Crystal structure of DMGO provides a prototype for a new tetrahydrofolate-binding fold

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Abstract
The crystal structure of DMGO (dimethylglycine oxidase) from Arthrobacter globiformis in complex with folate compounds has revealed a novel THF (tetrahydrofolate)-binding fold [Leys, Basran and Scrutton (2003) EMBO J. 22, 4038–4048]. This fold is widespread among folate-binding proteins. The crystal structures of aminomethyltransferase (T-protein), YgfZ and TrmE all reveal similar THF-binding folds despite little similarity in sequence or function. The THF-binding site is highly specific for reduced folate compounds and most members of this fold family enhance the nucleophilic character of the THF N10 position.

One-carbon (C1) metabolism is mediated by folate coenzymes and plays an essential role in cellular processes such as the biosynthesis of nucleotides, vitamins and amino acids [1]. Folates are based on the pteroic acid skeleton conjugated to one or more L-glutamate units, and exist in different oxidation states. The carbon unit is attached to the reduced pteroid acid by the N5 and N10 atoms, and can differ in oxidation state ranging from the highly oxidized formimino and formyl substituents to the reduced methylene and methenyl groups [2]. Carbon units are derived from a range of compounds such as serine, betaine degradation products, glycine or formate and are activated by attachment to THF (tetrahydrofolate) [3]. Cancer therapies use inhibition of dihydrofolate reductase by folate analogues such as methotrexate (2003) EMBO J.

DMGO crystal structure
The structure of DMGO was determined to 1.6 Å (1 Å = 10−10 m) and reveals that the protein is folded in two regions (Figure 1). The N-terminal region covalently binds FAD and has dimethylglycine dehydrogenase activity. There is a strong structural similarity between this domain and monomeric sarcosine oxidase [8], polyamine oxidase [9], and p-hydroxybenzoate hydroxylase [10]. Putative active-site residues can be identified by the presence of a bound acetate ion that mimics the carboxylate group of the substrate. Sequence alignment with related flavoproteins reveals that those residues postulated to be involved in the formation of the Michaelis complex are not conserved. However, two residues that reside close to the flavin N5, i.e. His225 and Tyr259, are strictly conserved. Both residues are unlikely to be involved in substrate binding and are thought to play an essential role in substrate oxidation. The mechanism of amine oxidation has been the subject of much debate in recent years, with both polar and radical mechanisms proposed [11]. Although there is no clear evidence to support either mechanism in DMGO, it is interesting to note that a stable tyrosyl radical has been detected in monooamine oxidase A [12]. Tyr259 might play a similar role in DMGO and related enzymes and could allow dimethylglycine oxidation to occur by a single electron transfer mechanism, although this remains to be established.

Oxidation of betaine compounds leads to the corresponding iminium ions, which undergo spontaneous hydrolysis in water to yield the product secondary (e.g. DMGO and DMGDH) or primary (e.g. SDH) amine and formaldehyde. In the mammalian enzymes DMGDH and SDH and also in bacterial DMGO, the production of toxic formaldehyde is avoided by production of 5,10-methylene-THF [6]. This activity resides in the C-terminal region of the protein. This folate-binding region comprises three domains positioned in a cloverleaf-like arrangement around a central hole, thus resembling a ring. Both domains A and B have a ferredoxin-like fold that consists of an anti-parallel β-sheet that contains a single Greek-key motif packed on one side by flanking

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Abbreviations used: DMGDH, dimethylglycine dehydrogenase; DMGO, dimethylglycine oxidase; SDH, sarcosine dehydrogenase; THF, tetrahydrofolate.

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α-helices. The two anti-parallel β-sheets from domains A and B are loosely packed against each other with the individual strands roughly aligned with the plane of the ring. Although there is no significant sequence homology between the polypeptide segments of A and B, the domains can be superimposed with a Z score of 6.3 and an r.m.s.d. (root mean square distance) of 1.8 Å for 76 Cα atoms [13]. This clearly suggests early evolution by domain duplication followed by insertion of an additional β-strand in domain A. The C-terminal domain C is folded in a distorted six-stranded jelly roll and packs perpendicular with the β-sheets of A and B, closing the protein ring-like structure.

The crystal structure of the DMGO–folinic acid complex reveals little change upon binding of folinic acid into the central hole (Figures 1 and 2). The position of the side chain of several amino acids is rearranged to provide better hydrophobic packing with the pterin component. The ligand is bound in a kinked conformation, with the pterin group facing the internal protein cavity between the N- and C-terminal regions. The pterin group is bound by a double hydrogen bond with Glu658; a very similar contact can be observed in other folate binding enzymes such as dihydrofolate reductase [14] and formiminotransferase [15]. Four additional hydrogen bonds are made between the pterin group and DMGO, including a hydrogen bond between the pterin N8 and the backbone of Gly566. The folinic acid N10 group is hydrogen-bonded to Asp552, increasing the N10 nucleophilic character. Further activation of N10 is achieved by a slight
Superimposition of the THF-binding sites of DMGO, the T-protein and TrmE

For clarity only the β-sheets of domains A and B (or both N-terminal domains of the TrmE dimer) have been shown respectively in blue and green. For clarity, only the folinic acid and folate bound to DMGO are depicted, by coloured atoms and grey sticks respectively. Residues essential for THF binding and selection are shown in sticks and labelled for DMGO.

Figure 2 | Superimposition of the THF-binding sites of DMGO, the T-protein and TrmE

Aminomethyltransferase crystal structure

The crystal structure of the aminomethyltransferase (T-protein) from the glycine cleavage system of Thermotoga maritima has recently been reported [16]. The overall structure of the protein is very similar to the DMGO C-terminal domain and superimposes with a Z score of 47.3 and an r.m.s.d. of 1.53 Å for 361 equivalent Cα atoms (Figure 1). No significant changes are observed upon binding of either THF and folinic acid, providing further support for the idea that this class of folate-binding proteins present a rigid binding site. The THF and folinic acid ligands bind in an almost identical fashion with that observed in the DMGO-folic acid structure. The contacts made with the pterin group are highly similar, with Glu195 making a double hydrogen bond. Again like DMGO, the THF N10 is activated by hydrogen-bonding with Asp96 (Figure 2). In contrast with DMGO, the substrate of the T-protein is the methylamine-bound form of the H-protein. The methylamine is covalently bound to the H-protein lipoamide arm and converted into ammonia and 5,10-methylene THF with release of the dihydrolipoyl H-protein. Although no detailed structure is available for the T-protein–H-protein complex, a binary complex of the T-protein with lipoic acid indicates that the molecule binds to a predominantly rigid hydrophobic funnel. A putative model of the ternary T-protein–THF–lipoic acid complex reveals two structurally conserved water molecules bound between the lipoic acid and THF that probably mimic the position of the methylamine group. This places the carbon atom of the methylamine group within van der Waals distance of both THF N5 and N10. The lipoic acid-binding site occupies the same position as postulated for the iminium ion in complex with active site 2 in DMGO. It is interesting to note that the postulated H-protein docking site is similar to the docking site of the FAD-domain in DMGO.

YgfZ crystal structure

The recent structure determination of YgfZ from Escherichia coli reveals high similarity to the C-terminal region of DMGO, despite marginal sequence similarity [17].
structural superimposition leads to a Z score of 23.6 with an r.m.s.d. of 3.0 Å for 296 equivalent Ca atoms (Figure 1). The three domains have slightly different orientations in YgfZ and can individually be superimposed with the corresponding DMGO domains leading to significantly lower r.m.s.d. values. Solution studies have demonstrated nanomolar-binding affinities for both folinic acid and THF to YgfZ. Although a binary complex of YgfZ with folate compounds is not available, structural modelling led to the identification of a putative THF-binding site highly similar to the binding sites observed in DMGO and the T-protein. Interestingly, the conserved glutamate residue involved in binding folinic acid in DMGO and the T-protein (respectively Glu<sup>658</sup>/Glu<sup>195</sup>) is absent from YgfZ and is replaced by a leucine residue. In addition, the activating residue Asp<sup>552</sup>/Asp<sup>96</sup> is only present in eukaryotic members of the YgfZ family as well as representatives of the alpha division of the Proteobacteria. It therefore seems that the widespread YgfZ family can be subdivided into families with enzymatic potential and others that are unlikely to catalyse similar reactions. A concave surface patch on YgfZ was noted as being predominantly covered by basic residues and it is postulated that this might serve to bind DNA. Although the exact function of YgfZ and related proteins remains to be firmly established, the possibility exists that YgfZ is a folate-dependent regulatory protein.

**Crystal structure of TrmE**

TrmE is a guanine nucleotide-binding protein involved in the modification of uridine bases at the first anticodon positions of tRNAs [18]. The crystal structure of TrmE from *T. maritima* revealed a dimeric protein comprising three domains. In addition to the N-terminal domain that is involved in dimerization, the protein contains a helical domain and the C-terminal nucleotide-binding domain. Unexpectedly, the N-terminal domain of TrmE is structurally similar to both domains A and B of the folate-binding region in DMGO and related molecules (Figure 1). The dimerization of the TrmE N-terminal domain leads to a similar topology as observed for the packing of domains A and B in DMGO, where both domains are present on a single polypeptide chain. The crystal structure of the folinic acid complex with TrmE indeed reveals a THF-binding site highly similar to DMGO between both N-terminal domains of the TrmE dimer. Owing to the local symmetry of the N-terminal domains, two rather than one folinic acid-binding sites are observed. The THF-binding site is similar to the other crystal structures; the pterin group is bound to an invariant glutamate (Glu<sup>78</sup>) whereas the N10 position is in close contact with a residue at position 59 that is conserved as Glu, Gln, Asp or Asn in the TrmE family (Figure 2). Although detailed mechanistic evidence for the exact role of TrmE is lacking, it is postulated by Wittinghofer and co-workers [18] that TrmE might catalyse the transfer of C<sub>1</sub> units from 5-formyl-THF to position 5 of uracil.

**Conclusions**

The THF-binding fold represented by the C-terminal DMGO region appears to be widespread among folate-binding proteins. The folate-binding properties of both TrmE and YgfZ were discovered by structural similarity with DMGO [17,18]. The N-terminal folate-binding domain of TrmE probably resembles an ancient member of the family that subsequently evolved by gene duplication with concomitant loss of one THF-binding site and addition of the third domain C. The presence of the third domain creates a ring-like protein structure with a central THF-binding funnel. The binding site is highly specific for reduced folates and members with catalytic function enhance the nucleophlic character of the folate N10 position.

**References**


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