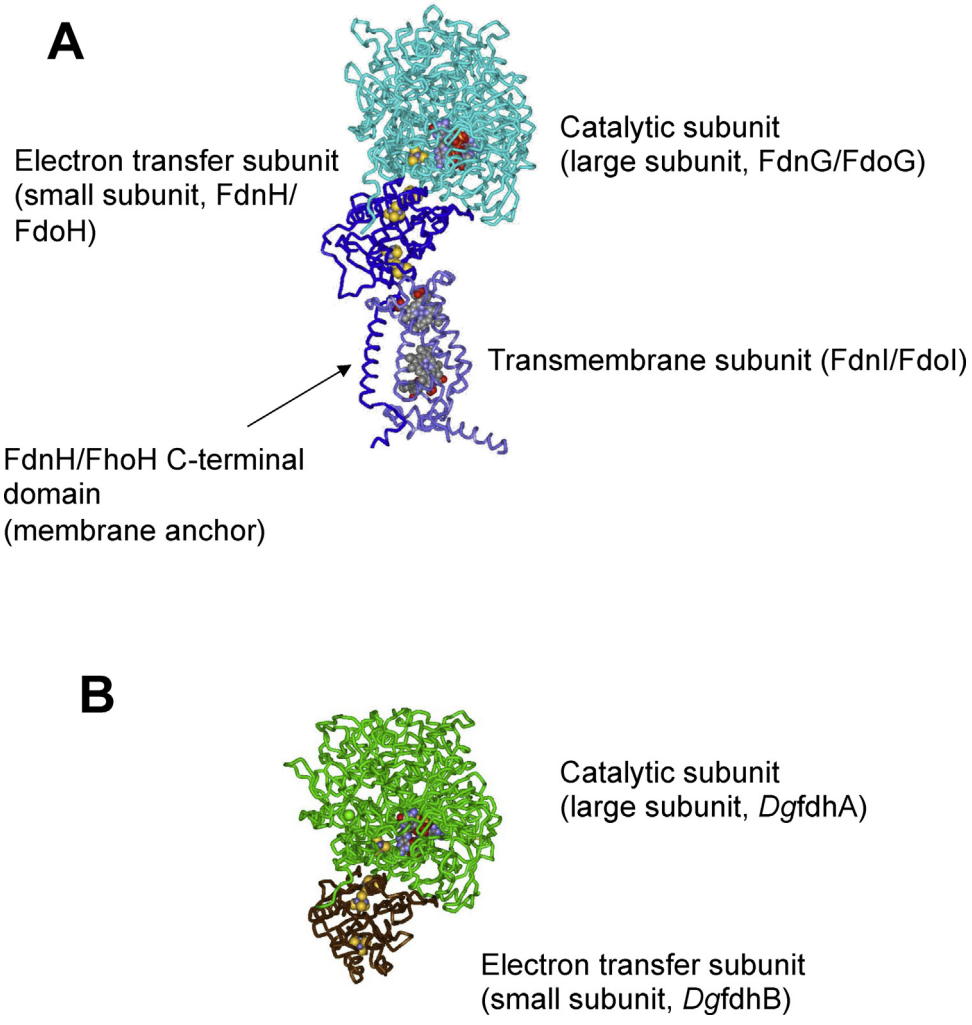

Fig. 2. (A) Crystal structure of E. coli FDH-N [10]. FDH-O exhibits high homology with FDH-N, and they are thought to have essentially the same structure. *E. coli* FDH-N and FDH-O contain a catalytic subunit, an electron transfer subunit and a transmembrane subunit. *E. coli* FDHs are tethered to membrane via the C-terminal domain of the electron transfer subunit and the transmembrane subunit. (B) Crystal structure of D. gigas FDH [8]. *D. gigas* FDH is composed of a catalytic subunit and an electron transfer subunit.

from aerobiosis to anaerobiosis [15]. The dimer of FdoG and FdoH is directed toward the cytoplasmic side, and with FdoH anchored to the periplasmic membrane and the FdoI subunit by a C-terminal hydrophobic domain [16]. Among these three metal-dependent FDHs from *E. coli*, EcFDH-H has been well-studied due to its structural simplicity and solubility [3], but it is intractable for practical applications due to its extremely high oxygen-sensitivity.

In contrast to *E. coli* FDHs, the metal-dependent FDHs from some anaerobic bacteria, such as sulfate reducing bacteria and *Syntrophobacter fumaroxidans*, are expressed in a soluble form and are thought to transfer electrons via several types of periplasmic electron carrier proteins by forming a complex in the periplasmic space [4,17–19]. Their subunit structures are relatively simple, containing a catalytic subunit and one or two electron transfer