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# How does binuclear zinc amidohydrolase FwdA work in the initial step of methanogenesis: From formate to formyl-methanofuran

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ABSTRACT

The initial step of methanogenesis is the fixation of $CO_2$ to formyl-methanofuran (formyl-MFR) catalyzed by formyl-MFR dehydrogenase, which can be divided into two half reactions. Herein, the second half reaction catalyzed by FwdA (formyl-methanofuran dehydrogenase subunit A), i.e., from formate to formyl-methanofuran, has been investigated using density functional theory and a chemical model based on the X-ray crystal structure. The calculations indicate that, compared with other well-known di-zinc hydrolases, the FwdA reaction employs a reverse mechanism, including the nucleophilic attack of MFR amine on formate carbon leading to a tetrahedral *gem*-diolate intermediate, two steps of proton transfer from amine to formate moieties assisted by the Asp385, and the C−O bond dissociation to form the formyl-MFR product. The second step of proton transfer from the amine moiety to the Asp385 is rate-limiting with an overall barrier of 21.2 kcal/mol. The two zinc ions play an important role in stabilizing the transition states and intermediates, in particular the negative charge at the formate moiety originated from the nucleophilic attack of the MFR amine. The work here appends a crucial piece in the methanogenic mechanistics and advances the understanding of the global carbon cycle.

## 1. Introduction

Carbon dioxide ($CO_2$) is the main greenhouse gas and the over-emission of $CO_2$ resulted from human activities and industrialization can lead to harmful climate change. Many attempts have been made to recycle $CO_2$ from atmosphere [1], among which biological utilization of $CO_2$ is considered to be an environmentally friendly and promising approach since $CO_2$ is the main carbon source for plants via photosynthesis and for microorganism such as microalgae and methanogens. In particular, the $CO_2$ fixation by methanogens is of significance and broad interest as the catabolic product of methane is a widely used natural gas for human activities [2,3]. *Methanogenic archaea*, which produces a large amount of methane (~1 billion tons/year) through energy catabolism [4], plays an important ecological role in the global carbon cycle because 60% methane created by methanogens is finally oxidized to $CO_2$ [5,6]. A typical methanogenic pathway with $H_2$ as electron donor (Fig. 1) is initiated by the fixation of $CO_2$ to formyl-methanofuran (formyl-MFR) catalyzed by formyl-MFR dehydrogenase termed Fwd$(ABCDFG)_2$ and Fwd$(ABCDFG)_4$ complexes [7]. Therefore, the scrutiny into the reaction mechanism of formyl-MFR dehydrogenase is crucial for the understanding of the methanogenic mechanistics and the global carbon cycle, and may benefit the biological utilization of $CO_2$.

The reaction of formyl-MFR dehydrogenase requires two electrons derived from ferredoxin ($Fd_{red}^{2-}$) and two additional protons [8,9] (Fig. 2A). This reaction was observed to be reversible [10], and it needs to be coupled energetically with the reduction of heterodisulfide CoM-S-S-CoB (CoM and CoB are referred to as coenzymes M and B respectively) [5]. The latter can assist in the reduction of ferredoxin by hydrogen, leading to the reduced ferredoxin required by the former (Fig. 1). In fact, *Methanothermobacter wolfeii* belongs to thermophilic species and the optimal reaction temperature for formyl-MFR dehydrogenase from *Methanothermobacter wolfeii* is about 65 °C [11]. This reaction was recently suggested by the crystallographic analysis of formyl-MFR dehydrogenase (PDB code: 5T61) [7] to proceed via two half reactions, i.e., the reduction of $CO_2$ with electrons and proton to formate (Fig. 2B), followed by the condensation of the resultant formate with MFR to form formyl-MFR (Fig. 2C). It was proposed that the first half reaction occurs in a tungsten-containing active site located at the FwdBD (formyl-methanofuran dehydrogenase subunits B and D, formate dehydrogenase), and then the resulting formate diffuses through a hydrophilic tunnel to a binuclear zinc active site at the FwdA (formyl-methanofuran dehydrogenase subunit A, amidohydrolase) triggering the second half reaction (see Fig. 3 for the protein and active-site structures) [7]. The reduction of $CO_2$ to formate is also found in acetogens as their first step of respiration [12,13]. Since the first half

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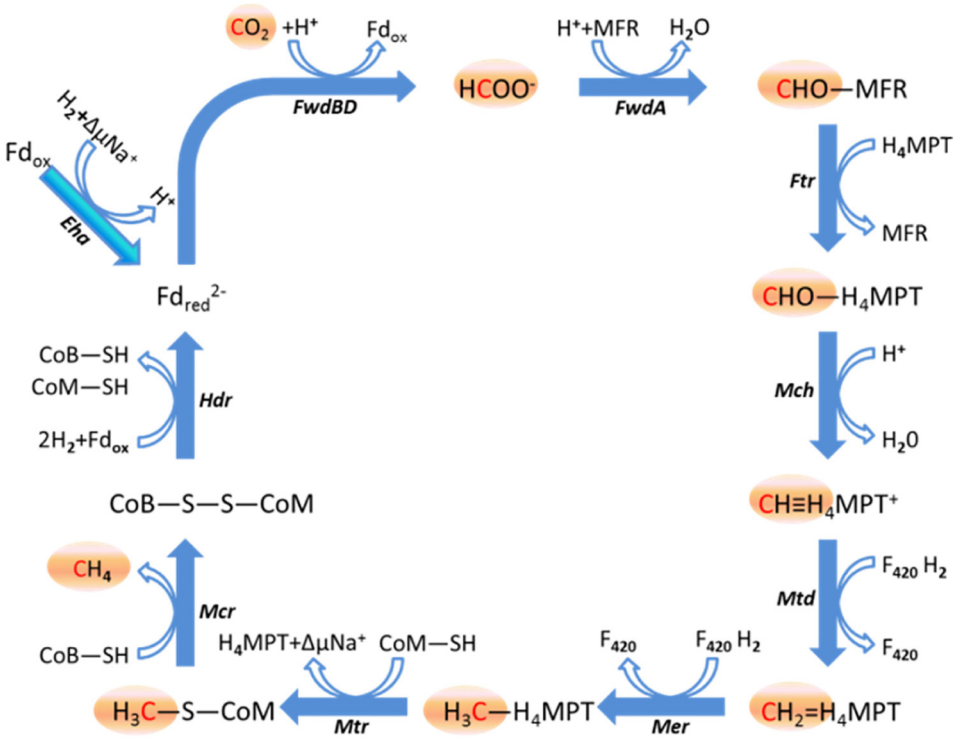


**Fig. 1.** The simplified diagram of methane formation from $CO_2$ and $H_2$ in *methanogenic archaea*. The carbon denoted by red color indicates the transferred carbon in the cycle. The italic bold represents various enzymes catalyzing the reactions involved. FwdBD: formate dehydrogenase. FwdA: amidohydrolase. Ftr: formylmethanofuran/$H_4$MPT formyltransferase. Mch: methenyl-$H_4$MPT cyclohydrolase. Mtd: methylene-$H_4$MPT dehydrogenase. Mer: methylene-$H_4$MPT reductase. Mtr: methyl-$H_4$MPT-CoM methyltransferase. Mcr: methyl-CoM reductase. Hdr: heterodisulfide reductase. Eha: energy-converting hydrogenase. Fd: ferredoxin. MFR: methanofuran. $H_4$MPT: tetrahydromethanopterin. $F_{420}$: coenzyme $F_{420}$. CoM: coenzyme M (HS-CoM). CoB: coenzyme B (HS-CoB).

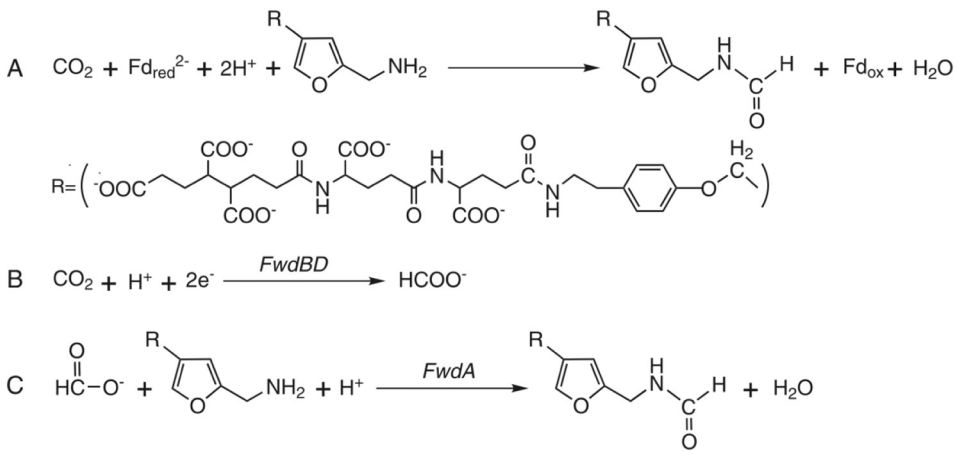


**Fig. 2.** The reaction catalyzed by formyl-MFRdehydrogenase (A), including the first half reaction occurring in the FwdBD subunits (B) and the second half reaction in the FwdA subunit (C).

reaction is predicted to be thermodynamically favorable with $\Delta G = -3.2$ kcal/mol (discussed in more detail later in Section 3.1.), in this work we will focus on the mechanism of the second half reaction happening in the FwdA subunit.

FwdA (63 kDa) is structurally classified as a member of the amidohydrolase superfamily, which includes urease, phosphotriesterase, dihydroorotase, dihydropyrimidinases, and so on, and has the character of a binuclear metal center placed inside a deep solvent-accessible cavity [14–16]. The X-ray crystal structure of FwdA (PDB code: 5T61) (Fig. 3) [7] reveals that the two divalent zinc ions are separated by ~3.0 Å from each other in the active site, and bridged by a carboxylated lysine (Lys178). Four histidines (His57, His59, His231, and His271) and an aspartate (Asp385) compose the rest of first-shell ligands. Most of dizinc enzymes known before are hydrolases. Considering the architectural similarity of binuclear zinc core between FwdA and other amidohydrolases like dihydroorotase [17–19] and aminopeptidase from *Aeromonas Proteolytica* (AAP) [19–21], it is conceivable that the FwdA reaction may employ a reverse mechanism relative to other dizinc amidohydrolases. A mechanism is thus hypothesized to generally involve the C—N bond formation between formate and MFR, the proton transfer from the MFR amido to the formate oxygen, and finally the dissociation of a C—O bond originally situated in the formate moiety (Fig. 4). However, such a mechanistic proposal lacks effective verification.

In the present work, using the density functional theory (DFT) with the hybrid functional B3LYP [22–24], we have investigated the reaction mechanism of FwdA on the basis of a chemical model built from the crystal structure of FwdA (PDB code: 5T61) [7]. The energetics and the characterization of stationary points involved are provided here. The calculations demonstrate that the FwdA reaction proceeds via a stepwise mechanism (Fig. 4) where the first-shell Asp385 functions as a key proton shuttle to deliver the proton from the MFR amido to the formate oxygen.

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