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FDH: an Aldehyde Dehydrogenase Fusion Enzyme in Folate Metabolism

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Abstract

FDH (10-formyltetrahydrofolate dehydrogenase, Aldh1L1, EC 1.5.1.6) converts 10-formyltetrahydrofolate (10-formyl-THF) to tetrahydrofolate and CO₂ in a NADP⁺-dependent reaction. It is a tetramer of four identical 902 amino acid residue subunits. The protein subunit is a product of a natural fusion of three unrelated genes and consists of three distinct domains. The N-terminal domain of FDH (residues 1–310) carries the folate binding site and shares sequence homology and structural topology with other enzymes utilizing 10-formyl-THF as a substrate. *In vitro* it functions as 10-formyl-THF hydrolase, and evidence indicate that this activity is a part of the overall FDH mechanism. The C-terminal domain of FDH (residues 400–902) originated from an aldehyde dehydrogenase-related gene and is capable of oxidation of short-chain aldehydes to corresponding acids. Similar to class 1 and 2 aldehyde dehydrogenases, this domain exists as a tetramer and defines the oligomeric structure of the full-length enzyme. The two catalytic domains are connected by an intermediate linker (residues 311–399), which is a structural and functional homolog of carrier proteins possessing a 4'-phosphopantetheine prosthetic group. In the FDH mechanism, the intermediate linker domain transfers a formyl, covalently attached to the sulfhydryl group of the phosphopantetheine arm, from the N-terminal domain to the C-terminal domain. The overall FDH mechanism is a coupling of two sequential reactions, a hydrolase and a formyl dehydrogenase, bridged by a substrate transfer step. In this mechanism, one domain provides the folate binding site and a hydrolase catalytic center to remove the formyl group from the folate substrate, another provides a transfer vehicle between catalytic centers and the third one contributes the dehydrogenase machinery further oxidizing formyl to CO₂.

Keywords

folate metabolism; 10-formyltetrahydrofolate dehydrogenase; aldehyde dehydrogenase; functional domains; 4'-phosphopantetheine; enzyme mechanism

1. Introduction

1.1. Overview of folate metabolism

The present review is focused on the folate enzyme, 10-formyltetrahydrofolate dehydrogenase (**FDH**, Aldh1L1, EC 1.5.1.6). The crucial role of folate coenzymes in cellular metabolism is

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well established. They are key molecules in transferring one-carbon groups in numerous reactions [1] including *de novo* nucleotide biosynthesis [1–3] and the regeneration of methionine from homocysteine [1,4,5]. The latter process is connected to production of S-adenosylmethionine [1,5,6], a universal methyl donor in more than a hundred methylation reactions in the cell [7]. It is not surprising, therefore, that disruption of folate metabolism has dramatic consequences for the cell [8–10], ultimately reducing growth rate and impairing cell division [1,11–13]. Of note, folate is a vitamin, meaning that humans are unable to synthesize it and strictly depend on diet to obtain this coenzyme [14]. For many years, folate metabolism attracted the attention of researchers due to strong correlations between folates status and several diseases. Epidemiological studies have linked low folate levels, or a low folate intake, with increased risk for several types of cancer [15], neural tube defects [16], and cardiovascular diseases [17]. At present, it is generally accepted that increased folate intake decreases the risk of these diseases in humans [15,18].

The intracellular folate pool consists of several major forms of folate that are interconvertible through multiple reactions catalyzed by more than a dozen enzymes [1,19]. The FDH substrate, 10-formyltetrahydrofolate (10-formyl-THF), represents a major folate pool in the cell [20] and serves as a formyl donor in three biosynthetic reactions (Fig. 1) [1]. It is an essential substrate for two reactions of *de novo* purine biosynthesis, catalyzed by glycinamide ribonucleotide formyltransferase (GARFT, EC 2.1.2.2) and aminoimidazole-4-carboxamide ribonucleotide formyltransferase (EC 2.1.2.3) [2]. It is also required for the third reaction, formylation of methionyl-tRNA, that is an initiation step of translation in bacteria [21]. This reaction is catalyzed by methionyl-tRNA formyltransferase (FMT, EC 2.1.2.9). FMT is also present in higher organisms, including humans, where it might be required for translation initiation in mitochondria [21,22]. While the importance of this reaction is not completely clear for mammalian cells [23], 10-formyl-THF-dependent reactions of purine biosynthesis are crucial for cellular proliferation. This was demonstrated in the experiments with inhibitors of GARFT [24], the first folate dependent enzyme in the *de novo* purine pathway: these inhibitors induce cytotoxicity by severe depletion of intracellular purine nucleotides [25–27]. In the cell, 10-formyl-THF is synthesized by one of two pathways (Fig. 1). The first includes two sequential reactions that convert 5,10-methylene-THF to 5,10-methenyl-THF and then to 10-formyl-THF [28]. The second is an ATP-dependent reaction that incorporates formate into the folate pool [28]. In the cytosol of mammalian liver, all three reactions are catalyzed by a trifunctional enzyme, C₁-synthase [29].

1.2. Disposition of FDH in cellular pathways

FDH converts 10-formyl-THF to THF and CO₂ in a NADP⁺-dependent dehydrogenase reaction (Fig. 2). This pathway, in contrast to other 10-formyl-THF utilizing reactions, removes one-carbon groups from the folate pool instead of incorporating them into biosynthetic processes. FDH is an abundant enzyme in mammals [30]. Its levels are especially high in liver and kidney, the two principal sites of folate metabolism [30]. In rat liver, for example, it comprises about 1.2% of the total cytosolic protein [19] suggesting an essential function. The FDH gene is present in animals, including *C. elegans* and *D. melanogaster*, but is not found in prokaryotes, fungi and plants. The protein sequence is relatively conserved among different species (Table 1), including its catalytic residues (see below). It has been proposed that the FDH reaction is important for replenishing the pool of THF at the expense of 10-formyl-THF [31], thus allowing re-use of the coenzyme by other reactions [1]. The finding of a low THF level in mice lacking FDH supports the importance of the enzyme in THF regeneration [31]. These mice also showed a dramatically decreased reproductive efficiency [32], indicating that disruption of the FDH gene has significant physiological consequences. The FDH-catalyzed reaction also regulates *de novo* purine biosynthesis [30,33] and formate degradation [34]. A recent study further implicated this reaction in regulation of methylation status of the cell

[35]. Interestingly, although the precise biological role of FDH is not settled yet, the enzyme appears to be crucial regulator of cellular metabolism in general: it is strongly down regulated at certain physiological and pathological conditions [30,36,37], while its up-regulation can produce drastic antiproliferative effects [30].

In vitro FDH catalyzes conversion of 10-formyl-THF to THF in two reactions: the NADP⁺-dependent dehydrogenase reaction with the formyl group ending up as CO₂ and the NADP⁺-independent hydrolase reaction, in which formyl group is released as formate (Fig. 1). The enzymatic activity that deformylates 10-formyl-THF and yields free formate was first reported by Osborne et al. [38] in a preparation from beef liver. It has been also observed that the formation of THF was accelerated by addition of NADP⁺ [39] although this particular reaction does not require the participation of nicotinamide dinucleotide coenzymes. Kutzbach and Stokstad later demonstrated the NADP⁺-dependent oxidative deformylation of 10-formyl-THF (dehydrogenase reaction) using a partially purified enzyme [40,41]. In this reaction the formyl group of 10-formyl-THF is converted to CO₂ rather than to formate. More detailed characterization of the enzyme has confirmed that both activities, the hydrolase and the dehydrogenase, are catalyzed by the same enzyme [42,43]. It has been also observed that the rate of the hydrolase reaction is 21% of the rate of the dehydrogenase reaction [42]. Despite these earlier findings, the hydrolase reaction was the subject of controversy for years. While Rios-Orlandi et al. showed that both dehydrogenase and hydrolase activities can take place simultaneously [44], Case et al. later reported that the two activities resided in two different but closely related enzymes, from cytosolic and mitochondrial fractions, respectively [45]. This controversy was resolved in favor of the original observation of an enzyme with dual catalytic activity after expression of recombinant rat liver FDH [46]. These studies have demonstrated that the recombinant enzyme indeed carries out both reactions, thus proving that both activities are associated with the product of a single gene, i.e. reside in one polypeptide [46].

2. Domain organization of FDH

2.1. Overview

Earlier studies have demonstrated that the enzyme is a tetramer of similar or identical subunits, each with a molecular mass of about 100 kDa [42]. Cloning of rat FDH cDNA by Cook et al. [47] confirmed that the protein is comprised of identical 902 amino acid residue subunits. Analysis of the FDH amino acid sequence performed in this study revealed the unexpected chimeric nature of the enzyme. Three distinct domains were discerned (Fig. 2): an N-terminal folate-binding domain (Nt-FDH, residues 1–310), an intermediate domain of unknown nature (residues 311–419) and an aldehyde dehydrogenase-like C-terminal domain (Ct-FDH, residue 420–902) [47]. The boundaries between the FDH domains are arbitrary and were first assigned based on the sequence similarity to other proteins. Thus, the N-terminal domain revealed identity with two enzymes utilizing 10-fFDH as a formyl donor in biosynthetic reactions: GARFT [48,49] and FMT [50]. The C-terminal domain was found to be similar to aldehyde dehydrogenase family members (reviewed in [51,52]). The discovery that FDH has high similarity with aldehyde dehydrogenases initiated a search for an aldehyde dehydrogenase activity of the enzyme. Unsurprisingly, studies have demonstrated that FDH possesses this activity towards short chain aldehydes, converting them to corresponding acids (Fig. 2) [47]. A later study employing proteolytic digestion of purified rabbit FDH has demonstrated that the 32 kDa N-terminal domain carries the folate binding site and the hydrolase activity while the 53 kDa C-terminal domain carries the NADP⁺ binding site and catalyses aldehyde dehydrogenase activity [53]. These findings have been further confirmed in experiments with expression of different recombinant constructs of rat FDH [54–56].

The intermediate domain was assigned a linker function between the two domains based on the absence of identity to either of the group of proteins [47]. Moreover, at that time, the

intermediate domain did not reveal any sequence similarity to known proteins. Interestingly, in the absence of the intermediate domain, the two catalytic domains are not capable of producing 10-formyl-THF dehydrogenase activity [53,54]. Therefore, the intermediate domain evidently couples the N-terminal and C-terminal domains into a functional enzyme.

2.2. Structure and function of the N-terminal domain of FDH

Nt-FDH (residues 1–310) can be expressed separately as a functional enzyme that allowed its detailed characterization [54]. The folate-binding site and the hydrolase activity reside in this domain, and two other domains of FDH do not contribute to folate binding or hydrolase reaction [53–55]. By itself, Nt-FDH exists as a monomer and is not involved in FDH tetramer formation. The crystal structures of the N-terminal domain of rat and human enzyme have been solved [57,58]. The sequence similarity between Nt-FDH of the two species is 93% that obviously defines very close superimposition of their structures. The Nt-FDH can be divided into two sub-domains, N-terminal (subdomain 1) and C-terminal (sub-domain 2) connected by a long stretch of polypeptide chain, which generally lacks ordered secondary structure (Fig. 3). Sub-domain 1 contains a Rossmann fold [59] while sub-domain 2 represents a slightly open β -barrel that is similar in topology to the OB fold [60].

The overall sequence identity between three 10-formyl-THF utilizing enzymes, Nt-FDH, GARFT and FMT, does not exceed 32%, but there is a region of about 70 amino acid residues in the middle of their sequences where identity increases to about 50% [61]. This region was originally predicted to be a folate binding site [47] that was later confirmed by crystal structure analysis [58,62,63]. Overall, sub-domain 1 of Nt-FDH shows a fold similar with both FMT and GART and superimposes closely in all three enzymes; with the closest overlap being the central seven-stranded sheet comprising the folate-binding site [57]. Two catalytically important and strictly conserved residues, aspartate 142 and histidine 106, have been identified in the active sites of FDH [61,64]. These residues are also essential for catalysis in GARFT and FMT [65,66]. They reside in the hydrophobic cleft that serves as a folate-binding pocket. The respective aspartates and histidines overlap very closely in superimposed structures of the three enzymes implying similarity of catalytic mechanisms. In the proposed mechanism of the hydrolase catalysis, Asp142 has two functions [57]: it activates a water molecule facilitating its nucleophilic attack on the carbonyl carbon of 10-formyl-THF and then it participate in the intermediate stabilization by compensating for a positive charge on quaternary amine at N10 of 10-formyl-THF. His106 in this mechanism participates in the stabilization of the other part of the intermediate, a negatively charged oxygen of a tetrahedral carbon [57].

Role of the C-terminal sub-domain of Nt-FDH—Compared to GARFT, Nt-FDH and FMT both have an additional sub-domain, which is very similar in topology in the two proteins. In FMT, it is responsible for binding methionyl-tRNA [67,68]. A nucleic acid binding activity for FDH has not been reported, but several other folate-metabolizing enzymes do act as autoregulatory translational repressors by binding to mRNA [69,70]. Unexpectedly, it has been revealed that this domain is important for the hydrolase catalysis: deletion experiments show that at least 300 residues are required for the functional hydrolase [57], although the folate-binding site and the hydrolase catalytic center are both located within the first 200 residues of the protein. Interestingly, sub-domain 2 of FMT is capable of substituting for the corresponding domain of Nt-FDH in the hydrolase catalysis [56]. The chimera did not reveal a detectable level of 10-formyl-THF dehydrogenase activity, though. This, however, was rather a result of distortion of the interface between the hydrolase and aldehyde dehydrogenase domains of FDH caused by differently positioned carboxyl-terminus of sub-domain 2 of FMT that oriented the aldehyde dehydrogenase domain of FDH away from the hydrolase catalytic center.

2.3. Structure and function of the C-terminal domain of FDH

Overall structure—The C-terminal domain of FDH (residues 420–902) is up to 50% homologous to enzymes from a family of aldehyde dehydrogenases [47,55] suggesting that this part of the FDH molecule is derived from an aldehyde dehydrogenase-related gene [51, 52]. The identity between FDH and aldehyde dehydrogenases is spread through the entire sequence and includes all of the regions conserved in the aldehyde dehydrogenase family [47,51,52,55]. All aldehyde dehydrogenases have a strictly conserved cysteine in the active site [51], which is a key catalytic residue serving as nucleophile [71,72]. Using site-directed mutagenesis, it has been shown that the corresponding cysteine of FDH, Cys707, is directly involved in the dehydrogenase catalysis [73]: it is crucial in both dehydrogenase reactions utilizing either 10-formyl-THF (full-length enzyme catalysis) or aldehydes [73]. The C-terminal domain can be expressed separately as a functional aldehyde dehydrogenase [55] proving that ALDH activity is carried out by this domain and does not require participation of the two other domains of FDH. Similar to other class 1 and class 2 ALDH, the C-terminal domain exists as a tetramer [53,55], indicating that this domain is responsible for the oligomerization of subunits of FDH. However, it is unable to oxidize 10-formyl-THF, due to its inability to bind the folate substrate: this domain has neither a folate-binding site nor participates in formation of such a site [53–55].

Since 1997, when the first two crystal structures of aldehyde dehydrogenases were solved [74,75], a large number of aldehyde dehydrogenases structures have been reported. These studies permitted the identification of residues that are important for aldehyde dehydrogenase function. It was not surprising to find that most of the functional residues are present in the sequence of FDH. The recently solved crystal structure of Ct-FDH [76] confirmed a fold typical for class 1 and class 2 aldehyde dehydrogenases [75,77] with four identical subunits arranged as a dimer of dimers [75,77,78] (Fig. 4A). Similar to other members of the family, the Ct-FDH subunit is comprised of three sub-domains (Fig. 4B): a NADP-binding sub-domain (residues 405–539, 564–675, and 867–893), a catalytic sub-domain (residues 676–866), and an oligomerization sub-domain (residues 540–563 and 894–902). The NADP-binding and catalytic domains are α/β in structure, whereas the oligomerization domain contains a three-stranded antiparallel β -sheet. While residues 423–902 of Ct-FDH can be superimposed closely into structures of other class 1 and 2 ALDHs, the rest of its amino-terminus is different from ALDH fold: it forms a β -hairpin, packed against the main body of the molecule (Fig. 4B). This region is not required for ALDH catalytic activity of Ct-FDH [55] and is likely a part of the interdomain interface in full-length FDH.

Active site and coenzyme specificity—The active site of Ct-FDH is similar to those of ALDH including the two residues essential for catalysis, Cys707 and Glu673 [75,77]. The substrate entrance tunnel is situated between the coenzyme-binding and the catalytic domains of the protein. The amino acid composition of the tunnel is closer to that of the ALDH1 group, which is more specific towards larger substrates [77,79]. Interestingly, while the overall mode of coenzyme binding is highly similar between Ct-FDH and class 1 and 2 ALDH, which are NAD⁺-specific, Ct-FDH, as well as the full-length enzyme, demonstrates a strong preference for NADP⁺: K_m for NAD⁺ is about three order of magnitude higher than K_m for NADP⁺ [55]. The structural basis for the shift in coenzyme specificity has been highlighted in our recent study [76]. Interestingly, while bacterial NADP⁺-specific ALDHs have been reported [80–82], and class 3 ALDH can use both NAD⁺ and NADP⁺ *in vitro* [83], it appears that FDH is the only example of strictly NADP⁺-specific ALDH in mammals. Comparison of two crystal structures of NADP⁺-specific bacterial ALDH, from *Streptococcus mutans* [81] and from *Vibrio harveyi* [82], with the structure of Ct-FDH showed that, in the latter, coordination of the 2'-phosphate of the coenzyme involves different residues, indicating a different mode for accommodating the extra phosphate.

Coenzyme-induced rearrangements in the active site and coenzyme mobility—

As in other ALDH enzymes [74,75], the coenzyme molecule is bound in Ct-FDH through a non-classical Rossmann fold. The nicotinamide moiety of the bound NADP⁺ is in the so-called hydride transfer, or extended conformation (the nicotinamide ring lies close to the cysteine nucleophile) and is buried in a pocket that approaches the active site from a direction opposite to the substrate entrance tunnel. Overall, binding of the cofactor does not result in large-scale rearrangements of the protein structure and the major differences between the apoenzyme and the NADP⁺-bound enzyme are observed in the active site. In the apoenzyme, the carboxylate group of Glu673 is within hydrogen bonding distance of the sulfhydryl of Cys707, whereas in the complex with NADP⁺, this side chain has rotated away from the cysteine and become hydrogen bonded to the amide group of the nicotinamide. In the complex with NADPH, Glu673 occupies the same position as in the apoenzyme, thus restoring the contact with Cys707. The nicotinamide moiety of the reduced coenzyme in this structure is distorted and displaced away from the cysteine and the catalytic center, similar to so-called hydrolysis position [74,75,77, 84,85]. The two conformations of the coenzyme observed in the Ct-FDH structures may correlate with two states of the reaction, acylation and deacylation, as was proposed for other ALDHs [75,77,85–87]. In support of this hypothesis, the transition from well-ordered to distorted conformation of the nicotinamide portion of NADP⁺, bound to Ct-FDH, was directly observed after soaking crystals of binary in propanal, that apparently converted an oxidized coenzyme to its reduced form [76].

Unusual feature of Ct-FDH structure: a covalent bond between catalytic cysteine and nicotinamide—

While the flexibility of the coenzyme bound to ALDH is a hallmark of this enzyme family and correlates with enzymatic activity [88], the Ct-FDH structure revealed an unusual mode of NADP⁺ binding. Similar to some other ALDH [86], two conformations of catalytic Cys707 were observed in crystal structures of both apoenzyme and its binary complex with NADP⁺ [76]. Surprisingly, in one of these conformations the distance between the sulfur of the cysteine and C4 atom of the nicotinamide ring is 1.6 Å, indicating a covalent bond between the two atoms. Furthermore, the electron density of the nicotinamide ring is distorted, which is consistent with the tetrahedral geometry of a covalent bond. The presence of such a bond was confirmed by absorption spectroscopy that revealed the appearance of an additional peak in the spectrum of NADP⁺ bound to Ct-FDH. The observed covalent bond is of a temporary (transient) nature and is easily reversible: NADP⁺ readily dissociates from the complex with Ct-FDH [76].

At first glance, such a covalent bond appears to be highly unusual. In general, however, nucleophiles, including the -S⁻ anion, can add reversibly to the *para* position (C4) of nicotinamide to form adducts, producing structures reminiscent of those of the reduced coenzymes [89,90]. Accordingly, covalent adducts of the coenzyme in other enzymes have also been reported [91–94], including demonstration of a covalent attachment of NADH to a catalytic cysteine in GAPDH [95]. Interestingly, prediction of such a bond by molecular dynamics simulations in ALDH3 [96] indicates that its formation is thermodynamically favorable. With this regard, re-examination of some existing structures of ALDH, as well as generation of high-resolution structures, could provide additional examples of the covalent adduct. Indeed, in two cases, such a bond has been already detected [76,97]: in the structure of the allosteric nonphosphorylating GAPDH from *Thermoproteus tenax* [87] and in ALDH2 [85]. It is not clear at present whether the covalent bond is a part of ALDH mechanism. In support of this possibility, chemical activation of the nucleophilic cysteine in non-phosphorylating GAPDH upon coenzyme binding was observed [98].

There are several reasons why the equilibrium of covalent/dissociated states might exist. (1) The interaction may serve to maintain the cysteine in an activated state after its proton has been abstracted by Glu673 and the glutamate has been displaced by NADP⁺. (2) It may also help

activate the C4 of the nicotinamide for hydride transfer. (3) It may hold the coenzyme in the hydride transfer position prior to substrate binding preventing it from occupying the hydrolysis position. It has been also proposed that the covalent bond may protect the cysteine from oxidation during the reaction [97]. Formation of a covalent bond between enzyme and coenzyme might also be a mechanism of activation of the esterase reaction by NAD⁺/NADP⁺ [99]. ALDHs display an esterase activity that is enhanced by a nicotinamide coenzyme even though it is not a participant in the esterase reaction [100]. FDH also displays this type of activity [101]. Thus, stabilization of a deprotonated cysteine by a positively charged coenzyme might facilitate a nucleophilic attack on the substrate. We have also hypothesized [76] that “half-of-the-site” reactivity of some ALDH [102–104] could be explained in the context of a covalent bond between enzyme and coenzyme: equilibrium between covalent and non-covalent states of NADP⁺, each with approximately 50% occupancy, could result in only half of the active sites being active at a specific time-point.

2.4. Role of the intermediate domain of FDH

Until recently, the origin of the intermediate domain (residues 311–399) and its precise function remained unknown. Indeed, it does not have sufficient sequence identity to known proteins to allow its assignment to a specific protein family. Early studies have demonstrated that this domain is important for catalysis: in its absence the mixture of two separately expressed catalytic domains of FDH, the N- and C-terminal, did not produce 10-formyl-THF dehydrogenase activity (34). It has been proposed that this domain is not directly involved in the enzymatic mechanism but serves as a linker, which brings two catalytic domains in close proximity to each other to allow catalysis (35). Therefore, it was surprising to find out that this domain is a structural and functional homolog of carrier proteins. These proteins are components of multi-enzyme complexes involved in fatty acid, polyketide, and non-ribosomal peptide biosynthesis (reviewed in [105,106]). Their characteristic feature is the 4'-phosphopantetheine (4'-PP) moiety, covalently attached to a conserved serine residue through a phosphodiester bond, functioning as a moving arm that holds a growing chain of fatty acids, polyketides, or peptides [107]. Another enzyme with a 4'-PP modification is α -amino adipate semialdehyde reductase, a component of lysine biosynthesis specific to fungi [108,109]. In higher organisms (including humans) the reversal of this pathway has been suggested to serve as a lysine degradation route [110].

All carrier proteins are similar in structure representing a three- or four-helix bundle connected by loops with 4'-PP arm covalently attached to a conserved serine located in one of these loops. The NMR structure of the intermediate domain of human FDH, recently solved by the RIKEN Structural Genomics/Proteomics Initiative (Protein Data Bank # 2CQ8), has demonstrated that the fold is similar to other carrier proteins (Fig. 5). While this structure does not have a prosthetic group, the alignment with other carrier proteins has identified Ser354 as the residue carrying this modification, a suggestion confirmed by site-directed mutagenesis experiments [111]. These studies add an additional layer of complexity to the FDH functional organization by highlighting the requirement for a post-translational modification. The presence of the 4'-PP group, which extends about 20 Å from the peptide part of the intermediate domain, provides a mechanism to access the catalytic centers of FDH, a feature especially crucial for the ALDH center which is situated at the end of 12 Å deep tunnel. How the 4'-PP arm transfers formyl between domains is not clear, but conformational changes within the intermediate domain [112], as well as the catalytic domains [57], are likely to assist in this process.

3. Mechanism of FDH catalysis

The site-directed mutagenesis studies provided evidence that the hydrolase reaction and aldehyde dehydrogenase reaction are parts of the overall dehydrogenase catalytic mechanism of FDH towards 10-formyl-THF. Thus, replacement of either of the two conserved catalytic

residues in the hydrolase active center, His106 and Asp142, resulted in mutant enzymes lacking both hydrolase and dehydrogenase activities and suggested a tight connection between the two mechanisms [61,64]. Likewise, mutation of catalytically important Cys707 [73] or Glu673 [76] in the aldehyde dehydrogenase C-terminal domain abolished both dehydrogenase activities but the hydrolase activity remained. The likely explanation of these results is that the 10-formyl-THF dehydrogenase reaction is not a separate reaction but a combination of two steps, the hydrolase reaction and aldehyde dehydrogenase reaction and that the 10-formyl-THF hydrolysis step should always precede the dehydrogenase catalysis. In this mechanism, uncoupling the Ct-FDH domain would stop the overall reaction after the hydrolase step releasing formate [112]. While it is not clear whether hydrolase and ALDH reactions take place *in vivo*, they are a reflection of the fact that the catalytic domains of FDH can function as separate enzymes [54,55].

Thus, to convert 10-formyl-THF to THF and CO₂ FDH employs a complex mechanism, which includes three general sequential steps, two catalytic and one transfer as schematically shown in Fig. 5. Each step is accomplished by a separate domain of the enzyme. In the first step, which takes place in the N-terminal hydrolase domain, the formyl group is removed by a hydrolytic cleavage from 10-formyl-THF. In the second step, generated formyl is transferred onto the sulfhydryl moiety of 4'-PP arm of the intermediate domain. The arm then swings from the hydrolase catalytic center into the ALDH catalytic center and donates the formyl to the ALDH active site Cys707. In the third step, carried out by the ALDH C-terminal domain, the formyl is oxidized to CO₂ through an ALDH-like mechanism, which involves reduction of NADP⁺ into NADPH.

The sequence of events proposed above reflects the overall mechanism of FDH but its details await future investigations. For example, studies of the hydrolase catalysis of FDH indicated that participation of a sulfhydryl moiety is essential to complete the reaction: *in vitro* it requires the presence of millimolar concentrations of 2-mercaptoethanol [46,101]. A molecule of 2-mercaptoethanol bridging two catalytic residues, His106 and Asp142, was seen in the crystal structure of rat Nt-FDH [57]. It occupies the same spatial position as the expected position of the formyl group of 10-formyl-THF bound in the active site [62], thus mimicking the formyl moiety. It has been proposed therefore that, in the absence of the rest of FDH molecule, THF and formate remain bound in the active site after the hydrolase reaction but displacement of formate by 2-mercaptoethanol brings the reaction to completion. In light of the function of the intermediate domain it is more likely, however, that 2-mercaptoethanol replaces a functionality lost in the absence of the intermediate domain thus forming a thiol ester with formate, which is then rapidly hydrolyzed yielding formic acid. Of note, 10-formyl-THF hydrolase, which shares some sequence similarity with Nt-FDH, GARFT and FMT, exists in bacteria [113, 114]. The fact that this enzyme does not require participation of a thiol for catalysis implies a feature of Nt-FDH mechanism that prevents hydrolase reaction if an assisted discharge of formyl is not available.

While the ALDH catalysis of FDH is similar to a common ALDH mechanism, the exact mechanism of oxidation of formyl to CO₂ as a part of overall 10-formyl-THF dehydrogenase reaction is not clear. With this regard it should be noted that the oxidation states of a substrate and a product of ALDH reaction (an aldehyde and an acid oxidation level, correspondingly) are different from those of 10-formyl-THF dehydrogenase reaction (an acid and CO₂ oxidation level, correspondingly). Therefore, oxidation of formyl should proceed through a different intermediate that, in turn, might require an altered ALDH mechanism. Curiously, an NAD⁺-dependent dehydrogenase oxidizing formate to CO₂, which exists in bacteria, yeast and plants, employs a mechanism different from that of ALDH: it directly transfers a hydride ion from the substrate to NAD⁺ without stages of acid-base catalysis [115].

4. Conclusion

Domain organization is an intrinsic element of protein structure and the majority of proteins consist of distinctive domains that can act independently or cooperatively to achieve a unique function [116,117]. The interaction between functional modules within a single polypeptide determines mechanisms by which multidomain proteins operate. Often evolution combines enzymes from the same metabolic pathway in one polypeptide thus compartmentalizing the synthesis of the final product and allowing channeling intermediates between reactions within the pathway [49,118–121]. In such multi-functional fusions each module serves its own catalytic purpose. In the case of FDH, however, the combination of three genes creates an end product with new enzymatic activity.

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Abbreviations

4'-PP	4'-phosphopantetheine
ALDH	aldehyde dehydrogenase
Ct-FDH	C-terminal domain of FDH
FDH	10-formyltetrahydrofolate dehydrogenase
FMT	methionyl-tRNA formyltransferase
GARFT	glycinamid rybonucleotide formyltransferase
Nt-FDH	N-terminal domain of FDH
THF	tetrahydrofolate

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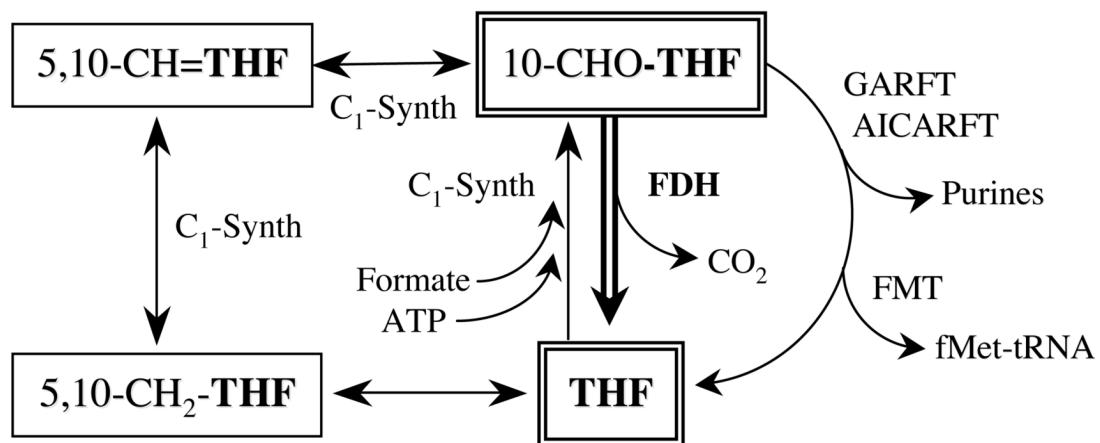
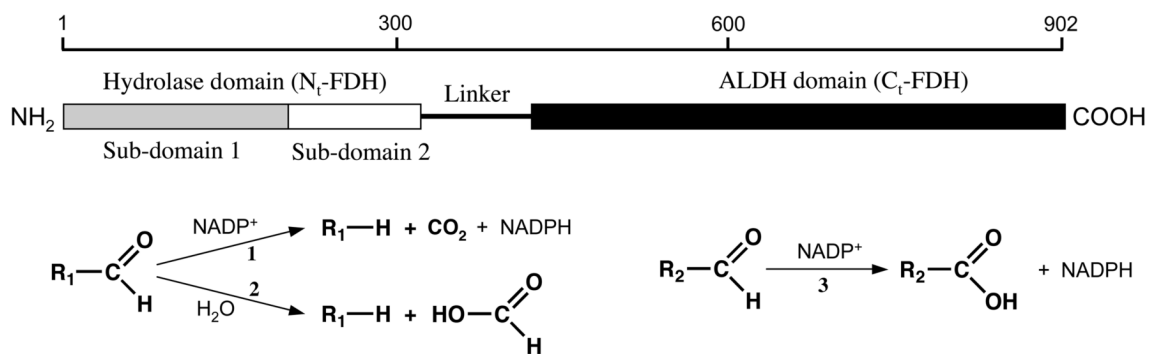


Fig. 1. Pathways of folate metabolism involving 10-formyl-THF. Abbreviations: THF, tetrahydrofolate; CH₂, methylene; CH, methenyl. Enzymes involved in 10-formyl-THF metabolism are shown. C₁-Synth, C₁-synthase.



$\text{R}_1 = \text{THF}$ $\text{R}_2 = -(\text{CH}_2)_n-\text{CH}_3$ 1, Dehydrogenase reaction; 2, hydrolase reaction; 3, ALDH reaction

Fig. 2.

Schematic diagram of domain organization of the FDH subunit and the reactions catalyzed by the enzyme. Dehydrogenase reaction (reaction 1) requires the full-length enzyme; hydrolase reaction can be catalyzed by the full-length enzyme or by the hydrolase domain (residue 1–310); ALDH reaction can be catalyzed by the full-length enzyme or by the C-terminal ALDH domain (residue 420–902).

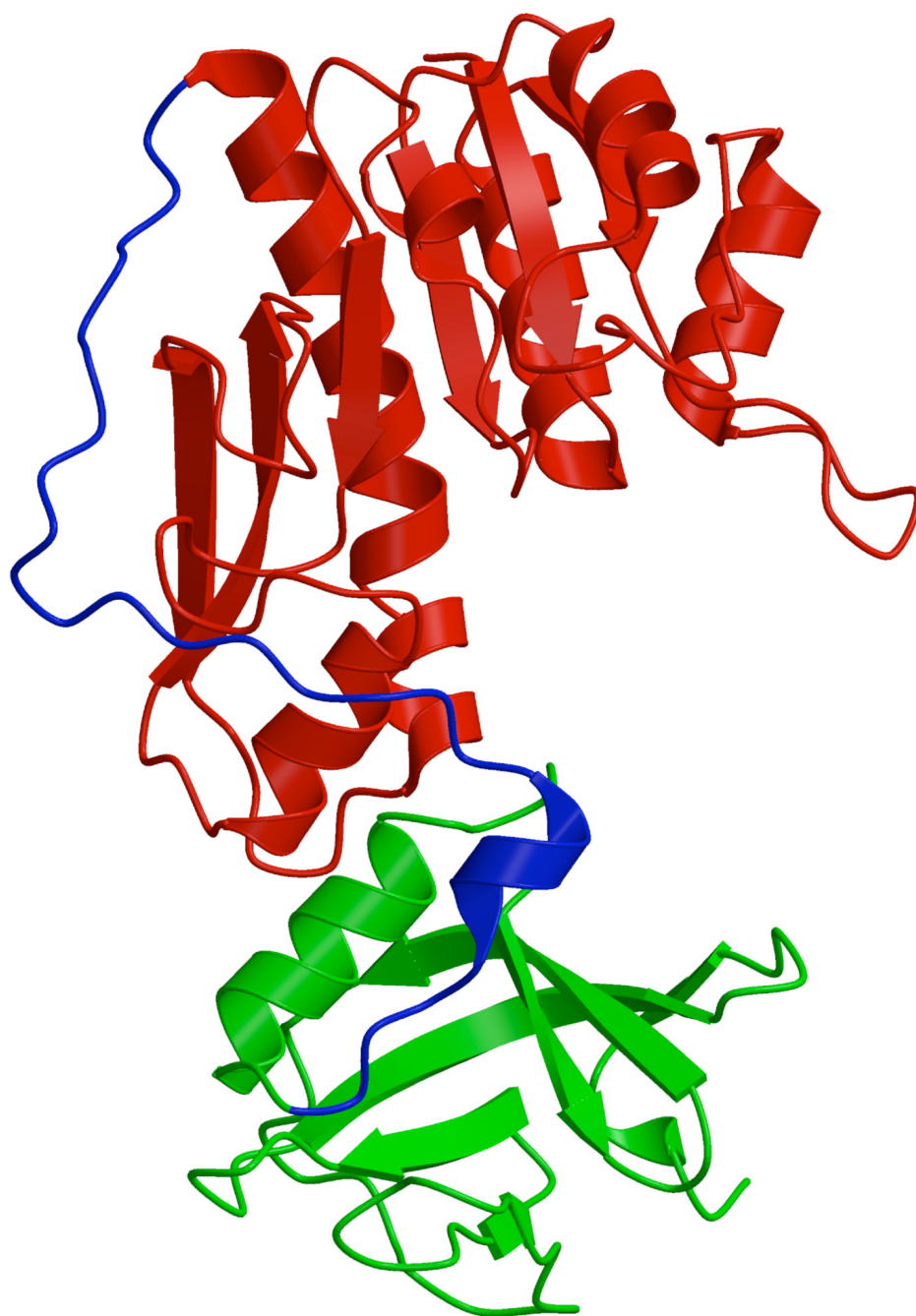


Fig. 3. Overall structure of the N-terminal domain of FDH (Nt-FDH). *Red*, the folate-binding sub-domain; *green*, the sub-domain similar to tRNA binding domain of FMT; *blue*, the linker connecting two sub-domains.

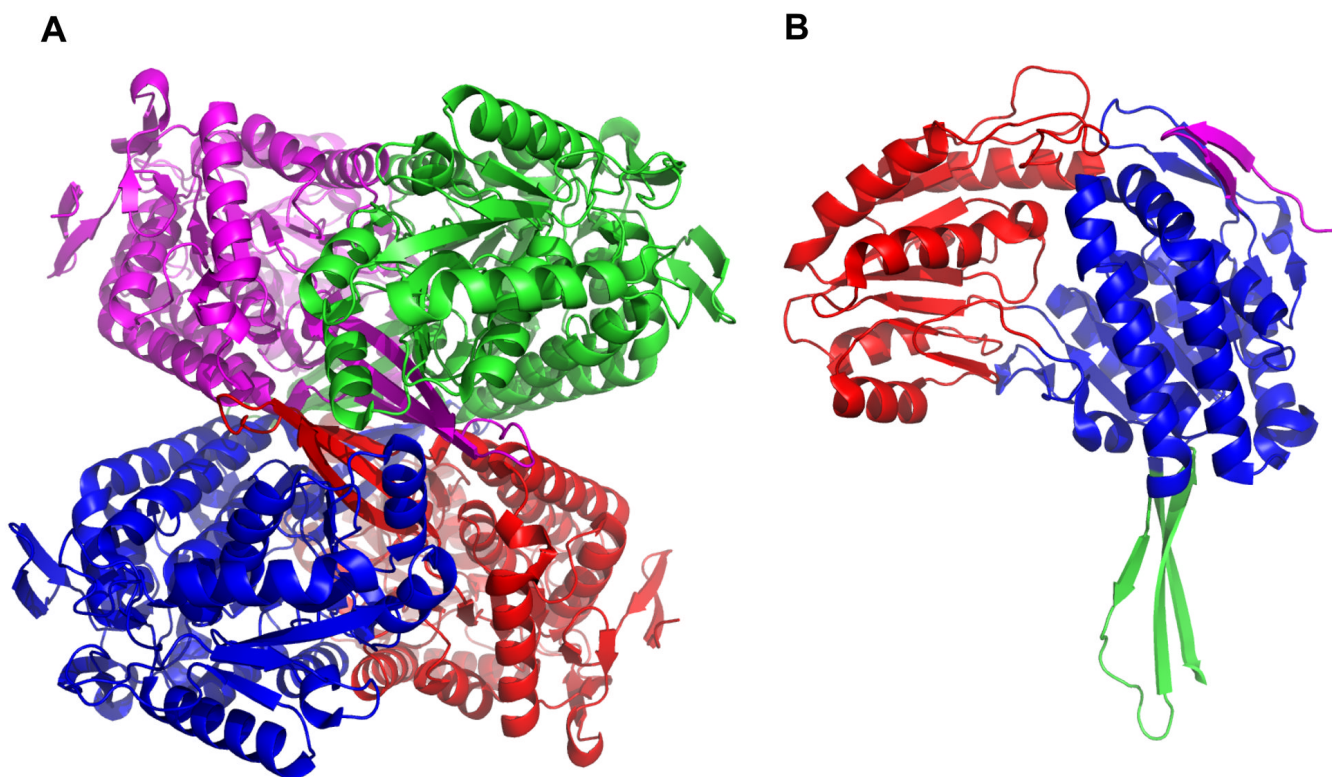


Fig. 4. (A) Ribbon diagram of the Ct-FDH tetramer. The four subunits are in different colors. (B) Ribbon representation of the Ct-FDH subunit: the NADP-binding domain (*blue*); the catalytic domain (*red*); the oligomerization domain (*green*); a hairpin representing a part of the Ct-FDH interface with the rest of FDH is shown in *magenta*.

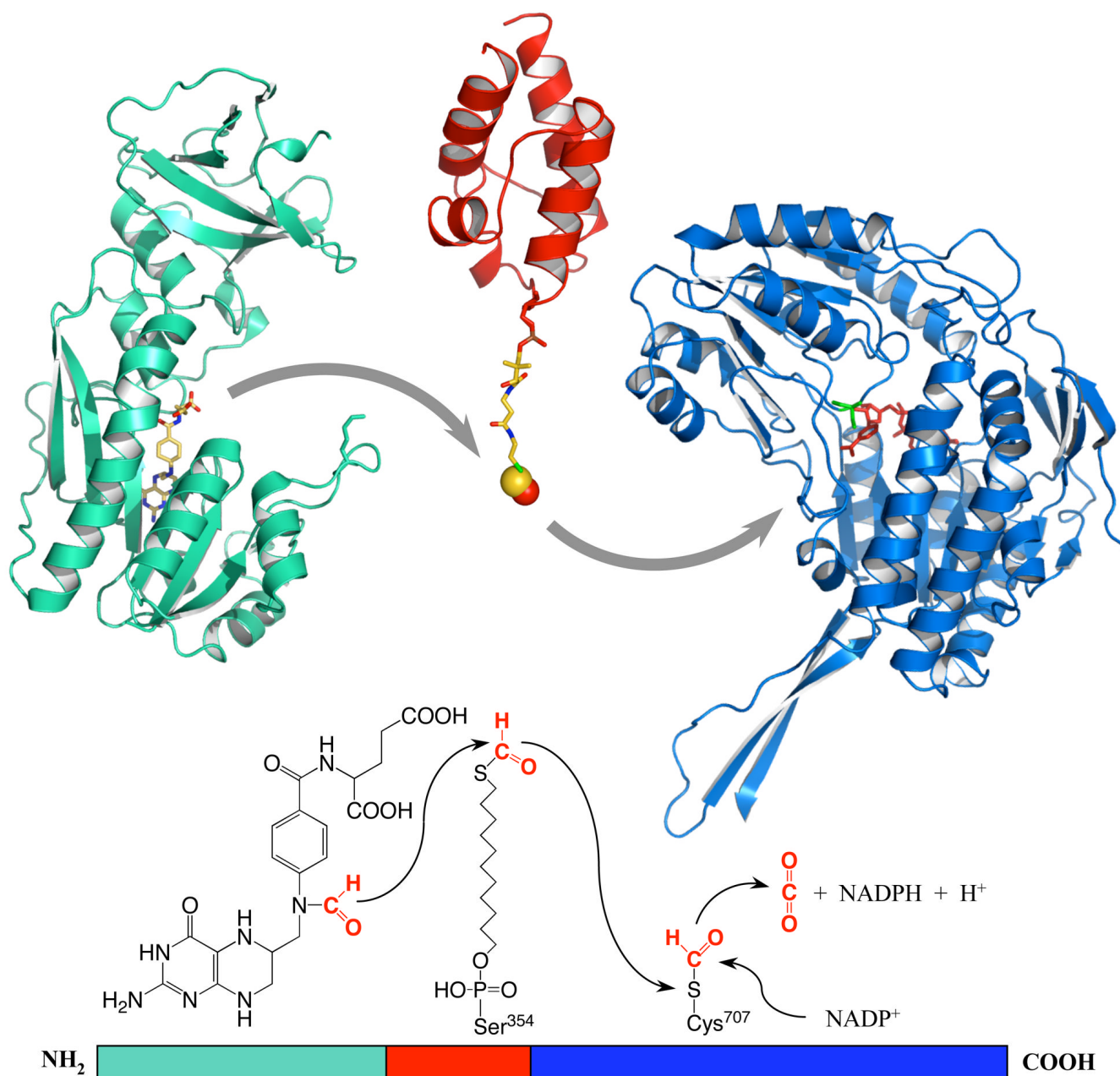


Fig. 5.

FDH mechanism. FDH subunit (a single polypeptide) consists of three domains: N-terminal (green), intermediate (red) and C-terminal (blue). FDH converts its substrate, 10-formyl-THF, to THF and CO₂ in three steps: (i) N-terminal domain hydrolytically cleaves the bond between formyl group and THF; (ii) 4'-PP prosthetic group (shown in stick mode), covalently bound to the intermediate domain at Ser354, transfers formyl (red-yellow space fill) from N-terminal to C-terminal domain; (iii) formyl is oxidized in the C-terminal domain to CO₂ by function of catalytic nucleophile Cys707 with participation of NADP⁺. For simplicity, Ct-FDH is shown as a monomer. Bound folate substrate and NADP⁺ molecule are shown in N- and C-terminal domains, correspondingly.

Table 1

Protein sequence similarity among FDHs from different species.

	<i>H. sapiens</i>	<i>R. norvegicus</i>	<i>M. musculus</i>	<i>C. elegans</i>	<i>D. melanogaster</i>
<i>H. sapiens</i>	100	92	92	58	59
<i>R. norvegicus</i>	92	100	97	58	57
<i>M. musculus</i>	92	97	100	59	58
<i>C. elegans</i>	58	58	59	100	54
<i>D. melanogaster</i>	59	57	58	54	100