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Molecular basis for dysfunction of some mutant forms of methylmalonyl-CoA mutase: Deductions from the structure of methionine synthase

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ABSTRACT **Inherited defects in the gene for methylmalonyl-CoA mutase (EC 5.4.99.2) result in the *mut* forms of methylmalonic aciduria. *mut*^{0} mutations lead to the absence of detectable mutase activity and are not corrected by excess cobalamin, whereas *mut*$^-$ mutations exhibit residual activity when exposed to excess cobalamin. Many of the mutations that cause methylmalonic aciduria in humans affect residues in the C-terminal region of the methylmalonyl-CoA mutase. This portion of the methylmalonyl-CoA mutase sequence can be aligned with regions in other B_{12} (cobalamin)-dependent enzymes, including the C-terminal portion of the cobalamin-binding region of methionine synthase. The alignments allow the mutations of human methylmalonyl-CoA mutase to be mapped onto the structure of the cobalamin-binding fragment of methionine synthase from *Escherichia coli* (EC 2.1.1.13), which has recently been determined by x-ray crystallography. In this structure, the dimethylbenzimidazole ligand to the cobalt in free cobalamin has been displaced by a histidine ligand, and the dimethylbenzimidazole nucleotide "tail" is thrust into a deep hydrophobic pocket in the protein. Previously identified *mut*^{0} and *mut*$^-$ mutations (Gly-623 → Arg, Gly-626 → Cys, and Gly-648 → Asp) of the mutase are predicted to interfere with the structure and/or stability of the loop that carries His-627, the presumed lower axial ligand to the cobalt of adenosylcobalamin. Two mutants that lead to severe impairment (*mut*^{0}) are Gly-630 → Glu and Gly-703 → Arg, which map to the binding site for the dimethylbenzimidazole nucleotide substituent of adenosylcobalamin. The substitution of larger residues for glycine is predicted to block the binding of adenosylcobalamin.**

Methylmalonyl-CoA mutase (EC 5.4.99.2) is one of two cobalamin (B_{12})-dependent enzymes found in humans. This enzyme uses the adenosylcobalamin (AdoCbl) cofactor to catalyze the isomerization of methylmalonyl-CoA to succinyl-CoA, a reaction required in the catabolism of methionine, valine, threonine, isoleucine, odd-chain fatty acids, and cholesterol. Although the well-characterized bacterial methylmalonyl-CoA mutases are heterodimers with subunits of M_r 80,100 and M_r 69,500 (1, 2), the human enzyme is a homodimer of M_r 78,489 subunits (3, 4). Cloning and expression of the human mitochondrial enzyme has made it accessible for study (5, 6) and has facilitated the characterization of mutations (7–14).

Methylmalonic aciduria, an inborn error of metabolism that can arise from functional impairment of methylmalonyl-CoA mutase, is associated with infant mortality and with developmental retardation (15). A survey of newborns in Massachusetts suggested that methylmalonic acidemia occurs in 1 in 48,000 births (16). This number is considered a minimum estimate (17). Patients with defects in methylmalonyl-CoA metabolism have elevated concentrations of methylmalonic acid in blood and urine and can suffer from extreme acidosis and die within the first few weeks of life (15). Although methylmalonic aciduria can result from defects either in the apoenzyme or in cofactor transport and processing (15), a common cause is mutation of the gene that codes for the methylmalonyl-CoA mutase apoprotein. Two types of apoenzyme defects are classically distinguished by studies of [^{14}C]propionate metabolism in fibroblasts from patients with methylmalonic aciduria (8, 15). *mut*^{0} mutations are defined by an absence of detectable mutase activity and by no detectable [^{14}C]propionate incorporation in cultured cells. *mut*$^-$ mutations lead to residual but abnormal mutase activity and exhibit [^{14}C]propionate incorporation in fibroblasts when excess hydroxocobalamin is added (9, 18–20). Enzymes in *mut*$^-$ fibroblasts typically show elevated apparent K_m‖ values for AdoCbl, suggesting some perturbation in the binding of the cofactor (11, 12, 18–20). In contrast, high concentrations of AdoCbl are unable to restore the activity of *mut*^{0} mutant proteins. In general, *mut*$^-$ patients have a milder phenotype than do *mut*^{0} patients (21).

Many of the mutations that cause methylmalonic aciduria in humans affect residues in the C-terminal region of the methylmalonyl-CoA mutase subunit. This portion of the protein sequence can be aligned (Fig. 1) with the small subunit of glutamate synthase (MutS) (27, 34), with the C terminus of methylene glutarate mutase (26), and with the C-terminal portion of the cobalamin-binding region of methionine synthase (EC 2.1.1.13) (27, 33, 35). The alignments allow us to map the mutations of human methylmalonyl-CoA mutase onto the structure of the cobalamin-binding fragment of methionine synthase from *E. coli*, which has recently been determined by x-ray crystallography (33), and thus to make inferences about how mutations in human methylmalonyl-CoA mutase lead to dysfunction of the enzyme.

RESULTS

Location of Methylmalonyl-CoA Mutase Mutation Sites in the Structure of Methionine Synthase. The cobalamin-binding fragment of *E. coli* methionine synthase comprises amino acids

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Abbreviation: AdoCbl, adenosylcobalamin.

Data deposition: The atomic coordinates have been deposited in the Protein Data Bank, Chemistry Department, Brookhaven National Laboratory, Upton, NY 11973 (reference 1bmt).

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‖Apparent K_m (AdoCbl) values for the *mut*$^-$ mutant enzymes can be obtained by measuring methylmalonyl-CoA mutase activity in the presence of different concentrations of AdoCbl (15).

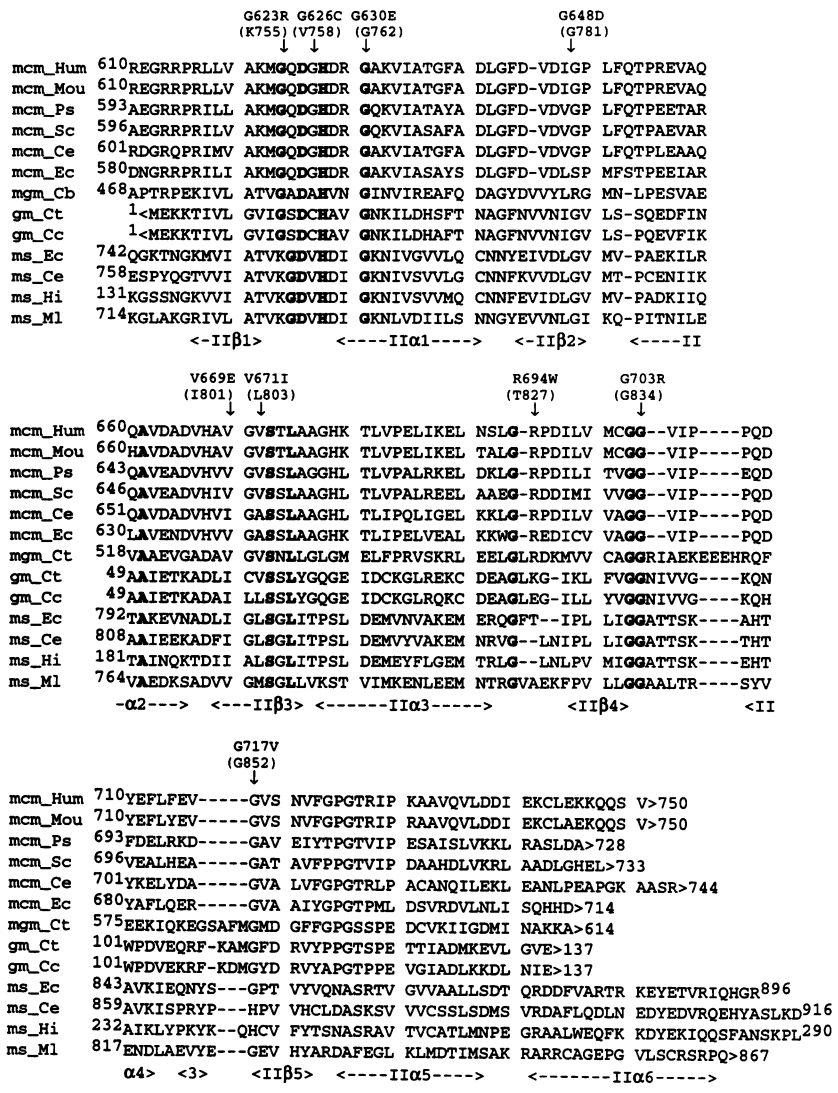

FIG. 1. Alignments of the sequences of methylmalonyl-CoA mutase with other B_{12}-dependent mutases that catalyze carbon skeleton rearrangements and with the cobalamin-binding fragments of methionine synthase. The alignments are based on the structure of *Escherichia coli* methionine synthase. Residues that are invariant are noted in bold, and mutations of human methylmalonyl-CoA mutase that occur within the aligned regions have been indicated by arrows; the corresponding residue numbers in methionine synthase are given in parentheses. Aligned sequences are residues 610–750 of the human methylmalonyl-CoA mutase, designated mcm_Hum (22); residues 610–750 of mouse methylmalonyl-CoA mutase, designated mcm_Mou (23); residues 593–728 of the MutB subunit from *Propionibacterium shermanii*, designated mcm_Ps (1); residues 596–733 of the MutB subunit from *Streptomyces cinnamonensis*, designated mcm_Sc (24); residues 601–744 of the deduced amino acid sequence of an open reading frame from *Caenorhabditis elegans*, designated mcm_Ce (GenBank accession no. Z35604; deposited by B. Mortimore in 1995); and residues 580–714 of an open reading frame (sbm) from *E. coli* with homology to methylmalonyl-CoA mutase, designated mcm_Ec (25). Also shown are residues 468–614 of methyleneglutarate mutase, designated mgm_Cb (26); sequences of the small subunit of glutamate mutase from *Clostridium tetanomorphum*, designated gm_Ct (27); and sequences from *Clostridium cochlearium*, designated gm_Cc (28). The final four lines give cobalamin-dependent methionine synthase sequences: residues 742–896 of *E. coli*, designated ms_Ec (29–31); residues 758–916 of an open reading frame from *C. elegans*, designated ms_Ce (GenBank accession no. Z46828; deposited by J. Swinburne in 1995); residues 131–290 of the deduced amino acid sequence from *Haemophilus influenzae Rd*, designated ms_Hi (32); and residues 714–867 of the deduced amino acid sequence of an open reading frame *metH2* from *Mycobacterium leprae*, designated ms_Ml (GenBank accession no. U00017; deposited by D. R. Smith in 1994). The numbering scheme for the human methylmalonyl-CoA mutase amino acid sequence shown here is that introduced by Andrews and coworkers (22). The secondary structure elements indicated below the aligned sequences are derived from the x-ray crystallographic analysis of the cobalamin-binding region of methionine synthase from *E. coli* (33).

651–896 of the 1227-residue chain. The N-terminal portion of the fragment is an α-helical domain that interacts with the upper (β) face of the bound methylcobalamin cofactor, where the methyl group is coordinated, whereas the $\approx$150 residues of the C-terminal part of the fragment, as depicted in Fig. 2, form an α/β (Rossmann) domain that interacts with the lower (α) face of the cofactor and with the dimethylbenzimidazole nucleotide. When cobalamin binds to methionine synthase, the dimethylbenzimidazole group, which is coordinated to the cobalt in the free cofactor, dissociates from the corrin and is replaced by a histidine contributed by the protein. Fig. 2 shows the resulting "open" conformation of the bound methylcobalamin and the extensive areas of contact between the cofactor and the α/β domain. The dissociated dimethylbenzimidazole nucleotide is inserted into a deep pocket that penetrates to the center of the domain and is presumed to contribute to the tight binding of the cofactor.

The sequence similarities that are evident in the sequences of several cobalamin-dependent enzymes are confined to the α/β domain. The sequence motif Asp-Xaa-His-$(Xaa)_2$-Gly-$(Xaa)_{41}$-Ser-Xaa-Leu-$(Xaa)_{26}$-Gly-Gly (26, 27, 33, 35) is conserved in methylmalonyl-CoA mutase and the other enzymes

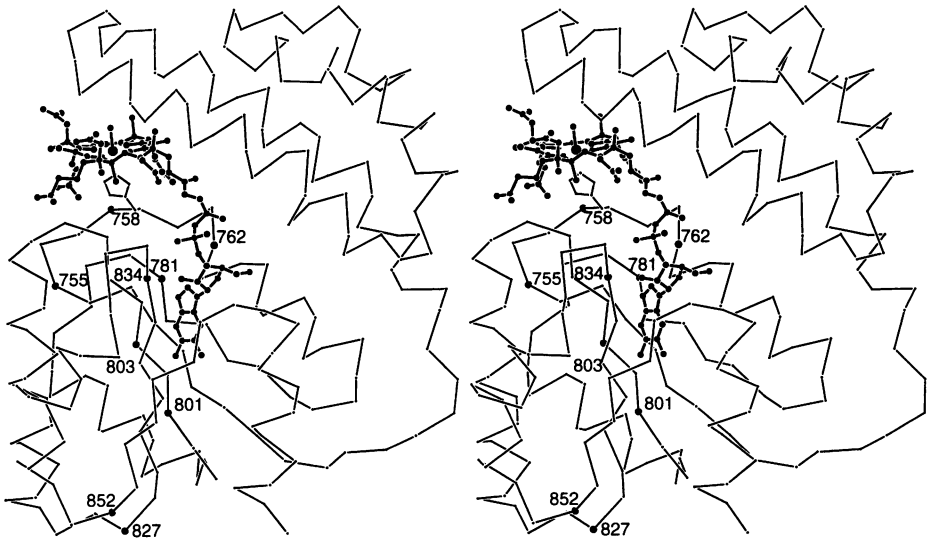

FIG. 2. A stereodrawing of the cobalamin-binding fragment of *E. coli* methionine synthase. The bound cofactor is drawn with thicker bonds. The "upper" helical domain is connected to the "lower" α/β domain by a flexible loop. This vantage point provides views of both the central parallel sheet of the α/β domain and the extended dimethylbenzimidazole tail of the cobalamin. Mutations in human methylmalonyl-CoA mutase (see Table 1) are indicated by enlarged C^{α} atoms and are labeled with the corresponding residue numbers from methionine synthase.

aligned in Fig. 1, and includes invariant residues located in regions that interact directly with cobalamin in methionine synthase (33, 35). Conservation of these sequences has suggested that the proteins included in Fig. 1 all bind cobalamin similarly, an idea that is supported by electron paramagnetic resonance spectroscopy. In experiments similar to that described by Stupperich and coworkers (36), it has been shown that histidine interacts with cobalt in methylmalonyl-CoA mutase from *P. shermanii* (37) and in glutamate mutase from *Cl. cochlearium* (38). Table 1 lists the mutations of human methylmalonyl-CoA mutase that are located in the region homologous to the α/β domain of methionine synthase. The positions of these mutations in the sequence and in the structure of the cobalamin-binding fragment are shown in Figs. 1 and 2, respectively. Fig. 2 indicates that many of the known mutations are clustered in the vicinity of the bound cobalamin (11).

Mutations That Block the Nucleotide Binding Pocket. Replacement of glycines at positions 630 and 703 of human methylmalonyl-CoA mutase results in the more severe *mut*^{0} phenotypes (11, 14). Unlike the *mut*$^-$ mutations, these mutations result in an enzyme whose activity cannot be even partially restored with increased amounts of AdoCbl. Although this phenotype could arise for other reasons, such as mutation of an essential catalytic residue, the structure strongly implies that failure to bind the cofactor is the primary defect in the Gly-703 → Arg and Gly-630 → Glu mutants. Gly-630 and Gly-703 (Gly-762 and Gly-834 in methionine synthase) are invariant residues in the sequence motif for cobalamin binding (Fig. 1). Mutations at these positions replace conserved glycines in the site that binds the dimethylbenzimidazole nucleotide in methionine synthase. As shown in Figs. 2 and 3, the nucleotide substituent is accommodated in a long pocket between two of the β-sheet strands and two helices. Comparison of the α/β domain of methionine synthase with the nucleotide-binding domain of lactate dehydrogenase reveals that the helices are not pried away from the β-sheet to make room for the cobalamin nucleotide tail, but rather glycines are substituted for residues with larger side chains (35). Thus, replacement of conserved glycines with bulky residues would be predicted to interfere with binding of AdoCbl to methylmalonyl-CoA mutase by occluding the tail-binding pocket. To explore the structural consequences of these mutations, the mutant side chains were modeled using interactive graphics, and the structures were subjected to energy minimization in X-PLOR 3.1 (40). The results, shown in Fig. 3, indicate that the dimethylbenzimidazole-binding pocket would be blocked by either of these mutations, consistent with the observation that excess AdoCbl does not restore activity. Both mutations are easily accommodated in the empty pocket, suggesting that the mutant apoproteins can fold correctly, as expected from their activity in complementation experiments (14). The models further imply that enzymatic activity might be restored by using modified cobalamins as cofactors.

Table 1. Mutations of human methylmalonyl-CoA mutase

Mutation	Corresponding residue in *E. coli* methionine synthase	Phenotype	Expression	Ref.
Gly-623 → Arg	Lys-755	*mut*^{0}	+(IC)	(14)
Gly-626 → Cys	Val-758	*mut*$^-$	+(IC, EA, WB)	(11)
Gly-630 → Glu	Gly-762	*mut*^{0}	+(IC, WB)	(11)
Gly-648 → Asp	Gly-781	*mut*$^-$	+(IC, EA, WB)	(11)
Val-669 → Glu	Ile-801	Uncharacterized	ND	(39)
Val-671 → Ile	Leu-803	Polymorphism	+	(14)
Arg-694 → Trp	Thr-827	*mut*$^-$	+(IC, EA, WB)	(11)
Gly-703 → Arg	Gly-834	*mut*^{0}	+(IC)	(14)
Gly-717 → Val	Gly-852	*mut*$^-$	+(IC, EA, WB)	(12, 13)

IC, expression established by interallelic complementation of phenotype with *mut*^{0} strain Arg-93 → His; EA, expression established by enzyme assay; WB, expression established by Western blotting; ND, not determined.

Mutant Methylmalonyl-CoA Mutases with Blocked Nucleotide Pockets May Bind Cofactors with Truncated Side Chains. As shown in Fig. 3, modifying the cobalamin to fit the mutant proteins offers a potential route to the formation of molecules that are competent catalysts. Modeling using the structure of the α/β domain of methionine synthase suggests that a cobalamin whose tail is truncated before the phosphate group (cobinamide) would be able to bind to the Gly-630 → Glu mutant protein (Fig. 3*B*), whereas truncating the tail after the phosphate may restore binding to the Gly-703 → Arg mutant protein (Fig. 3*C*). AdoCbl analogs, including adenosylcobinamide, have been shown to bind relatively tightly to methylmalonyl-CoA mutases (37, 41, 42), and it is conceivable that the cobalamin analogs displayed in Fig. 3 might in fact bind better to mutant protein than to wild-type protein. For example, the side chain of Glu-630 could potentially hydrogen bond to the terminal hydroxyl group of the truncated cobalamin, increasing affinity for the analog, and interactions between the positively charged Arg-703 side chain and the

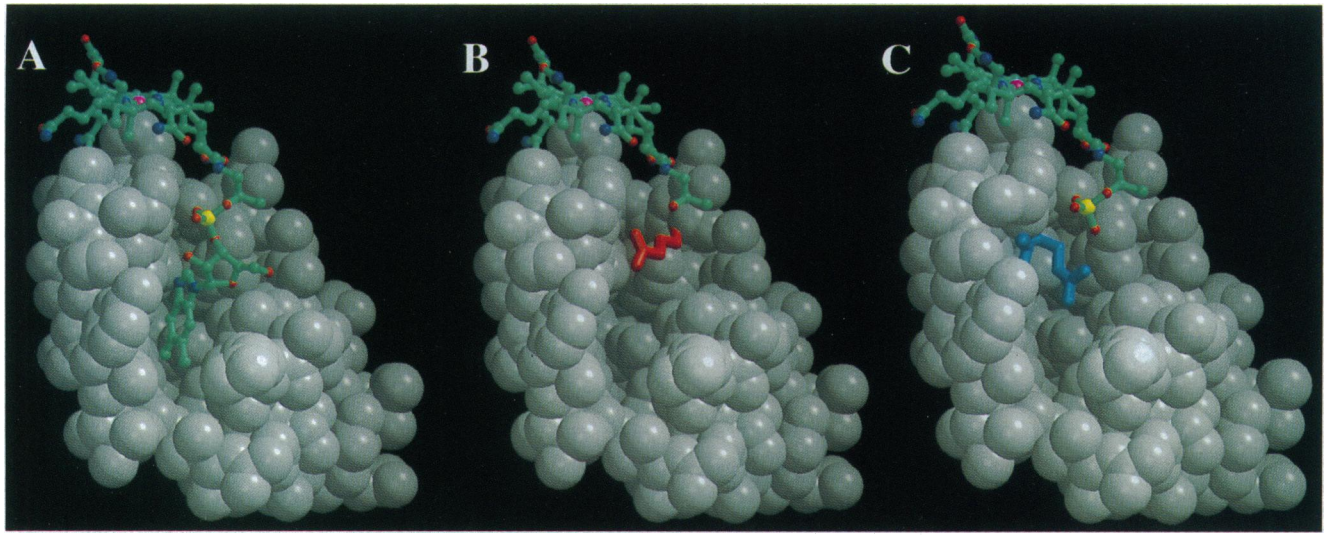

FIG. 3. The nucleotide-binding pocket is blocked by the Gly-630 → Glu and Gly-703 → Arg mutations. (*A*) Bound cobalamin (in ball-and-stick mode) and van der Waals surfaces outlining the pocket that binds the dimethylbenzimidazole nucleotide in wild-type protein. The "front cover" of the pocket, consisting of strand 5 and helix 5, has been stripped away to reveal the crevice that holds the nucleotide tail. (*B*) A model of the Gly-630 → Glu mutant with the altered amino acid residue in red. This model predicts that cobinamide (cobalamin analog in which the nucleotide side chain is terminated before the phosphate) can be bound to this mutant species. (*C*) A model of the Gly-703 → Arg mutant with the altered amino acid residue in blue and a bound cofactor truncated at the phosphate group.

negatively charged cobalamin phosphate could similarly enhance the affinity for the cobalamin analog.

Mutations Affecting the "Histidine Loop" Lead to Impaired Function. The most surprising aspect of the structure of the cobalamin-binding domains of methionine was the replacement of the dimethylbenzimidazole ligand to cobalt by a histidine from the protein. The loop that contributes the histidine ligand adopts an unusual conformation, and comparison with structures of other nucleotide-binding proteins suggests that it may be unique to Rossmann folds that bind cobalamin. Interactions between the histidine loop and the cobalamin are extensive (Fig. 4) and are expected to be important both for catalysis and for binding of the cofactor. It is perhaps no surprise that mutations in and around this loop lead to dysfunction. Two mutations that cause methylmalonic aciduria in humans, Gly-623 → Arg and Gly-626 → Cys, directly affect residues in the loop (11, 14). Gly-623 of methylmalonyl-CoA mutase corresponds to Lys-755 in methionine synthase, which is in the second position of a β-turn. Glycines are often found in β-turns for steric reasons (43). In all of the mutase sequences listed in Fig. 1, glycine occurs in position 2 of the putative β-turn, and in all the methyltransferase sequences, glycine is found in position 3. We suspect that accommodation to the arginine side chain in the Gly-623 → Arg mutant may induce deleterious changes in the conformation of the loop carrying the histidine ligand and may, in addition, affect interactions of the α/β domain with other domains of the enzyme.

The Gly-626 → Cys mutation at the residue between aspartate and histidine in the Asp-Xaa-His-$(Xaa)_2$-Gly motif decreases the enzymatic activity, resulting in a *mut*$^-$ phenotype (11). Comparison of sequences would not predict a major effect of this substitution. Valine, with a similar volume to cysteine, is found in this location in methionine synthase, and cysteine occurs at this position in the glutamate mutases. Why then is the activity of the enzyme so affected? Although this mutation appears conservative, the residue is positioned in a critical place in the structure. In methionine synthase, the backbone nitrogen of this residue directly contacts the cobalamin. Thus, even a conservative change at this location may affect interactions at the lower face of the corrin.

A third mutation, Gly-648 → Asp, replaces the glycine residue that aligns with Gly-781 in methionine synthase and displays a mut$^-$ phenotype (11). This residue is not in direct contact with the cobalamin but makes interactions that stabilize the loop carrying the histidine ligand. In methionine synthase, Gly-781 is located in a β-bulge at the C terminus of strand βII. As can be seen in Fig. 5, a series of backbone hydrogen bonds from the βII–αII connector helps to position the histidine loop. Two ways in which a mutation at Gly-648 could affect the structure and cobalamin affinity of methylmalonyl-CoA mutase are suggested by the related structure of methionine synthase (Fig. 5). First, the bulge glycine adopts a backbone conformation that would be high energy for any other residue. If substitution of the glycine disrupts the

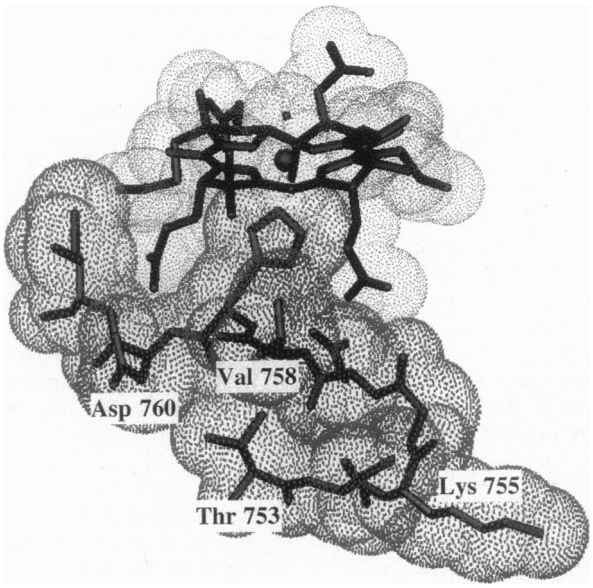

FIG. 4. A view of the loop carrying the His-759 ligand to cobalt in *E. coli* methionine synthase. Residues 755–761, from the sequence Gly-Asp-Val-His-Asp-Ile, pack tightly against the cobalamin. van der Waals surfaces around the protein are shown in yellow, and van der Waals surfaces around the cobalamin are shown in blue. Propionamide side chains from the B and C rings of the cobalamin are in position to hydrogen bond with backbone atoms of residues Gly-756, Val-758, Asp-760, and Ile-761.

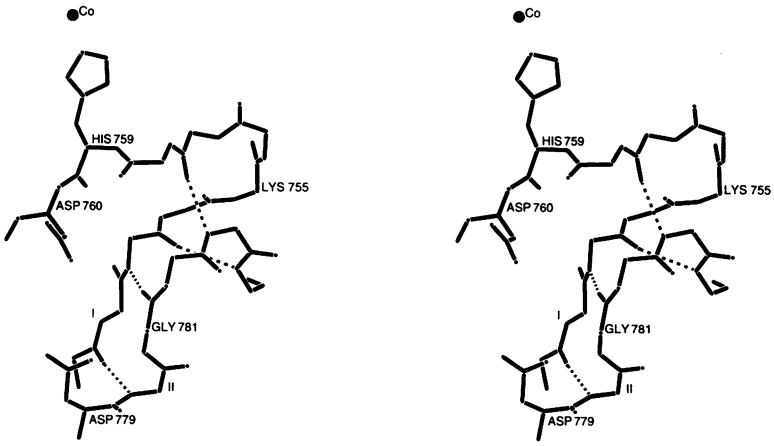

FIG. 5. A stereoview of the loops in methionine synthase that connect sheet strands βI and βII with helices αI and αII. The hydrogen bonding that stabilizes and orients these connectors is displayed as dashed lines. To simplify the view, most side chains have been deleted, and only cobalt of the cobalamin is shown. The irregularity in the β-sheet (the β-bulge) is visible at residues 780 and 781. In a standard β-sheet configuration, the carbonyl of residue 780 would be pointed toward βI, and the carbonyl of Gly-781 would be pointed away and would be unable to form the indicated hydrogen bond. The β-bulge at Gly-781 is secured by interaction with Asp-779. The carboxylate of Asp-760 is a potential hydrogen bonding partner of the corrin ring C propionamide.

β-bulge, this could in turn disrupt the hydrogen bonds that stabilize the histidine loop. Second, substitution of the glycine by aspartate places an additional negative charge in this region, not far from conserved aspartate residues at positions 779 and 760 (methionine synthase numbering).

In contrast to wild-type methylmalonyl-CoA mutases, cobalamin binding is pH-dependent in the Gly-648 → Asp mutant. From measurements of product formation vs. AdoCbl concentration, the apparent K_m (AdoCbl) was estimated to be ≈25–30 μM at pH 5.5–7.5, as compared with ≈200 μM at pH 9.5 (11). Despite this dramatic difference, the introduction of the negative charge is clearly not the whole story of the *mut*$^-$ phenotype of Gly-648 → Asp. The K_m (AdoCbl) for wild-type enzyme is ≈100-fold smaller than the value for the mutant at pH 5.5 (11, 15). Thus, characterization of the Gly-648 → Asp mutant gives some weight to the argument that the additional hydrogen bonds formed in the vicinity of the β-bulge are important for the structural integrity of the cobalamin-binding site.

Other Mutations in the β-Strands May Impair Function. At the core of the α/β domain of methionine synthase are the five parallel β-strands. One mutation that leads to methylmalonic aciduria, Val-669 → Glu, and one polymorphism, Val-671 → Ile, affect residues predicted to lie in strand βIII. Since the polymorphism (14) merely substitutes one hydrophobic residue for another in strand βIII, it is not surprising that the substitution is well-tolerated. In contrast, the mutation Val-669→ Glu that leads to disease (39) involves substitution of a charged residue for an uncharged one. The mutant protein has not yet been expressed, so details of the phenotype of this mutation are not known. The basis for the *mut*$^-$ phenotypes of the mutants Gly-717 → Val and Arg-694 → Trp (11–13) is more difficult to discern. Both residues are located in turns at the edge of the β-sheet that is remote from the corrin ring of cobalamin. Their replacement may somehow distort the sheet packing, which would impact the dimethylbenzimidazole-binding pocket (see Fig. 2) or may affect interactions of the α/β domain with other domains of the enzyme. It is interesting to note that Gly-717 is highly conserved in the sequence alignment shown in Fig. 1.

DISCUSSION

A Structural Explanation for Interallelic Complementation. Human methylmalonyl-CoA mutase is a dimer of identical subunits. Extensive studies by the laboratories of Rosenblatt and Ledley (11, 12, 14, 44) have established that a point mutation at the N terminus of the protein subunit that leads to complete abolition of activity in a homozygote, Arg-93 → His, can be complemented by any one of a cluster of mutations in the C terminus of the subunit that themselves abolish activity. These observations led Crane and Ledley (11) to suggest that the C-terminal portion of human methylmalonyl-CoA mutase was critical for cobalamin binding and could be complemented in *trans* by the N-terminal portion of another subunit (Fig. 6). This idea can be rationalized in view of the structure of the cobalamin-binding fragment of methionine synthase. As shown in Fig. 2, the cobalamin in methionine synthase is sandwiched between the α-helical region contacting the top face of the cobalamin and a Rossmann fold interacting with the lower face of the cobalamin and the nucleotide side chain. These two domains are connected by a single peptide bridge that appears disordered in the crystal structure, indicating conformational flexibility. The structure suggests that the interallelic complementation arises because the AdoCbl prosthetic group of human methylmalonyl-CoA mutase can be bound by the C terminus of one subunit, which interacts with the bottom face of cobalamin and with the nucleotide tail, and also interacts, in the vicinity of the top face, with the N terminus of the other subunit. Since the bacterial methylmalonyl-CoA mutases are heterodimers of chains with distinctly different sequences, and only one chain shows clear homology with the cobalamin-binding region of methionine synthase, the forthcoming structure of the dimeric bacterial methylmalonyl-CoA mutase from *P. shermanii* (2, 47) may not display a mode of dimerization that accounts for the complementation observed in the human enzyme (see Note).

Implications for Treatment of Human Disease. These structure-based predictions provide the rationale for dysfunction of some methylmalonyl-CoA mutases and suggest initial strategies for therapy of patients carrying these mutations. For those patients with mutations predicted to block cobalamin binding, treatment with cobalamin will probably not be successful, but treatment with analogs is an avenue worthy of further investigation. A first step is to determine whether the mutant human enzymes are active *in vitro* with AdoCbl analogs. Whether or not AdoCbl analogs are able to restore high levels of activity is a matter of some disagreement (37, 41, 42). Transport and processing of the analogs *in vivo* present further possible obstacles. Kolhouse, Allen, and coworkers (48) showed that analogs that stimulate methionine synthase activity *in vitro* can inhibit activity *in vivo*. They suggested that the *in vivo* inhibition by analogs may be due to inhibition of intracellular cobalamin transport or to inhibition of one of the enzymes involved in cobalamin processing (48). We know that intrinsic factor, the protein responsible for cobalamin absorption in the small intestine, has a much higher specificity for cobalamin than it does for analogs (49), whereas transcobalamin II, the major plasma transport protein for cobalamin, is less selective and can bind analogs with high affinity (48). Unfortunately, cobinamide, the analog displayed in Fig. 3*B*, does not bind tightly to transcobalamin II (48). Despite the potential problems with analog therapy, results from Willard and Rosenberg (19) and Ledley and coworkers (9, 10, 13) provide encouragement that restoration of even low levels of methylmalonyl-CoA mutase activity in patients with *mut*^{0} phenotypes could have significant clinical benefit.

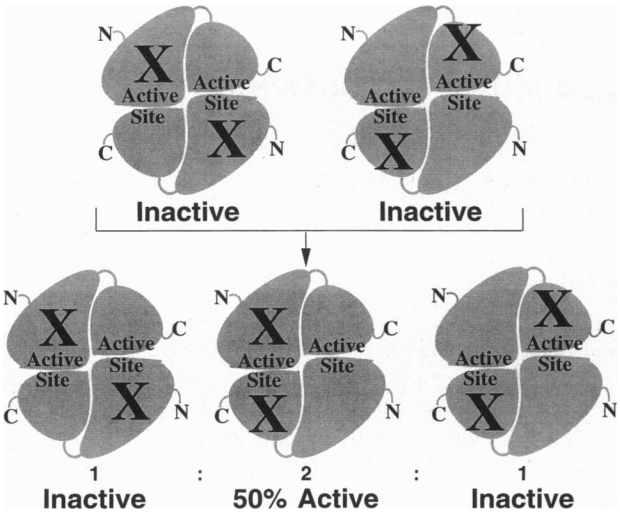

FIG. 6. Diagram of the simplest model by which interallelic complementation might occur. A mutation at the N terminus of a homodimer (*Upper, Left*) can be complemented by a mutation at the C terminus of a homodimer (*Upper, Right*) if the active site is formed from portions of the N terminus of one monomer and the C terminus of the other monomer (45, 46). Mixing together molecules homozygous for a mutation (X) in the N terminus with molecules homozygous for a mutation (X) in the C terminus will theoretically result in 50% heterozygous molecules (*Lower, Center*), which will have one doubly mutated active site and one normal active site.

Note. A paper by Mancia and coworkers (50) that describes the structure of methylmalonyl-CoA mutase from *Propionibacterium shermanii* was published after the submission of this manuscript.

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