The Crystal Structure of the Hydrolase Domain of 10-Formyltetrahydrofolate Dehydrogenase

MECHANISM OF HYDROLYSIS AND ITS INTERPLAY WITH THE DEHYDROGENASE DOMAIN

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10-Formyltetrahydrofolate dehydrogenase (FDH) converts 10-formyltetrahydrofolate, a precursor for nucleotide biosynthesis, to tetrahydrofolate. The protein comprises two functional domains: a hydrolase domain that removes a formyl group from 10-formyltetrahydrofolate and a NADP+-independent hydrolase domain that reduces the formyl to carbon dioxide. As a first step toward deciphering the catalytic mechanism of the enzyme, we have determined the crystal structure of the hydrolase domain of FDH from rat, solved to 2.3-Å resolution. The structure comprises two domains. As expected, domain 1 shares the same Rossmann fold as the related enzymes, methionyl-tRNA-formyltransferase and glycinamide ribonucleotide formyltransferase, but, unexpectedly, the structural similarity between the amino-terminal domain of 10-formyltetrahydrofolate dehydrogenase and methionyl-tRNA-formyltransferase extends to the C terminus of both proteins. The active site contains a molecule of β-mercaptoethanol that is positioned between His-106 and Asp-142 and that appears to mimic the formate product. We propose a catalytic mechanism for the hydrolase reaction in which Asp-142 polarizes the catalytic water molecule and His-106 forms a hydrogen bond to the formyl group of formyl. The structure also provides clues as to how, in the native enzyme, the hydrolase domain transfers its product to the dehydrogenase domain.

10-Formyltetrahydrofolate dehydrogenase (FDH)3 (EC 1.5.1.6) is an abundant enzyme in several tissues (1), comprising up to 1% of total cytosolic protein (2). In vitro, FDH converts 10-formyltetrahydrofolate (10-formyl-THF) to tetrahydrofolate in either an NADP+-dependent dehydrogenase reaction or an NADP+-independent hydrolase reaction (Fig. 1). The substrate for FDH, 10-formyl-THF, forms a major intracellular folate pool (3) and serves as a formyl donor in two reactions of de novo purine biosynthesis (4). The product of the FDH-catalyzed reaction, THF, can be converted to 5,10-methylene-THF, which is required for thymidine biosynthesis, and then to 5-methyl-THF, which is required for S-adenosylmethionine biosynthesis. The precise metabolic role for FDH is unclear, but one possibility is that it converts excess 10-formyl-THF from the de novo purine pathway back to the THF pool (5). Another may be a protective one against formate toxicity by removing formate, in the form of CO2 (6).

Folate derivatives are required for the biosynthesis of the nucleotide precursors of DNA and RNA, and hence folate-metabolizing enzymes are critical for proper cell functioning. Elevated levels of these enzymes, including the key folate enzyme, dihydrofolate reductase, occur in rapidly proliferating cancer cells (1, 7, 8). By contrast, FDH is expressed at much lower levels in tumors compared with corresponding normal tissues (1). In fact, elevation of intracellular levels of FDH in cultured cancer cell lines inhibits proliferation and results in cytotoxicity (1). This suppressor effect of FDH is dependent upon its enzymatic activity (1). These studies imply that FDH may possess a growth-regulatory function and that cancer cells lower FDH expression to enhance proliferation.

FDH is a homotetramer of 902-amino acid residue monomers (9, 10) where each monomer comprises two distinct functional domains connected by a 110-amino acid residue linker (9). The binding site for folate is located in the N-terminal region of the enzyme (termed N1-FDH, residues 1–310) (11). This domain shows sequence similarity with folate-binding domains in other proteins, including methionyl-tRNA-formyltransferase (FMT) and glycinamide ribonucleotide formyltransferase (GART) (9, 11). The carboxyl-terminal domain of FDH (residues 420–902) shows up to 50% sequence identity with aldehyde dehydrogenases (9, 12, 13). Interestingly, when the N-terminal domain is expressed separately, it functions as a 10-formyl-THF hydrolase, resulting in the production of formate (11), whereas the dehydrogenase reaction is catalyzed only by the full-length FDH (11, 12). This suggests that the hydrolase activity of the N-terminal domain contributes to the overall dehydrogenase reaction and, therefore, some functional communication between the two domains must exist. Thus, FDH is an interesting example of a natural fusion of two unrelated proteins that creates a new enzymatic activity.

A further issue is whether the hydrolase reaction can occur in vivo independently of the dehydrogenase domain or whether...
crystals used for data collection were passed through a cryo-}
mechanism of FDH, we are pursuing structural studies of this}
activity could maintain THF levels when NADP$^+$ is low. Inter-
estingly, an enzyme called formyltetrahydrofolate hydrolase
catalyzes the same conversion of 10-formyl-THF to THF and
formate in E. coli (16), suggesting the importance of this
reaction under some circumstances. Enzymes similar to
E. coli hydrolase are not apparent in eukaryotes, so this func-
tion might be performed by FDH. Contrary to this argument, if
the hydrolase reaction were to occur in tandem with the syn-
thesis of 10-formyl-THF, which is the opposite reaction, cata-
lized by 10-formyl-THF synthase and coupled with ATP hy-
drolysis, the result will be a “futile cycle” that simply consumes
energy. Moreover, in vitro, the hydrolase reaction requires
millimolar concentrations of β-mercaptoethanol (17), and,
given that β-mercaptoethanol is not present naturally in the
cell, it is unlikely that this reaction occurs in vivo independ-
ently of the overall dehydrogenase activity of FDH (although it
remains possible that other reducing agents such as glutathi-
one could act in a similar manner).

Previously, we have proposed a sequence of steps to describe
the dehydrogenase mechanism of FDH (18). In this scheme, the
hydrolase domain catalyzes the removal of the formyl group
from 10-formyl-THF, followed by its transfer to the aldehyde
dehydrogenase domain by an unknown mechanism where it is
oxidized to CO$_2$. As a first step toward deciphering the catalytic
mechanism of FDH, we are pursuing structural studies of this
enzyme. In this paper, we report the crystal structure of N$_t$-
FDH from rat solved to 2.3 Å resolution.

**MATERIALS AND METHODS**

**Data Collection**—The expression and crystallization of N$_t$-FDH has
been described previously (19). Briefly, N$_t$-FDH crystallizes in space
group P2$_1$2$_1$2 with cell dimensions $a = 100.0$ Å, $b = 64.6$ Å, and $c = 64.6$
Å. All crystals used for data collection were passed through a cryoprot-
tectant solution containing 1.3 M ammonium sulfate, 0.1 M sodium
acetate, pH 4.6, 27% glycerol and then flash-frozen
using an X-Stream cryostat (Rigaku MSC).

Diffraction data were collected on an RAXISIV+ image plate de-
tector mounted on a RU-H3R rotating anode x-ray generator fitted with
Osmic Conofocal optics (Rigaku MSC) and operating at 50 kV and 100
mA. Data were collected at a crystal-to-plate distance of 150 mm, with
an oscillation angle of 0.5° and an exposure time of 5 min/frame.
Typically 100° of data were sufficient to generate essentially complete data sets. Data were collected and processed using Crystal Clear (20).
Friedel pairs were not merged in order to retain the anomalous signal.
To improve the phasing (and for refinement purposes), a new native set
was collected in place of one described previously (19).

**Phasing**—A search for derivatives was made by soaking crystals in
solutions of heavy atom compounds in stabilizing buffer (1.3 M amno-
nium sulfate buffered in 0.1 M sodium acetate, pH 4.6). The degree
of substitution by heavy atoms in each data set was evaluated using
difference Patterson maps. For the first derivative so identified, heavy atom positions were determined from the Patterson maps and refined
prior to phasing at 3.0 Å. Subsequent derivatives were identified by
difference Fouriers using the current best phases. Where measurable,
 contributions from the anomalous signal of the heavy atom were in-
cluded in the phasing calculations. All calculations were performed
using PHASES (21).

**Model Building and Refinement**—Electron density maps were calcu-
lated using CCP4 programs (22) and displayed using the O program
(23). Starting with the crystal structure of FMT (24) (Protein Data Bank
code 1FMT) as a guide, a model was built and then refined by alternat-
ing rounds of XPLOR (25) and manual revision using O. In later rounds,
water molecules were included where visible in both the 2 F$^{-}$ – F$^+$ and
F$^{-}$ map and with suitable hydrogen binding partners. The final
ronds of refinement were performed using REFMAC (26). The stereo-
chemistry of the final model was evaluated using PROCHECK (27).
The coordinates and structure factors have been deposited with the
Protein Data Bank (1S3I).

**Preparation of Cysteine to Alanine Mutants**—The QuikChange™
site-directed mutagenesis kit (Stratagene) was used to generate five
mutants of N$_t$-FDH, each replacing a cysteine with an alanine. Mu-
tagenesis was performed on cDNA cloned into the pRSET vector. Each
construct containing the mutation was then transformed into E. coli
XL-1 Blue cells, as described previously (28).

After selection on LB-ampicillin plates, plasmids were isolated from
individual colonies using the Quantum Prep kit (Bio-Rad) and the
presence of the mutation was confirmed by sequencing. The entire
coding region of each clone was also sequenced to ensure the absence
of random mutations. Each mutant protein was expressed in E. coli and
purified in the same manner as described previously for wild-type
N$_t$-FDH (28). The hydrolase activity of each mutant enzyme was as-
sayed using 10-formyl-DDF as a substrate, as described previously (17).
Each assay was performed twice, and the numbers reported are an
average of these two experiments.

**RESULTS**

**Structure Determination**—The structure of N$_t$-FDH was de-
termined by multiple isomorphous replacement with anomalous
scattering. Data from crystals soaked in mercury, plati-
num, and uranyl compounds were used for the phasing. The
best derived model was obtained using thimerosal, and this was
used to identify other weaker derivatives by difference Fouriers
(18). The data collection and phasing statistics are shown in
Tables I and II, and portions of the experimental, calcu-
lated at 3.0 Å, and final 2F$^{-}$ – F$^+$ electron density maps at 2.4 Å,
are shown in Fig. 2. The refinement of this structure was
rather complicated, because several regions of the molecule
were difficult to fit due to weak density, and this is one con-
tribution to the comparatively high R and R$_{free}$ values of the
structure. In particular, the region around residues 85–88 was
difficult to place correctly. In fact, the density appears to en-
compass at least two conformations in this region, and this
suggests that it may become ordered upon binding substrate.
Another contributor is the inherent quality of the crystals,
which grow as thin plates and do not diffract equally well in all
directions. The final structure comprises residues 1–307 and 53
water molecules and has a crystallographic R factor of 24.5%
(R$_{free}$ = 30.5%) at 2.3 Å resolution (Table III). As defined by
PROCHECK (27), 88.6% of the residues lie within the most
favored region of the Ramachandran plot with one outlier (Ser-
87). The two C-terminal residues of the N$_t$-FDH construct are
not visible in the electron density and were omitted from the
model. Interestingly, four of the five cysteines present in the
structure appear to be modified by β-mercaptoethanol (βME).
This was probably caused by the high concentration of βME
present in the crystallization solution (10 mM). Thus, four mol-
ecules of βME were included in the model, modifying cysteines
86, 152, 191, and 238 (the exception is Cys-17). One of these
cysteines (Cys-86) is near the active site (see below).

**Site-directed Mutagenesis of Cysteine Residues**—Since most of
the cysteines in the crystal structure were covalently modi-
fied by βME, it was important to determine the effect of these
 modifications on the catalytic activity of the enzyme. Modification of Nt-FDH with N-ethylmaleimide, an agent that is directed toward cysteines, did not inhibit the hydrolase activity (data not shown). This implies that a cysteine residue is not involved in the hydrolase catalysis. However, a buried cysteine may be protected from chemical modification; hence, using site-directed mutagenesis, we replaced each of the cysteines involved in the hydrolase catalysis. However, a buried cysteine may be protected from chemical modification; hence, using site-directed mutagenesis, we replaced each of the cysteines involved in the hydrolase catalysis. However, a buried cysteine may be protected from chemical modification; hence, using site-directed mutagenesis, we replaced each of the cysteines involved in the hydrolase catalysis.

Structure Description—The structure comprises two domains (Fig. 3). Domain 1 contains a seven-stranded sheet of mixed parallel and antiparallel β-strands, comprising β1–β6 and β7–β9, packed on both sides by α-helices, and is immediately recognizable as a Rossmann fold (29). β6 is separate from this sheet and forms a small two-stranded sheet with part of the loop that connects domains 1 and 2. Domain 2 is mostly all β in structure and from one angle appears as a sandwich of two β-sheets, one five-stranded and the other four-stranded. However, two central strands, β12 and β14, bend at approximately right angles and link the two sheets to create a single sheet. The resulting arrangement is barrel-like and reminiscent of the OB fold (30). Between the two domains lies a long stretch of intervening polypeptide chain, comprising residues 185–218, which is predominantly extended in structure. The secondary structure assignments are shown in Fig. 3c.

As expected, since it contains the folate-binding site, domain 1 of the structure shows similarity with both FMT (24) and GART (31). This domain superimposes closely in all three enzymes, with the closest overlap being the central seven-stranded sheet and the greatest differences being in the relative positions of the surrounding helices as well as the length and structure of the connections between the elements of secondary structure. For instance, the r.m.s. deviation between the main chain atoms of 145 common residues of Nt-FDH and GART is 3.5 Å, whereas for the seven equivalent strands it is 0.8 Å. The same analysis for 156 common residues between Nt-FDH and FMT yields a r.m.s. deviation of 3.7 Å, but for the β sheet it is 1.0 Å. The divergence between Nt-FDH and GART begins at residue 187 (of both enzymes), near the C terminus of GART, whereas for FMT, the structural similarity extends all the way to the C terminus of both proteins. In fact, domain 2 of Nt-FDH and the C-terminal domain of FMT are topologically equivalent except for the connection between β13 and β14, which is much longer in Nt-FDH and includes an additional strand β13, whereas in FMT this connection, between β13 and β10, is much shorter (Fig. 4). Interestingly, although there is no topologically equivalent strand for β13 in FMT, the edge position in the sheet is occupied instead by β14 in FMT, which is at the C terminus of the protein. Given the lack of sequence similarity in the C-terminal half of these proteins, the structural overlap between Nt-FDH and FMT in this region was unexpected. The functional significance of these structural similarities is discussed below.

Active Site—The location of the active site can be determined by the sequence similarity of Nt-FDH to FMT and GART and, in particular, the presence of highly conserved residues that form the active sites in these enzymes. It is situated at the base of the deep cleft between domains 1 and 2. The substrate-binding site is formed by the divergence of β strands (β8 and β9) in the central β sheet of domain 1. As viewed in Fig. 5, the active site is formed by several loops including residues 113–117 at the base, 85–89 at the top, 104–108 to the left (part of β6), 139–144 to the right, and Phe-135 from β7. Of note is the relatively poor density of the 85–89 region, making it difficult
to fit these residues with high confidence (see above). Much of
the active site is markedly hydrophobic in character due to
residues such as Phe-35, Phe-135, Leu-141, Ala-114, and Ile-
104. The lack of large side chains in residues 115–117 (Gly-
Ala-Ser) may permit the close approach of the substrate
molecule.

Two key residues in the active site are His-106 and Asp-142.
These are conserved in both FMT and GART and mutation of
either of these renders Nt-FDH inactive as a hydrolase (18, 32).
Interestingly, we observe electron density bridging between
these two residues and have interpreted this as a molecule of
\[\beta ME\] (Fig. 5). It has been fitted such that the sulphydryl group
is within hydrogen bonding distance of His-106 and the hy-
droxy group within hydrogen bonding distance of Asp-142. The
involvement of \(\beta ME\) in the \textit{in vitro} hydrolase mechanism of
Nt-FDH is discussed below.

Comparison with the Active Sites of FMT and GART—To
compare the similarity of the active site with those of FMT and
GART, the crystal structure of Nt-FDH was superimposed onto
those of FMT and GART using several conserved residues in
the active site (Fig. 6). The close correspondence of many of the
loops in the active sites again shows the overall similarity of
the three proteins, but it also reveals some distinctive differ-
ences. Overall, there is closer similarity of Nt-FDH with FMT
than GART. Most notably, the loop in GART that is topologi-
cally equivalent to residues 113–117 in Nt-FDH and residues
117–121 in FMT adopts a wholly different conformation and
occupies a different position in that enzyme.

GART have been solved in a ternary complex with glycini-
amide ribonucleotide and the folate inhibitor, 5-deaza-5,6,7,8-
tetrahydrofolate (5dTHF) (33). 5dTHF is very similar to THF,
except that a carbon is substituted for nitrogen at position 5 of
the pteridine ring. The superimposition of Nt-FDH with the
GART-glycinamide ribonucleotide-5dTHF complex provides a
view of how ligands may fit into the active site of Nt-FDH (Fig.
7). Specifically, it shows that \(\beta ME\) occupies the same spatial
position as the expected position of the formyl group, adjacent
to N-10 of THF. This suggests that in our structure \(\beta ME\) is a
mimic of formate, such that the O-C-C backbone of \(\beta ME\) is
equivalent to O-C-O of formate. More pertinently, it shows that
Asp-142 is best placed to orient a hydrolytic water between N-10
and the carbonyl carbon of formyl group and that His-142 may
orient the carbonyl oxygen of the formyl group. This has impli-
cations for the mechanism of hydrolase, which is discussed below.
Table III
Statistics of the final model
The figures in parentheses are for the highest resolution shell of the data.

| Parameter                                  | Value          |
|--------------------------------------------|----------------|
| Resolution range (Å)                       | 15.0–2.3 (2.36–2.30) |
| No. of reflections used in refinement      | 18,079 (1278)  |
| Percentage of reflections used in Rmerge   | 5.0            |
| No. of protein atoms                       | 2380           |
| No. of water molecules                     | 53             |
| No. of β-mercaptoethanol molecules         | 5              |
| Crystallographic R-factor (%)              | 24.5 (30.3)    |
| R-work (%)                                 | 24.2 (29.9)    |
| Free R-factor (%)                          | 30.5 (36.2)    |
| Bond lengths (Å)                           | 0.010          |
| Mean B-factor (main chain) (Å²)            | 33.1           |
| r.m.s. deviation in main chain bonds B-factor (Å²) | 0.46          |
| Mean B-factor (side chains) (Å²)           | 34.2           |
| r.m.s. deviation in side chain B-factors (Å²) | 1.20          |
| Ramachandran plot                          |                |
| Residues in most favored region (%)        | 88.6           |
| Residues in additionally allowed regions (%)| 10.2           |
| Residues in generously allowed regions (%) | 0.8            |
| Residues in disallowed regions (%)         | 0.4            |

Table IV
Activity of mutant Nt-FDH enzymes relative to wild-type Nt-FDH

| Nt-FDH mutant | Hydrolyase activity, percentage of wild type* |
|---------------|-----------------------------------------------|
| C17A          | 94                                            |
| C86A          | 110                                           |
| C152A         | 95                                            |
| C191A         | 96                                            |
| C238A         | 102                                           |

* Average of two independent experiments.

Discussion

Similarity of Nt-FDH, GART, and FMT—There are several enzymes in the cell that use 10-formyl-THF as a substrate. Three of these enzymes, GART, AICART, and FMT, use 10-formyl-THF as a formyl donor in biosynthetic reactions. Although GART and FMT show very low sequence identity with each other (19%), they share the same fold for the folate-binding domain (24). By contrast, AICART shows no sequence similarity with either GART or FMT (34), and indeed its crystal structure reveals a different fold (35). FMT is 314 residues long (36) and contains a Rossmann fold, comprising -200 amino acids, and a separate carboxyl-terminal domain of around 100 residues (24). GART is a shorter protein and contains only the Rossmann fold (37, 38). Nt-FDH shows sequence similarity with both of these Rossmann folds but not to the C-terminal domain of FMT (11). As expected then, the fold in this region of Nt-FDH is highly similar to that for FMT and GART. Unpredicted, though, is the extension of this structural similarity in FMT to encompass the C-terminal domains of FMT and Nt-FDH.

Role of the C-terminal Domain of Nt-FDH—One issue that arises from the structural similarity noted above is the role of the C-terminal domain in Nt-FDH. In FMT, this domain is responsible for binding methionyl-tRNA (24, 39) and raises the possibility that this domain of Nt-FDH can also bind nucleic acids. 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 (40, 41). Examination of the electrostatic surface of this region of Nt-FDH, however, does not show a preponderance of basic charges that would be typical of a protein that binds nucleic acids but rather an equal distribution of positive and negative charges (data not shown). Interestingly, this domain is required for function, because deletion experiments show that at least 300 residues of this domain are required for the hydrolyase activity of FDH, although the folate-binding site and active site for the hydrolyase activity are both located within the first 200 residues of the protein. This is surprising given that E. coli 10-formyl-THF hydrolase, the catalytic domain of which is shorter than 200 amino acid residues, can function as a hydrolase (42). Moreover, GART, which has about 200 amino acid residues, also removes the formyl group from 10-formyl-THF as part of its transferase reaction (43). How the C-terminal region of Nt-FDH contributes to hydrolyase activity, however, remains an unanswered question.

Conservation of Active Site Architecture in Nt-FDH, FMT, and GART—Three important residues, asparagine, histidine, and an aspartate, have been identified in the active sites of GART and FMT (44, 45), and these residues are strictly conserved among all known GARTs and FMTs. Nt-FDH contains only two of these residues, His-106 and Asp-142, and these residues reside in the hydrophobic cleft that serves as a folate-binding pocket. Both of these are strictly required for the hydrolyse and dehydrogenase activities of FDI (18, 32), suggesting that the hydrolyase reaction is an essential part of the dehydrogenase mechanism. The superimposed structures of FMT, GART, and Nt-FDH showed that positions of the aspartate and histidine overlap very closely in all three enzymes (Fig. 6), suggesting that the removal of formyl from 10-formyl-THF is likely to proceed through a similar mechanism in all three enzymes. The third residue, however, differs in FDH; in place of an asparagine, there is an isoleucine. This is an interesting difference, because in GART this residue helps to orient the formyl group (46). The absence of this asparagine suggests that Asp-142 and His-106 are the most crucial residues for the function of Nt-FDH.

Role of β-Mercaptoethanol in the Hydrolase Mechanism—Nt-FDH catalyzes the hydrolysis of 10-formyl-THF to formate and THF and the same reaction can also be assayed in full-length FDH by excluding NADP (17). In both cases, however, the hydrolyase reaction requires millimolar concentrations of βME (17). In our crystal structure, five molecules of βME are observed. Four of these are modifying cysteine residues and, as discussed above, are unlikely to have a significant impact on hydrolyase activity. The remaining βME, however, is positioned between both of the catalytic residues, His-106 and Asp-142, such that the sulfhydryl group is hydrogen-bonded to His-106, and the hydroxyl group is hydrogen-bonded to Asp-142. This suggests that βME may be involved directly in the hydrolyase mechanism rather than simply serving as a reducing agent (although it cannot be ruled out that the binding of βME to the active site of Nt-FDH is nothing more than happenstance, as indeed happens in many protein crystal structures, and has no bearing on how βME acts in hydrolyase activity). Since Nt-FDH is an “artificial” enzyme, the most likely explanation is that βME replaces a functionality lost in the absence of the dehydrogenase domain. A number of hypotheses can be proposed to explain the requirement for βME in the hydrolyase mechanism. 1) Cys-707, an essential residue of the aldehyde dehydrogenase domain, has a direct role in the hydrolyase reaction, and in the absence of the dehydrogenase domain in Nt-FDH, this function is replaced by the sulfdryl group of βME. Examination of the structures of Nt-FDH and of aldehyde dehydrogenase (47, 48), however, suggests that this is very unlikely, because both active sites are located at the base of relatively deep clefts and Cys-707 could not access the active site of the hydrolyase domain.

a S. Krupenko, unpublished observations.
without major conformational changes in both domains of FDH. 2) In the absence of the aldehyde dehydrogenase domain, hydrolysis in Nt-FDH proceeds via an entirely novel mechanism that requires βME, perhaps as a nucleophile to attack the carbonyl carbon of the formyl group. Again, this seems unlikely, because it is hard to envisage what property of the dehydrogenase domain is being replaced by βME and, furthermore, it would mean that the hydrolase reaction in Nt-FDH can operate by two different mechanisms. 3) Finally, our preferred hypothesis is that βME is required for displacement of the products of the hydrolase reaction. In this mechanism, the interaction between the hydrolase and aldehyde dehydrogenase domains in the full-length enzyme lowers the binding affinity of the products, formate and/or THF, in the hydrolase active site. Since this domain-domain interaction is absent in Nt-FDH, either formate or THF remains bound after the hydrolase reaction, but these can be displaced by βME in vitro. Given the overlap of βME with the expected position for formate, we suggest that βME is required to displace formate that is positioned between His-106 and Asp-142. Further support to this hypothesis arises from the apparent flexibility in the 110-residue linker region between the hydrolase and dehydrogenase domains, which may act as a hinge to bring the two active sites in close proximity (49). In fact, given that formate is a
substrate for the dehydrogenase reaction, it would be logical that the hydrolase domain retains this group until the active site of the dehydrogenase domain is brought alongside. The end result may be a protected channel connecting the two active sites that prevents loss of this group to the solvent and thus enhances the overall reaction rate.

Mechanism of Hydrolase Catalysis—The superimposition of Nt-FDH with GART complexed with the folate substrate ana-

FIG. 4. The structural similarity of the C-terminal regions of Nt-FDH (left) and FMT (right). a, ribbon representation of each domain in which spatially equivalent elements of secondary structure are colored the same. This figure was prepared using MOLSCRIPT (51) and Raster3D (52). b, topology diagram of each domain using the same color scheme as a. Note the spatial similarity of β₁₃ in Nt-FDH to β₁₄ in FMT (both colored pink) although these strands are not topologically related.

FIG. 5. A stereoview showing the active site residues of Nt-FDH. A molecule of β-mercaptoethanol, situated between Asp-142 and His-106, is shown set against its 2(F₀ - F₁) electron density, contoured in blue at 1 σ. This figure was prepared using PyMOL (W. L. DeLano; available on the World Wide Web at www.pymol.org).
log, 5-deazafolate (33), revealed the likely position of the formyl group in Nt-FDH, and the close proximity of His-106 and Asp-142 to this group is consistent with a role for these residues in the hydrolase mechanism (18). The proposed mechanism for the hydrolase reaction is shown in Fig. 8. In this scheme, a water molecule is polarized by Asp-142, facilitating a nucleophilic attack at the carbonyl carbon of formyl-THF. This generates an intermediate comprising a quarternary amine adjacent to a tetrahedral carbon center, in which the charge of the oxyanion is stabilized by His-106, whereas Asp-142 stabilizes that of the cationic nitrogen. In the next step, the intermediate collapses to form the products, THF and formate. By polarizing the catalytic water molecule, Asp-142 also facilitates the abstraction of a proton by the tetrahydrofolate product. After the reaction is complete, formate is held in place by hydrogen bonds with His-106 and Asp-142. In the full-length enzyme, this formate is then passed to the dehydrogenase domain by an unknown mechanism (see below), but in Nt-FDH it can only be displaced by βME.

Hence, in our crystal structure, the view is after the hydrolase reaction is completed and βME now occupies the same position as formate. A similar mechanism for the removal of formyl from the folate substrate is likely to operate in both FMT and GART, which also contain the same aspartate and histidine residues, and indeed this has been proposed for GART (50). A potential catalytic water molecule has been observed in each crystal structure of GART and FMT (24, 50).

Communication between the Hydrolase and Dehydrogenase Domains—Since the product of the hydrolase reaction is probably a substrate for the dehydrogenase reaction, a key question remaining is how the hydrolase and dehydrogenase domains communicate in the full-length enzyme. As described above, the two active sites are both quite buried and lie within concave regions of their respective domains. Given this, it would appear improbable that residues of the hydrolase active site can access the active site of the dehydrogenase domain (or vice versa) to facilitate direct transfer of the formyl group. However, one mechanism of communication is suggested by the architecture of the β-sheet in the hydrolase domain. Interestingly, β7 and β8 form a hairpin that lies distinctly out of the plane of the sheet (Fig. 9), and Asp-142 is located at the pinnacle of the turn between these strands. Were this hairpin to fall back toward the plane of the sheet, Asp-142 would become increasingly exposed on the surface of the protein, making it possible for this residue, at least, to reach the active site of the dehydrogenase domain. How an aspartate would facilitate transfer of a formyl group is unclear, but in support of this hypothesis, the equivalent loop in GART undergoes a conformational transition in...
response to changes in pH (46). This hypothesis, of course, is highly speculative, and a better idea of how the formyl passes between domains must await a crystal structure of the full-length enzyme.

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Fig. 8. Proposed mechanism for the hydrolase reaction of FDH. In this scheme, Asp-142 polarizes a water molecule for hydrolysis of the bond between the carbonyl carbon of formyl and N-10 of THF, and His-106 helps orient the carbonyl group. After catalysis, formate remains bound in the active site until displaced by a molecule of βME.

Fig. 9. A possible mechanism of communication between the hydrolase and dehydrogenase domains of FDH. It is postulated that the loop between β₇ and β₈, which carries the active site residue Asp-142, can flip back into the plane of the sheet. In so doing, Asp-142 would become exposed on the surface of the hydrolase domain and thus be accessible to the active site of the dehydrogenase domain. This figure was prepared using MOLESCRIPT (51) and Raster3D (52).
Crystal Structure of the Hydrolase Domain of FDH

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The Crystal Structure of the Hydrolase Domain of 10-Formyltetrahydrofolate Dehydrogenase: MECHANISM OF HYDROLYSIS AND ITS INTERPLAY WITH THE DEHYDROGENASE DOMAIN

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