Expression, Purification, and Properties of the Aldehyde Dehydrogenase Homologous Carboxyl-terminal Domain of Rat 10-Formyltetrahydrofolate Dehydrogenase*

(Received for publication, September 12, 1996, and in revised form, January 17, 1997)

Sergey A. Krupenko‡§, Conrad Wagner‡, and Robert J. Cook‡

From the Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, Tennessee 37232 and the Research Service, Department of Veterans Affairs Medical Center, Nashville, TN 37212

The liver cytosolic enzyme, 10-formyltetrahydrofolate dehydrogenase (FDH) (EC 1.5.1.6) catalyzes two reactions: the NADP⁺-dependent oxidation of 10-formyltetrahydrofolate to tetrahydrofolate and CO₂ and the NADP⁺-independent hydrolysis of 10-formyltetrahydrofolate to tetrahydrofolate and formate. The COOH-terminal domain of the enzyme (residues 420–902) is about 48% identical to a family of NAD-dependent aldehyde dehydrogenases (EC 1.2.1.3), and FDH possesses aldehyde dehydrogenase activity. We expressed the COOH-terminal domain (residues 420–902) of FDH in insect cells using a baculovirus expression system. The recombinant protein was released from insect cells to the culture medium and was purified from the medium by a two-step procedure: precipitation with 35% saturated ammonium sulfate followed by chromatography on hydroxyapatite. The purified COOH-terminal domain displayed aldehyde dehydrogenase activity similar to that of native FDH but had neither dehydrogenase nor hydrolase activity toward folate substrates. Aldehyde dehydrogenase activity of the COOH-terminal domain and FDH was independent of the presence of 2-mercaptoethanol while 10-FDDF dehydrogenase activity of FDH occurred only in the presence of 2-mercaptoethanol. The COOH-terminal domain existed as a tetramer showing that the sites for oligomerization of subunits in native FDH resides in this domain. Using titration of tryptophan fluorescence, it was found that the COOH-terminal domain bound NAD⁺ to the same extent as FDH (Kₐ 0.2 and 0.3 μM, respectively) but did not bind folate. Both FDH and its COOH-terminal domain also bound NAD⁺ (Kₐ 11 and 16 μM, respectively) as measured by fluorescence titration. Both proteins were able to catalyze the aldehyde dehydrogenase reaction utilizing NADP⁺ or NAD⁺, but the Kₐ for NAD⁺ was three orders higher than that for NADP⁺ (2 μM and 1.5–2.0 μM, respectively). The concentration of NAD⁺ required for the reaction was high compared with the physiological level of NAD⁺, suggesting that the reaction does not occur in vivo. NAD⁺ at physiological concentrations stimulated the aldehyde dehydrogenase reaction performed by FDH or its COOH-terminal domain using NADP⁺.

The liver cytosolic enzyme, 10-formyltetrahydrofolate dehydrogenase (FDH) (EC 1.5.1.6) catalyzes two reactions: the NADP⁺-dependent oxidation of 10-formyltetrahydrofolate to tetrahydrofolate and CO₂ and the NADP⁺-independent hydrolysis of 10-formyltetrahydrofolate to tetrahydrofolate and formate (1–3). The functional form of the enzyme is a tetramer consisting of identical 99 kDa subunits (2–5). The physiological role of the enzyme is not clear, but it probably serves to recycle 10-formyltetrahydrofolate not required for purine synthesis back to tetrahydrofolate where it is available for other one-carbon reactions (6). Although it was reported that dehydrogenase and hydrolase activities were products of separate cytosolic and mitochondrial forms of this enzyme (7), we have subsequently shown that both activities are associated with the product of a single gene (8). The amino acid sequence of FDH contains 902 residues and can be divided into three domains (9). The amino-terminal sequence of the sequence (residues 1–203) is 24–30% identical to a group of glycaminide ribonucleotide transformylases (EC 2.1.2.2) from different species. The intermediate domain (residues 204–416) does not show extensive homology to any known protein sequence. The carboxyl-terminal sequence (residues 420–902) has about 48% identity with a series of NAD⁺-dependent aldehyde dehydrogenases (EC 1.2.1.3) (9, 10), and the enzyme is able to oxidize aldehydes (9, 11). At present, all aldehyde dehydrogenases classify into four groups (12). FDH has a higher identity with class I (cytoplasmic aldehyde dehydrogenases) and class II (mitochondrial aldehyde dehydrogenases) enzymes (13). An error in reading the original cDNA sequence (9) was recently noted which changes a 23-amino acid sequence from residue 566 to 588. The changes have been made in the data base files. This region is one of the highly conserved domains in the aldehyde dehydrogenase family (10), which is also conserved in FDH (Fig. 1). Recently (5), we have shown that cysteine 707 of rat FDH, which is homologous to a strictly conserved cysteine of NAD-dependent aldehyde dehydrogenases is directly involved in the dehydrogenase active site, apparently serving as the catalytic nucleophile.

Existence of three putative domains in the sequence of FDH, two of which have identity with different classes of enzymes suggests that the enzyme activities derive from the two functional domains. The third, intermediate, domain located between the NH₂- and COOH-terminal domains was predicted to connect the functional domains together (4, 9) and does not participate directly in the mechanism of enzyme action. Re-
Fig. 1. Sequence alignment of the COOH-terminal domain of FDH and mouse liver cytosol aldehyde dehydrogenase (class 1). The alignment was generated with GENALIGN (Intelligenetics Inc.) using the Needleman-Wunsch algorithm (28). Identical residues are indicated by vertical lines, and conservative changes are shown as +. Rat FDH sequence, residues 420–902, was taken from SWISS-PROT P28037. This sequence, residues 420–902, was taken from SWISS-PROT P28037. The underlined sequence was taken from SWISS-PROT P24549. The conserved active site cysteine residue is in boldface type.

(c)ently Schirch et al. (4) using limited proteolytic digestion of rabbit liver FDH have shown that the aldehyde dehydrogenase activity resides in a 53-kDa COOH-terminal domain (4). Neither this domain nor a bigger COOH-terminal proteolytic fragment (63 kDa) had 10-FTDH dehydrogenase activity (4).

A comparison between the molecular mass of the proteolytic COOH-terminal fragments obtained in the study of Schirch et al. (4) and the amino acid composition of these fragments suggests that the fragments were truncated from the carboxyterminal end. In the present study, we expressed the entire COOH-terminal domain of FDH to investigate its properties and to learn whether it is able to fold into a functional protein.

MATERIALS AND METHODS

Materials—10-formyl-5,8-dideazafolate (10-FDDF) was obtained from Dr. John B. Hynes, Department of Pharmaceutical Chemistry, Medical University of South Carolina. (6–8, 10–FTHF was prepared by the method of Rabinowitz (14). Oligonucleotides were synthesized on an Oligo 1000 DNA Synthesizer (Beckman). Restriction enzymes were purchased from New England BioLabs, Inc. (Beverly, MA) or from Stratagene (La Jolla, CA). Grace’s insect cell medium was purchased from Invitrogen (San Diego, CA). Fetal bovine serum was purchased from HyClone ( Logan, UT) or from New England BioLabs, Inc. (Beverly, MA) or from Bio-Rad. Standards and nitrocellulose membranes were purchased from Bio-Rad. Oligonucleotides were synthesized on an Applied Biosystems dideoxynucleotide chain-termination method (17) using a model 373A fluorescence sequenator.

Protein Expressions—Sf9 and High-five insect cells (Invitrogen) were grown as earlier described (15). The constructed transfer plasmids and linearized viral DNA were co-transfected into Sf9 cells using the BaculoGold transfection kit (Pharmingen, San Diego, CA) according to the manufacturer directions. Recombinant viral stocks were amplified in Sf9 cells (15) to produce high titer virus stock. High-five cells were seeded as a monolayer in 25-cm2 flasks (about 2 × 10^6 cells/flask) and grown overnight. Each flask was infected with 0.1 ml of high-titer virus stock. Culture medium and cells were collected separately 2–4 days post-infection and the recombinant protein production was detected by SDS-PAGE (18) followed by western immunoblot analysis (19) with rabbit polyclonal antiserum raised against pure rat liver FDH and goat anti-rabbit IgG conjugated to alkaline phosphatase (Bio-Rad) (20). Protein concentration was determined using the Bradford protein assay (21). For large scale recombinant protein production High-five cells were seeded in 225-cm2 flasks (about 2 × 10^6 cells/flask) and grown overnight. Each flask was infected with 0.1 ml of high titer virus stock. The culture medium, 96 h post-infection, was centrifuged to remove detached cells, and was used to isolate recombinant protein.

Purification of Recombinant Proteins—All buffers used in purification steps contained 10 mM 2-ME and 1 mM NaN3. FDH was purified as described earlier (15). To purify the COOH-terminal domain, ammonium sulfate was added to cell-free culture medium (about 100 ml) to 35% (20 g/100 ml) saturation at 4 °C. The solution was centrifuged (15 min, at 10,000 × g), and the pellet was dissolved in 5 mM potassium phosphate buffer, pH 7.0. Insoluble material was removed by centrifugation (10 min at 12,000 × g), and the solution was dialyzed overnight against the same buffer at 4 °C. The solution was then passed through a gel filtration column (5 × 10 cm) equilibrated with 5 mM potassium phosphate buffer, pH 7.0, and then a linear gradient of phosphate (5–100 mM) was applied. The peak of the COOH-terminal domain was eluted at about 200 mM phosphate.

Molecular Size Determination—Size-exclusion chromatography was...
done on a Sephacryl S-300 (Pharmacia) column (2.5 × 70 cm) at 4 °C. The column was calibrated using a gel filtration calibration kit (Pharmacia). The sample (10 ml) was applied to the column equilibrated with 50 mM potassium phosphate buffer, pH 7.0, containing 10 mM 2-ME and 1 mM NaN3 at a flow rate of 18 ml/h. Elution from the column was carried out with the same buffer at the same flow rate. Protein was detected using a flow through monitor (Pharmacia). Fractions of 4 ml were collected, and aldehyde dehydrogenase activity of the fractions was measured. The fractions were also analyzed by SDS-PAGE followed by immunoblot analysis.

**Assay of Enzyme Activity**—All assays were performed at 30 °C in a Perkin-Elmer 4B double beam spectrophotometer (Norwalk, CT). For measurement of hydrolytic activity, the reaction mixture contained 0.05 M Tris-HCl, pH 7.8, 100 mM 2-ME, and varying amounts of substrate, either 10-FTHF or 10-FDDF (0.5–18 μM). The enzyme (1 μg) was added in a final volume of 1.0 ml. The reaction was started by the addition of enzyme and read against a blank cuvette containing all components except enzyme. Appearance of product was measured at either 295 nm for 5,6-dideazafolate (DDF) or 300 nm for THF using molar extinction coefficients of 18.9 × 103 and 21.7 × 103 for DDF (22) and THF (7), respectively. Addition of NADP+ to the reaction mixture provided a measure of both dehydrogenase and hydrolytic activity, i.e. total activity of the enzyme. Hydrolytic activity measured in the absence of NADP+ was subtracted from the total activity to give the dehydrogenase activity. Dehydrogenase activity was also measured independently using the increase in absorbance at 340 nm due to production of NADPH and the molar extinction coefficient of 6.2 × 105 nmol/min.

Aldehyde dehydrogenase activity was assayed using propenal essentially as described by Lindahl and Evces (23). The reaction mixture contained 50 mM CAPS buffer, pH 9.4, 5 mM propenal, 1 mM NAD+, and enzyme in a total volume of 1 ml. Activity was estimated from the increase in absorbance at 340 nm. Enzyme activity is given as nmol/min.

**Analysis of Kinetic Data**—Initial reaction rates were used to determine the respective enzyme activities. KCat (BioMetallics Inc., Princeton, N.J.) was used to determine kinetic parameters from the Michaelis-Menten equation by nonlinear regression.

**Fluorescence Studies**—Binding of NADP+ and NAD+ to FDH and to its COOH-terminal domain and binding of 10-FDDF and DDF to the COOH-terminal domain were detected by measuring the quenching of enzyme fluorescence. These experiments were done on a Perkin-Elmer model 650–40 Fluorescence Spectrophotometer. Protein samples (about 10.0 μg) were in 50 mM Tris-HCl, pH 7.8, and 50 mM 2-ME. In other experiments where binding of 10-FDDF and DDF was studied, 2-3 times more protein was added to the samples. The NADP+ concentration was varied from 0.02 μM to 10 μM and concentration of NAD+ was varied from 0.5 μM to 100 μM. The 10-FDDF and DDF concentrations were varied from 50 nM to 2500 nM. The experiments were done at 25 °C. Fluorescence excitation was at 291 nm, and the emission was monitored at 340 nm (5, 24). The Ks for the ligands were calculated from data on fluorescence quenching in the presence of ligands that were plotted in a linear form. The value (1/F0)−1 was plotted against the inverse of ligand concentration (5, 25). This is a modified form of the classical Stern-Volmer plot that relates the decrease in fluorescence to the concentration of a collisional quencher (26). F is intrinsic fluorescence observed at a quencher concentration; F0 is fluorescence in the absence of quencher. The slope of the line (least squares fit) gave a Ks. Variation of the measured values was about 5%.

**Sequence Compilation and Analysis**—Nucleotide sequences were compiled and analyzed using DNA Strider 1.0. Data base searches were performed by BLASTP (27). The sequence alignment was achieved by GENALIGN (IntelliGenetics Inc.) using the Needleman-Wunsch algorithm (28).

**RESULTS**

**Expression and Purification of the COOH-terminal Domain of FDH**—The expressed COOH-terminal domain was present in the culture medium as well as in the cells (Fig. 2). Maximum protein production was achieved at 4 days postinfection (Fig. 2) and coincided at the level up to 6 days postinfection (data not shown). Distribution of the protein between cells and medium was almost equal, i.e. about 50% of the protein was released from cells to the medium (5 μl from a total volume of 2 ml of medium and 10 μl from a total volume of 2 ml of cell lysate were applied on the gel, i.e. for direct comparison, it is necessary to increase intensity of the bands in Fig. 2 corresponding to the COOH-terminal domain from the medium by 40 times).

We previously used affinity chromatography on immobilized 5-formyltetrahydrofolate (29, 30) to easily purify recombinant FDH (5, 15). In the case of the COOH-terminal domain, the affinity chromatography was ineffective with less than 10% of the protein retained on the column (data not shown). To purify the COOH-terminal domain, we used a two-step procedure that included precipitation with ammonium sulfate followed by chromatography on hydroxyapatite. We purified the COOH-terminal domain from the culture medium since 50% or more of the expressed protein was released from cells. Ammonium sulfate at 35% saturation in our experiments almost completely precipitated the COOH-terminal domain (Fig. 3). This step gave a good purification of the COOH-terminal domain and separated it from the major protein of the culture medium, bovine serum albumin, which precipitates from solution only at a high ammonium sulfate concentration (70–100% of saturation) (31). Chromatography of the 35% ammonium sulfate saturation fraction on hydroxyapatite showed that the COOH-terminal domain was retained by the column in 5 mM phosphate buffer and was eluted at a concentration of 200 mM phosphate. After this step, we obtained a preparation of the protein which was about 95% pure (Fig. 3).

**Properties of the COOH-terminal Domain**—Analysis of enzyme activities of the COOH-terminal domain showed that the protein possesses aldehyde dehydrogenase activity similar to that of FDH. The specific activity, Km for propenal and Kcat for NADP+ were very close for these two proteins (Table I). Both proteins were able to oxidize substrate only in the presence of physiological concentrations of NADP+, but not NAD+ (concentrations of NAD+ up to 0.5 mM were tested in these experiments). At higher NAD+ concentrations (above 0.5 mM), we did observe aldehyde dehydrogenase activity with a Kcat for NAD+ of about 2 mM (Fig. 4). The quenching of tryptophan fluorescence with NADP+ showed that binding of NADP+ was very similar for the COOH-terminal domain and the entire protein (data not shown). Similar results were obtained after quenching of tryptophan fluorescence with NAD+, but affinity of the
proteins for NAD$^+$ was about 50-fold lower than for NADP$^+$. The $K_d$ calculated from these data are presented in Table II. In all cases, the quenching was saturable and revealed the presence of only a single type of binding site for each of the coenzymes. No further quenching of the fluorescence was observed in the presence of only a single type of binding site for each of the coenzymes. To study whether NAD$^+$ would inhibit the aldehyde dehydrogenase reaction through competition for the NAD$^+$ binding site, we carried out the aldehyde dehydrogenase reaction in the presence of 5.0 $\mu$M NADP$^+$ and much higher concentrations of NAD$^+$ in the range of 10 $\mu$M to 1.0 mM. NAD$^+$ did not inhibit aldehyde dehydrogenase reaction in these experiments. On the contrary, stimulation of the reaction in the presence of NAD$^+$ was observed (Fig. 5). The maximum activation achieved was at 250–500 $\mu$M NAD$^+$, and the increase in activity was about 70% for COOH-terminal domain and about 50% for FDH (Fig. 5). We calculated the activation constants for NAD$^+$ from the difference between reaction rates in the presence of NAD$^+$ and in the absence of NAD$^+$ as a function of NAD$^+$ concentration. This gave a value of 46 $\pm$ 12 $\mu$M of NAD$^+$ for FDH and 92 $\pm$ 9 $\mu$M of NAD$^+$ for the COOH-terminal domain.

**Dependence of aldehyde (Ald DH) dehydrogenase activity of FDH and its COOH-terminal (C-term.) domain on concentration of NAD$^+$**. Curves with open circles represent COOH-terminal domain; curves with dark circles represent FDH. Insert shows the Lineweaver-Burk plot for the data. The assay was done as described under “Materials and Methods,” but samples did not contain NADP$^+$, which was replaced with NAD$^+$, 1.0–10 mM, and about 2.5 $\mu$g of enzyme was used in the assay.

**Table II**

<table>
<thead>
<tr>
<th>Coenzyme</th>
<th>FDH</th>
<th>COOH-terminal domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>NADP$^+$</td>
<td>0.3</td>
<td>0.2</td>
</tr>
<tr>
<td>NAD$^+$</td>
<td>11</td>
<td>16</td>
</tr>
</tbody>
</table>

**Analysis of the Oligomeric Structure of the COOH-terminal Domain**—We studied the oligomeric structure of the COOH-terminal domain using size-exclusion chromatography on a Sephacryl S-300 column. For this purpose, we applied to the column the medium after COOH-terminal domain expression without any preceding purification steps and measured the aldehyde dehydrogenase activity of collected fractions. Fig. 8 shows that maximum aldehyde dehydrogenase activity eluted with a protein of about 220 kDa molecular mass. SDS-PAGE and western immunoblot analysis of the eluted fractions confirmed that the COOH-terminal domain was present in the fractions corresponding to a protein of about 220 kDa molecular mass (Fig. 8, inset). Since the molecular mass of the expressed COOH-terminal domain is 52,707 Da, we concluded that it exists as a tetramer.
The fact that FDH has three catalytic activities suggests that the enzyme has several catalytic sites and that these sites could reside in different domains or that two or even three domains together could be involved in formation of a single catalytic site and be responsible for the activities. However, a combination of these two possibilities cannot be excluded, and some activities could belong to a single domain while other activities could be the result of several domains acting in concert. Thus, sequence identity between the COOH-terminal domain of FDH (residues 420–902) and a series of NAD-dependent aldehyde dehydrogenases suggests that the aldehyde dehydrogenase activity of FDH probably is carried out by the COOH-terminal domain alone, and the other two domains do not participate in this activity. Moreover, 77% of the glycine residues and 78% of the proline residues are conserved in sequences of the COOH-terminal domain of rat FDH and class I aldehyde dehydrogenase from mouse (Fig. 1), suggesting a similarity of tertiary structure and shape of these molecules. Recently, we showed that cysteine 707 of FDH is directly involved in the dehydrogenase active site (5) where it probably plays the role of a catalytic nucleophile. It has also been shown that this residue is crucial in both dehydrogenase reactions utilizing either 10-FTHF or aldehydes. This also suggests that the dehydrogenase catalytic site for 10-FTHF resides in the aldehyde dehydrogenase homologous COOH-terminal domain of FDH. Aldehyde dehydrogenases also have sites for binding aldehydes and the coenzyme, but there have been no reports about the presence of a folate binding site in these proteins. Aldehyde dehydrogenases act on a broad variety of aldehyde substrates (32–34), suggesting that the aldehyde binding site is not highly selective. It has been reported that aldehyde dehydrogenases bind and oxidize even very complicated compounds, e.g. steroids (35–38), thromboxane (39), and retinal (40, 41). Nevertheless, the question of whether aldehyde dehydrogenases bind these compounds in a general aldehyde binding site or whether there are separate binding sites for the various substrates has not been determined. It might be possible that the aldehyde dehydrogenase homologous COOH-terminal domain of FDH binds the folate substrate through the aldehyde binding site. On the other hand, its identity with aldehyde dehydrogenases does not exceed 49%, and so there is a mismatch in sequence of about 250 amino acid residues that could be enough to form a folate binding site.
that aldehyde dehydrogenase activity is indeed carried out by this domain and does not require participation of the two other domains. The kinetic parameters of the reaction were similar for both proteins, and both proteins displayed the same pH maximum for the reaction. The position of the pH maximum at basic pH corresponds to results reported for aldehyde dehydrogenases (42) and is in agreement with the fact that the cysteine residue is an active site nucleophile in the aldehyde dehydrogenase reaction as rates of reactions with participation of thiols increase with increasing pH (43). However, the COOH-terminal protein completely lost its ability to oxidize 10-FTHF or 10-FDDF and did not interact with a folate affinity column, suggesting absence of the folate binding site. Titration of tryptophan fluorescence using 10-FDDF or DDF confirmed that the COOH-terminal domain has no folate binding site. Similar to other class 1 and class 2 aldehyde dehydrogenases (42, 44, 45), the COOH-terminal domain exists as a tetramer proving that this domain is responsible for oligomerization of protomers of FDH.

Surprisingly, we found that although NAD$^+$ was binding to FDH and its COOH-terminal domain, it did not inhibit the aldehyde dehydrogenase reaction as a competitive inhibitor. Moreover, in our experiments, NAD$^+$ activated the aldehyde dehydrogenase reaction performed by both FDH and its COOH-terminal domain. A similar effect of NAD$^+$ on 10-FTHF hydrolase reaction performed by FDH was reported by Scrutton and Beis (3). At that time, high identity of FDH with aldehyde dehydrogenases was not known, and, therefore, the effect of NAD$^+$ on aldehyde dehydrogenase activity was not studied. These results evoke two questions. First, what is the mechanism of such activation, and, second, is there any physiological significance of the NAD$^+$ stimulation? Concerning the biological significance of the stimulation of dehydrogenase activity by NAD$^+$, we would like to note that this effect was present at near physiological NAD$^+$ concentrations (46, 47), suggesting it could take place in the cell. At present, we have no reasonable explanation of the mechanism of NAD$^+$ activation. It was reported that esterase activity of aldehyde dehydrogenase from horse liver was activated in the presence of NAD$^+$ (48), although the reaction does not require the coenzyme. The esterase activity of aldehyde dehydrogenase is associated with the aldehyde dehydrogenase catalytic center (49), and it was proposed that the presence of NAD$^+$ increases nucleophilicity of an active site amino acid and makes the active site nucleophile chemically more reactive (50). Although it is attractive to suggest that NAD$^+$ activates dehydrogenase reactions of FDH by the same mechanism as the esterase reaction of aldehyde dehydrogenase, our study did not reveal presence of a separate binding site for NAD$^+$ as necessary for such a mechanism. The aldehyde dehydrogenase reaction utilizing NAD$^+$ instead of NADP$^+$ probably has no physiological significance, and it may have occurred because of similarity between NADP$^+$ and NAD$^+$ structures. Theoretically, the activity of the enzyme under conditions where the concentration of NAD$^+$ is at least four times lower than the $K_{m}$ should not exceed 20%. Although the concentration of NADP$^+$ in liver is about 5–80 times lower than the concentration of NAD$^+$ (46), the $K_{m}$ for NADP$^+$ is three orders lower than the $K_{m}$ for NAD$^+$. Therefore, at physiological concentrations of the coenzymes, 95% or more of the enzyme activity should result from NADP$^+$ and 5% or less from NAD$^+$.

Earlier, we showed that the dehydrogenase and hydrolase reactions carried out by FDH on folate substrates are strictly dependent on the presence of 2-ME (8). We suggested that one of the possible reasons for 2-ME requirement could be the necessity to reduce the active site cysteine 707 (5, 8). However, as cysteine 707 is a key residue in both dehydrogenase reactions utilizing aldehydes or a folate substrate (5), the fact that the aldehyde dehydrogenase reaction does not require presence of 2-ME supports another role for 2-ME. Thus, it seems more likely that 2-ME is directly involved in the reaction utilizing folate substrates through formation of an intermediate complex with the enzyme and/or substrate. These results raise questions about the action of the FDH in vivo. Can some natural sulfurhydryl compounds such as glutathione substitute for 2-ME inside cells? Is there any physiological significance in the reaction performed on aldehyde substrates, and if so, how much of the enzyme is devoted to 10-FTHF dehydrogenase activity and how much devoted to aldehyde dehydrogenase activity? These questions are yet to be answered.

In contrast to FDH, the $K_{m}$ for NADP$^+$ for different aldehyde dehydrogenases is about 10–100-fold higher than the $K_{m}$ for NAD$^+$ (42, 51), and thus, all aldehyde dehydrogenases use NAD$^+$ in vivo (52) producing NADH. NADH is an electron donor in pathways producing ATP while NADPH serves as electron donor in biosynthetic reactions. So, it is possible that the existence of two enzymes with different preferences for NAD$^+$ or NADP$^+$ serves to separate the electron flux and to direct energy release in reactions utilizing the same substrates into different pathways, energetic or synthetic. It is very likely that the COOH-terminal domain of FDH originated from some pro-aldehyde dehydrogenase gene, but during evolution, the NAD$^+$ binding site changed to an NADP$^+$ binding site. The use of NADP$^+$ by FDH also makes biological sense for folate substrates. NADPH produced by FDH can be used by other enzymes of folate metabolism for synthesis of other folate coenzymes, forming a folate/NADP$^+$ cycle in which NADP$^+$/NADPH may be channeled as well as the folate (53).

Finally, we conclude that the present study answers several important questions. It is shown that the COOH-terminal domain of FDH can be folded by itself into a functional enzyme. The domain has sites responsible for protein oligomerization and bears an NADP$^+$ binding site and aldehyde dehydrogenase catalytic center, but it has no folate binding site and does not bind folate substrates through the aldehyde binding site. Therefore, it cannot utilize the folate substrate in the absence of the rest of FDH. Apparently, the entire enzyme applies the aldehyde dehydrogenase machinery of this domain to perform the dehydrogenase reaction on a new substrate. This supports the hypothesis about the origin of FDH as a result of fusion of separate genes (9).

REFERENCES

COOH-terminal Domain of 10-FDH

Expression, Purification, and Properties of the Aldehyde Dehydrogenase Homologous Carboxyl-terminal Domain of Rat 10-Formyltetrahydrofolate Dehydrogenase

Sergey A. Krupenko, Conrad Wagner and Robert J. Cook

doi: 10.1074/jbc.272.15.10266

Access the most updated version of this article at http://www.jbc.org/content/272/15/10266

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 45 references, 15 of which can be accessed free at http://www.jbc.org/content/272/15/10266.full.html#ref-list-1