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The Radical Use of Rossmann and TIM Barrel Architectures for Controlling Coenzyme B₁₂ Chemistry

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Abstract

The ability of enzymes to harness free-radical chemistry allows for some of the most amazing transformations in nature, including reduction of ribonucleotides and carbon skeletal rearrangements. Enzyme cofactors involved in this chemistry can be large and complex, such as adenosylcobalamin (coenzyme B₁₂), simpler such as “poor man’s B₁₂” (*S*-adenosylmethionine and an iron-sulfur cluster), or very small such as one iron atom coordinated by protein ligands. While the chemistry catalyzed by these enzyme-bound cofactors is unparalleled, it does come at a price. The enzyme must be able to *control* these radical reactions, preventing unwanted chemistry and protecting the enzyme active site from damage. Here we consider a set of “radical” folds: the (β/α)₈ or TIM barrel, combined with a Rossmann domain for coenzyme B₁₂ dependent chemistry. Using specific enzyme examples, we consider how nature employs the common TIM barrel fold and its Rossmann domain partner for radical-based chemistry.

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INTRODUCTION

Although the standard building blocks of nature (carbon, nitrogen, oxygen, sulfur) form peptide scaffolds that are capable of catalyzing a range of chemical reactions, there are some, such as those that are radical-based, that cannot be achieved using these functionalities alone. For this reason, nature employs a mixture of cofactors and prosthetic groups to assist either in catalysis or in generating the catalytic unit, and many of these cofactors contain metals. One of the most complex of these cofactors is coenzyme B₁₂ (AdoCbl, Figure 1). This cofactor is distinguished in possessing a C-Co bond, which is sufficiently weak that homolysis is relatively facile, with a bond dissociation energy measured for free coenzyme at $\sim 130 \text{ kJ mol}^{-1}$ ($31.8 \pm 0.7 \text{ kcal mol}^{-1}$) (29). Enzymes that utilize AdoCbl do so to generate a reusable radical. In particular, the reversible homolytic cleavage of the C-Co bond yields cob(II)alamin and a putative 5'-deoxyadenosyl radical (Ado•), a species that has never been directly observed but is widely accepted to exist (2). Such an Ado• species would be highly reactive and thus capable of initiating remarkable transformations, including cleavage of otherwise unreactive carbon-hydrogen bonds, leading to significant structural reorganizations of carbon skeletons (Figure 2). Importantly, AdoCbl-dependent enzymes are designed such that the generation of radical species is limited in the absence of substrates or effectors, protecting the enzyme from potential damage. For each enzyme, substrate or effector binding somehow modulates the reactivity of the AdoCbl, enhancing the rate of C-Co bond cleavage by as much as 10^{12+2} fold (reviewed in (1) and (11)). As described in more detail below, AdoCbl-dependent enzymes typically employ (β/α)₈ triosephosphate isomerase (TIM) barrel architectures for substrate binding and often use Rossmann domains for AdoCbl binding. They are commonly found as part of bacterial fermentative pathways, some of which have industrial relevance (38), and in fatty acid and amino acid metabolism, with associated medical implications (19, 21).

With recent reviews describing computational and kinetic studies of AdoCbl-dependent enzymes (8, 12, 17, 22, 24, 32, 44, 46, 47, 59), here we consider the molecular mechanism by which AdoCbl

enzymes “sense” substrate binding, focusing on AdoCbl-dependent mutases, a subset of AdoCbl enzymes that use Rossmann domains and TIM barrels for their radical-based chemistry. Structures of methylmalonyl-CoA mutase (MCM), lysine 5,6-aminomutase (5,6-LAM), and ornithine 4,5-aminomutase (4,5-OAM) suggest how substrate binding in each case triggers a large conformational change affecting the active site configuration, presumably leading to the enhanced rate of C-Co bond homolysis. In addition, we use structural data on MCM and glutamate mutase to help us understand the molecular process by which an Ado• species can rearrange to abstract a hydrogen atom from substrate, initiating catalysis. Conformational reorganizations appear to be key to the reactivity of many AdoCbl enzymes, and this review describes the structural information as well as our current thinking about how the AdoCbl-dependent mutases use the ubiquitous TIM barrel fold and the common Rossmann fold to harness radical chemistry.

FORMS OF COBALAMIN AND COBALAMIN-DEPENDENT CHEMISTRY

The cobalamin cofactor is a complex organometallic molecule, with a cobalt ion in the center of a tetrapyrrolic-derived macrocycle, which is known as the corrin ring or macrocycle (Figure 1). Cobalamin is highly decorated, with acetamide, propionamide, and methyl groups adorning the ring. The largest substituent is the dimethylbenzimidazole (DMB) tail, which can act as a lower ligand to the cobalt in the free cofactor. Although this review focuses on coenzyme B₁₂ or adenosylcobalamin (AdoCbl), a related form of the vitamin is methylcobalamin, where the adenosyl group on the upper face of the corrin is replaced by a methyl moiety (i.e. R = CH₃, Figure 1). Enzyme systems that use methylcobalamin include methionine synthase, one of two Cbl-dependent enzymes that are found in humans. The other human Cbl-dependent enzyme is AdoCbl-dependent MCM. Interestingly, both MCM and methionine synthase utilize a similar protein fold for binding their cofactors (18). In contrast to

AdoCbl enzymes, the C-Co bond of methylcobalamin undergoes heterolytic cleavage to methylate the enzyme substrate with a methyl cation, cycling the Cbl cofactor between cob(I)alamin and methylcob(III)alamin redox states.

While methylcobalamin-dependent enzymes catalyze methyl transfer reactions, AdoCbl-dependent enzymes catalyze rearrangement reactions using radical-based chemistry. These reorganizations include carbon skeleton mutase reactions (carried out by Class I enzymes), elimination reactions (Class II enzymes), and aminomutase reactions (Class III enzymes, also requiring pyridoxal phosphate (PLP) as an additional cofactor) and are summarized in Supplementary Table 1 with examples of reactions shown in Figure 2. In the case of the carbon skeleton mutases, a hydrogen atom from substrate is first abstracted by Ado• to create a reactive substrate radical, which rearranges to generate the product radical. Two sorts of rearrangement mechanisms have been proposed for these reactions: an addition/elimination mechanism, whereby the radical adds to an unsaturated group within the substrate, forming a cyclopropyl-like reactive species that then opens to afford the product radical (26, 64, 66); and a fragmentation/recombination mechanism, whereby the substrate radical fragments to an unsaturated species and intermediate radical that recombine to form the product radical (5, 14, 15, 44, 56). There has been much debate on which of these mechanisms is active for various mutases, with the current proposal of an addition/elimination mechanism for methylmalonyl CoA-like substrates (Figure 3a) and a fragmentation/recombination mechanism for substrates without a double bond, such as glutamate (Figure 3b) (5, 14, 15, 44, 56). Aminomutases follow a very similar reaction mechanism to the carbon-skeleton mutases. Here, however, the amino group is first anchored via PLP by formation of a Schiff-base, before following an addition/elimination mechanism (Figure 3c). Eliminases also undergo a 1,2-shift, typically of an alcohol or amino group. However, once the product gem-diol (or α -amino alcohol) is formed, release of water (or ammonia) is favored, driving the reaction to completion.

Regardless of the exact reaction catalyzed, all AdoCbl enzymes must exert a high level of control over the reactive radical, both to prevent unwanted side reactions and to ensure that the coenzyme can be reformed for subsequent turnovers. Below we consider how the structural folds of these proteins are used to exert this control.

OVERVIEW OF STRUCTURAL MOTIFS FOR ADOCBL-DEPENDENT CHEMISTRY

AdoCbl-dependent enzymes share a number of structural features (7, 42, 55, 61-63, 81, 82) (Supplementary Table 1, Figures 4,5). In each AdoCbl-dependent enzyme with a known structure, substrate binds in a β/α barrel (7, 42, 55, 61-63, 81, 82), which is typically a $(\beta/\alpha)_8$ TIM barrel (Supplementary Table 1, Figure 5b,d). The notable exception is ribonucleotide reductase (RNR), in which the active site is located in a 10-stranded β/α barrel that resembles much more closely other classes of RNR (63), as well as enzymes like pyruvate formate lyase that are members of the glycol radical enzymes (GREs) superfamily (6, 36, 37, 40, 50).

AdoCbl can exist in two enzyme-bound forms; the “base-on” form, which has DMB as the lower axial ligand for cobalt (61); and the “base-off/His-on” form (Figure 1), where the DMB is displaced by up to 25 Å from the cobalt, which is coordinated by a histidine from the protein (18). All AdoCbl-dependent enzymes that bind AdoCbl in the base-off mode use a fold that resembles flavodoxin: a Rossmann fold with a central sheet of five parallel β -strands and helices on either side (Supplementary Table 1, Figure 5a,c). This latter mode of binding is characterized by a “DXHXXG SXL GG” motif, which includes a loop containing the axial histidine ligand (18, 45). Also within this base-off motif, larger amino acids are substituted with two glycine residues (GG) in a β -strand, leaving room between the strand and an outside helix for the nucleotide tail (Figure 5c). For glutamate mutase, NMR studies show that the presence of this cavity and an extended His-ligand-containing loop lead to a

Rossmann domain that is reasonably dynamic in the absence of AdoCbl, with Cbl binding stabilizing the structure (74). This finding is consistent with X-ray structures of apo- and AdoCbl-bound human MCM that show movement of a helix and two loops of the Cbl-binding Rossmann domain, including the His-loop, in the absence of cofactor (23).

While carbon skeleton mutases and aminomutases bind AdoCbl in the “base-off/His-on” form using a Rossmann fold, the eliminases, including RNR, maintain the coenzyme in the “base-on” form. Structures of enzymes that bind AdoCbl “base-on” vary in terms of their binding motifs, with some enzymes using separate domains composed of β -strands and helices, but not in a Rossmann arrangement (see Figure 5a), while others like RNRs use loops from the substrate-binding 10-stranded β/α barrel (Supplementary Table 1). Irrespective of whether AdoCbl is bound “base-on” or “base-off”, Cbl-binding domains typically position the AdoCbl onto the rim of the substrate-binding barrel in close vicinity of the active site (Figure 5e), affording an appropriate distance for hydrogen-atom abstraction as well as providing a sealed environment. Importantly, the enzyme modulates cleavage of the C-Co bond of AdoCbl such that radical generation is favored when either a substrate (2) or an allosteric effector molecule (34) is bound. By using substrate or effector binding as a trigger to initiate C-Co bond cleavage, the enzyme can exert exquisite control over these highly reactive intermediates.

CARBON-SKELETAL MUTASES: OVERALL STRUCTURE AND REACTIONS

Methylmalonyl-CoA mutase

MCM is one of the best-characterized mutase enzymes with structures of a bacterial (*Propionibacterium shermanii*) and human enzyme demonstrating the use of the TIM barrel for substrate binding (pink and blue in Figure 4) and use of the Rossmann domain for AdoCbl binding (orange and yellow in Figure 4). The TIM barrel at the N-terminus is connected to the Rossmann domain at the C-terminus by a so-called

“belt” region (gray in Figure 4). While both bacterial and human enzymes are dimers, bacterial MCM is a heterodimer with one active protomer (containing Cbl, orange in Figure 4) and one inactive protomer (no Cbl, yellow in Figure 4), whereas the human enzyme is a homodimer with each protomer containing a Cbl cofactor (Figure 6). The functional significance, if any, of the inactive protomer in the bacterial enzyme is unknown.

MCM catalyzes the conversion of methylmalonyl-CoA to succinyl-CoA (Figure 2), allowing for the breakdown products of odd-chain fatty acids, several hydrophobic amino acids (isoleucine, valine, methionine, and threonine), and cholesterol to enter into primary metabolism (21). MCM is a mitochondrial enzyme and, as mentioned above, one of two human enzymes that require vitamin B₁₂ (the other is methionine synthase). Genetic defects in MCM can lead to a relatively rare (16, 35) condition known as methylmalonic aciduria (MMA), in which conversion of methylmalonyl-CoA to succinyl-CoA is blocked, resulting in production of methylmalonic acid. In severe cases of this disease, up to two grams of acid can be excreted per day (80), affecting the pH of the patient’s blood and leading to developmental disorders (21).

Like other AdoCbl-dependent mutases, the rearrangement of substrate into product involves a 1,2-migration (Figure 3a). Model studies pointed to an addition/elimination mechanism early on, wherein it was demonstrated that a cyclopropyl intermediate was thermodynamically accessible (26). Additional support for a cyclopropyl intermediate came from electron paramagnetic resonance (EPR) spectroscopy studies with substrate analogues as well as enzyme kinetic studies demonstrating that cyclopropyl compounds can act as competitive MCM inhibitors (72). Further computational studies have shown that a cyclopropyl intermediate is thermodynamically preferred and that the overall rearrangement can be facilitated by ‘partial protonation’ through a hydrogen-bond to the substrate carbonyl (64, 66, 78). Based on the crystal structure and on mutagenesis studies, His244 (numbering

based on bacterial enzyme) in the MCM active site is the most likely candidate to be involved in this putative partial proton transfer (41, 42, 73).

Glutamate mutase

Glutamate mutase (MutES), another well-studied mutase enzyme, is responsible for the reversible conversion of *S*-glutamate to (2*S*,3*S*)-3-methylaspartate during fermentation of *S*-glutamate to acetate, butyrate, carbon dioxide, and ammonia in various species of *Clostridium* (3, 13). In contrast to MCM, which contains both the TIM barrel and the Rossmann domain on the same polypeptide chain, the Rossmann Cbl-binding domain of glutamate mutase is on one polypeptide chain (MutS from *C. cochlearium* or GlmS from *C. tetanomorphum*), and the substrate-binding TIM barrel is on another (MutE or GlmE) (4). Although MutE subunits are always dimeric, MutE and MutS only form the catalytically active heterotetramer (MutES) in the presence of cofactor (30, 83) (Figure 4). Interestingly, the MutS protein on its own displays no significant binding affinity for Cbl, although it binds a synthetic construct of the Cbl nucleotide tail with low mM affinity (74); the heterotetramer binds Cbl with low μM affinity when MutS is in 5-fold excess (30). Crystal structures of glutamate mutase from *C. cochlearium* with various forms of cobalamin and substrates bound (25, 55) reveal that the heterotetramer binds two molecules of AdoCbl in the “base-off/His-on” orientation, sandwiched in-between the Rossmann and TIM barrel domains of each MutES heterodimer. The net result of these interactions between cofactor and protein creates an overall structure that closely resembles that of MCM (Figure 4).

Similar to the MCM reaction discussed above, MutES catalyzes the carbon skeleton isomerization of *S*-glutamate with a 1,2 migration of the entire amino acid backbone (i.e. a glycyl residue) (Figure 3b). This rearrangement is particularly interesting considering that the migrating carbon is sp^3 hybridized, compared with the corresponding sp^2 hybridized center in the MCM reaction.

Identification of the intermediates, C-4 glutamate radical by EPR, and acrylate and glyceryl radical by stopped-flow and HPLC analysis, supports the concept of fragmentation and recombination in the MutES reaction (9, 14, 15); the formation of the less stable methylaspartyl radical is not observed.

AMINOMUTASES: OVERALL STRUCTURE AND REACTIONS

Lysine 5,6-aminomutase and Ornithine 4,5-aminomutase

5,6-LAM and 4,5-OAM are two other AdoCbl enzymes that use a Rossmann domain to bind their AdoCbl cofactor and a TIM barrel to bind their substrate. They differ from other AdoCbl enzymes in that they have an additional cofactor, PLP. This second cofactor also binds in the TIM barrel, although the lysine residue (144 in 5,6-LAM) that provides its covalent attachment to the protein is derived from the Rossmann domain. 5,6-LAM is a heterotetramer, made up of two ($\alpha\beta$) dimers (7). Unlike MCM, which has both the TIM barrel and Rossmann domain on the same polypeptide chain, for 5,6-LAM the TIM barrel is on one chain (α -chain) and the Rossmann domain is another (β -chain). This arrangement is similar to MutES, for which the TIM barrel and Rossmann domains are on different polypeptide chains. Also on the α -chain of 5,6-LAM is a region of the structure called the accessory clamp (gray in Figure 4), which folds around the TIM barrel and contacts the AdoCbl that is bound to the Rossmann domain. The β -chain also has a second domain, thought to be involved in the dimerization of the two ($\alpha\beta$) units (Figure 4) since it makes multiple contacts at the dimer interface.

Only one structure is available for 5,6-LAM, from *Clostridium sticklandii*. However, the structure of a related enzyme is available, 4,5-OAM, also from *C. sticklandii* (81), showing high homology (28% and 35% sequence identity and 39% and 47% similarity to the 5,6-LAM α and β subunits, respectively) (75) and suggesting that AdoCbl-dependent aminomutases will all use similar structural motifs. 4,5-OAM consists of two separate proteins, an 83 kDa OraE subunit and a 13 kDa

OraS subunit. While both 5,6-LAM and 4,5-OAM are heterotetramers, 4,5-OAM is different from 5,6-LAM in that the Rossmann and TIM barrel domains are located on the same protein chain within the OraE subunit. The additional OraS subunit contains part of the accessory clamp observed in 5,6-LAM. Interestingly, although the overall structures of these enzymes are very similar (Figure 4), the positioning of the domains within the polypeptide chains is different (Figure 6). While the dimerization domain in 5,6-LAM is located at the N-terminus of the Rossmann domain, in 4,5-OAM, this domain is positioned between the TIM and Rossmann domains within the same protein chain, and its location at the dimer interface is swapped in comparison to 5,6-LAM. In 5,6-LAM, the dimerization domain (brown in Figure 6) is proximal to its Rossmann domain (also brown), while in 4,5-OAM the addition of a protein linker allows the dimerization domain (blue in Figure 6) to be proximal to the neighboring chain's Rossmann domain (yellow). Even more of a surprise, a domain swap between two OraE proteins of the heterotetramer results in exchange of the Rossmann domains between each OraE subunit, such that the Rossmann domain of monomer A (yellow in Figure 6) binds AdoCbl with the TIM barrel of monomer B (blue in Figure 6), and vice versa. The last unique component of the 4,5-OAM structure is the presence of the OraS subunit, which constitutes the remainder of the accessory clamp (gray in Figure 6). Thus, nature arrived at a similar overall architecture using a cut and paste approach.

Aminomutases catalyze the reversible migration of an amino group, with the best-studied reaction being of the 5,6-LAM enzyme from the laboratory of Perry Frey (49, 69, 70) (Figure 3c). Binding of the substrate lysine to the PLP cofactor causes a transaldimination reaction in which the covalent attachment through Lys144 is severed and a new covalent link between substrate and PLP is formed. Upon substrate binding, the C-Co bond of the AdoCbl is cleaved, generating Ado• first, and substrate radical second, through hydrogen-atom abstraction. During the subsequent rearrangement of the substrate radical into the product radical, the PLP plays a role in stabilizing an aziridylcarbinyl

intermediate species (Figure 3c). Following the formation of the product radical, hydrogen-atom abstraction back from AdoH will generate product and regenerate Ado•, which then can recombine with the cob(II)alamin forming AdoCbl.

The exact role of the PLP in this rearrangement reaction has been the subject of computational and model studies (27, 77, 79). Computational experiments indicate that this 5,6-aminomutase rearrangement is not facile in the absence of a covalent modifier (77, 79), and might instead proceed through a fragmentation/recombination pathway in a similar fashion as that proposed for glutamate mutase (Figures 3b). Unlike the prediction for other mutase reactions (64-67, 76), partial protonation of the substrate does not help in reducing the barrier for this type of rearrangement. In contrast to free lysine, computational experiments with PLP-bound lysine indicate that migration becomes favorable, proceeding through an aziridylcarbinyl intermediate species (77). The PLP hydroxyl group could also play a role in ensuring the intermediate is not overstabilized, which would trap the aziridylcarbinyl intermediate species in an energy well, preventing further reaction (79). Non-enzymatic model studies provide further support for this type of radical rearrangement (27).

LARGE CONFORMATIONAL CHANGES INDUCED BY SUBSTRATE BINDING

Case 1: Methylmalonyl-CoA mutase

For MCM, as for many other AdoCbl-dependent enzymes, the binding of substrate is required for the $10^{12\pm 2}$ -fold acceleration of C-Co bond homolysis to generate the Ado• radical, and initial production of a substrate radical (51). With radical generation limited in the absence of substrate, methylmalonyl-CoA, the enzyme is protected from damage. As mentioned above, major questions in the Cbl field are how the binding of substrate triggers C-Co bond homolysis and how the structure of the enzyme is designed such that substrate binding initiates AdoCbl cleavage.

In the case of MCM, structural studies show that substrate binds in the center of the TIM barrel, interacting with a number of small, hydrophilic residues (23, 43). The methylmalonyl moiety of the substrate is positioned near the C-terminal end of the barrel adjacent to the upper face of the AdoCbl, which is in turn placed into the C-terminal end of the TIM barrel by the Rossmann domain (Figure 5e). The CoA moiety of the substrate stretches across the full length of the barrel, from the exterior of the barrel to the Cbl. The positioning of the substrate in the center of the barrel is atypical for TIM barrel enzymes. With most family members, substrate binds in a pocket formed by loops at the C-terminal end of the TIM barrel and the center of the barrel is inaccessible, packed with hydrophobic residues (10). In these cases, the barrel provides a stable framework for chemistry to occur in the shallow active site formed at the barrel's C-terminal end. However for MCM, substrate lines the inside of the barrel, and this use of TIM barrel architecture is associated with another unusual barrel property, which is that this barrel can 'open' and 'close.' Two conformations of the barrel have been observed by crystallography: an open state in which three strands of the eight-stranded barrel and their associated helices are splayed out, away from the barrel interior; and a closed state in which those three strands join the other strands to form a typical $(\beta/\alpha)_8$ fold (Figure 7a) (23, 42). By linking this dramatic conformational change of the barrel to substrate binding and product release, the enzyme has an inherent signaling mechanism. Substrate binding triggers a conformational change from a resting state into an active state of the enzyme, which in turn triggers radical generation.

Such a dramatic conformational change is surprising and raises the question of how many turnovers an enzyme can accomplish before some type of structural failure occurs. Also, the C-Co bond of AdoCbl is cleaved and then re-formed on each turnover. If oxidation occurs during this cycle, the bond can remain forever cleaved and the enzyme can no longer function. These issues plague all AdoCbl enzymes and each enzyme seems to have evolved a different coping mechanism. For diol

dehydratases, for example, an ATP-dependent chaperone assists in the removal of a damaged AdoCbl, inserting a fresh cofactor (39, 48), whereas for MCM, a metallochaperone known as MeaB in the bacterial systems and MMAA (named for the link to methylmalonic aciduria type A) in humans, remains associated with MCM during catalysis, protecting it from damage (23, 31, 68). MeaB/MMAA proteins, which are in the GTPase family of metallochaperones, also appear to have a gating function, preventing Cbls that are missing the Ado group from being inserted into their target enzymes (53). Such Cbls would, of course, be inhibitory to MCM. Determining the mechanisms by which MeaB/MMAA function is an active area of investigation (33, 52-54). With published structures of both MeaB and MMAA (23, 31), the missing piece is a structure of a complex between the metallochaperone and its target enzyme. Without such a structure, it is impossible to know how the metallochaperone exerts its protective functions.

Case 2: Lysine 5,6-aminomutase and Ornithine 4,5-aminomutase

Before crystallographic analysis of any AdoCbl-dependent aminomutase, it was unclear how the binding of a small substrate like lysine or ornithine could send the requisite signal to the AdoCbl, enhancing homolysis of the C-Co bond. As described above for MCM, the substrate methylmalonyl-CoA is relatively substantial in size, and threads through the TIM barrel. This threading of substrate yields a large conformational change in the barrel itself. A small substrate, like lysine or ornithine, would not be expected to invoke such a dramatic response by its binding alone. How then does lysine or ornithine binding to 5,6-LAM or 4,5-OAM trigger C-Co bond homolysis?

A clue came from the structural analysis of 5,6-LAM (7) and the observation, mentioned above, that in the resting enzyme, PLP is covalently attached to the Rossmann domain. In particular, it is attached to Lys144, a residue that resides in a loop that disrupts helix 1 of the Rossmann domain (see

Figure 5d,f). Helix 1 follows the His-ligand loop, and in 5,6-LAM, this helix is disrupted after two helical turns by another loop that extends across the α and β interface, placing Lys144 into the TIM barrel where it attaches to the PLP. The net result of the covalent connection between the Rossmann domain and the PLP of the TIM barrel is that the side of the Rossmann domain rests against the TIM barrel, placing its AdoCbl cofactor off to the side as opposed to placing it in the barrel (Figure 5f). The 4,5-OAM structure, which was solved after 5,6-LAM, shows the same type of covalent attachment, again using a lysine from the Rossmann domain (Lys629) and depicts the same orientation of the Rossmann domain with respect to the TIM barrel (81). A comparison of the orientations of the Rossmann domain with respect to the TIM barrels for MCM and 5,6-LAM is shown in Figure 5e,f. Whereas the Rossmann domain of MCM places the AdoCbl into the active site of its TIM barrel, the AdoCbl bound to the Rossmann domain in 5,6-LAM is approximately 25 Å away from its active site. The AdoCbl in this “resting” or noncatalytic position makes contacts with the so-called accessory clamp, a structural modification of aminomutase enzymes that is not found in other families of AdoCbl enzymes. Although both 5,6-LAM and 4,5-OAM have accessory domains that interact with the “resting” AdoCbl, the exact fold and position in the sequence is not the same (Figures 4 and 6) (7, 81). Also, the adenosyl moiety in these aminomutases has a different orientation compared to other AdoCbls. The adenine ring adopts a *syn* conformation with respect to the ribose as opposed to the typical *anti* conformation (7, 81). Although the reason for adopting this conformation can be rationalized from the structure, the significance, if any, of this difference is not understood.

The net result of this different positioning of the Rossmann domain and of its AdoCbl is that neither 5,6-LAM nor 4,5-OAM can damage their active sites through unwanted radical chemistry in the resting state, since their cofactors are far from the active sites in the absence of substrate. How then does the binding of substrate trigger a conformational change to bring the AdoCbl into position for hydrogen-

atom abstraction? To answer this question, we return to the PLP. A space-filling model of 5,6-LAM in the resting state shows that lysine can enter the TIM barrel in a gap between the Rossmann domain and the dimerization domain (Figure 7b). Once the transaldimination reaction has occurred, substrate lysine is bound to the PLP and Lys144 is released. With the covalent attachment broken, the Rossmann domain is free to rotate and can position the AdoCbl into the active site of the TIM barrel, just as it does in the MCM structure. A model of this configuration is shown in Figure 7c. With AdoCbl positioned correctly, catalysis can take place. Thus, one role for the PLP seems to be in signaling that substrate is bound, or later that product is released. Through the formation and cleavage of covalent bonds to and from the PLP, a small substrate like lysine or ornithine can achieve a large conformational change in these enzymes. With this large subunit rearrangement associated with C-Co bond homolysis, a rationale is available for the large rate enhancement in C-Co bond cleavage upon substrate binding. Notably, both MCM and these aminomutases use TIM barrels and Rossmann domains, but MCM opens and closes the TIM barrel, whereas the TIM barrel remains intact in these aminomutases, and instead 5,6-LAM and 4,5-OAM must swing their Rossmann domains. Unfortunately, for both 5,6-LAM and 4,5-OAM, only structures in the “resting” states have been solved and thus only models are available for the closed conformations that have AdoCbl positioned in the barrel for catalysis.

As mentioned above, all AdoCbl enzymes must protect themselves from unwanted chemistry, and although 5,6-LAM and 4,5-OAM have one mechanism in place (keep AdoCbl out of the active site in the absence of substrate), this mechanism does not protect against all forms of damage. Once the C-Co bond is cleaved in the presence of substrate, the cycle can be interrupted by oxidation (71). Thus, even with a protective mechanism, 5,6 LAM is not perfectly designed to minimize side effects.

MOLECULAR BASIS FOR HYDROGEN-ATOM ABSTRACTION

Case 1: Methylmalonyl-CoA mutase

In addition to the large movements observed in the TIM barrel domains of both *P. shermanii* and human MCM, much smaller movements of AdoCbl are observed (23, 43). In structures of both bacterial and human MCM, electron density for an intact AdoCbl is observed in addition to electron density for cleaved AdoCbl (Figure 8). Since the structures of human and bacterial MCMs are similar with respect to AdoCbl binding and the human MCM structures are of higher resolution, we will focus on structures of the human MCM here. For human MCM, the structure with intact AdoCbl represents the resting state of the enzyme, before substrate binds, before the barrel closes, and before the C-Co bond becomes activated for cleavage (Figure 8a). Here the C-Co bond length observed is 2.2 Å, consistent with the bond length for intact free AdoCbl (32). In this structure, the upper Ado ligand makes no direct contacts to protein, as it hydrogen-bonds to protein only via water molecules. In total, seven hydrogen bonds are made, four by the ribose and three by the adenine ring. In the structure with cleaved AdoCbl and substrate analog malonyl-CoA (Figure 8b), the Ado moiety is observed in two conformations (C-Co bond lengths observed, 3.5 Å and 3.7 Å), refined at 50% occupancy each, with the ribose moiety hydrogen-bonding directly with side chains of Asn388, Gln352, Glu392, and Tyr264, and with both conformations of the adenine ring hydrogen-bonding with water molecules and a backbone carbonyl. In comparing cleaved with uncleaved Ado moieties, a significant rearrangement can be observed, in which the entire Ado group appears to have rotated by nearly 90°, bringing the catalytic radical-containing carbon (C5') within 3.4 Å and 3.9 Å of the bound malonyl-CoA C-2 atom (Figure 8c,d). With the intact cofactor, the distance between the C5' position of AdoCbl and the C-2 position of malonyl-CoA would be much farther (5.1 Å). Importantly, the binding sites observed for malonyl-CoA and the Ado moiety of intact AdoCbl overlap, with a close distance between the O3' of the Ado ribose and C-2 of malonyl-

CoA of 1.9 Å. Thus, binding of substrate should prompt movement of the Ado moiety. Further, accompanying the binding of malonyl-CoA and rotation of the cleaved Ado moiety, protein side chains (Asn388, Gln353, Gly392, and Tyr264) all move toward the cofactor and form direct hydrogen bonds with it, displacing at least seven water molecules from around the Ado moiety. Although the human MCM structure is not with the natural substrate (malonyl-CoA instead of methylmalonyl-CoA), structures of bacterial MCM show the same movements of the cleaved Ado moiety and of protein side chains with the natural substrate (43). Together these data suggest that hydrogen-atom abstraction by Ado• in MCM enzymes involves the movement of key side chains, the formation of a tighter binding pocket for the Ado group, and a 90° rotation of the adenosine moiety to bring the C5' radical into van der Waals contact with substrate. Thus large conformational changes of the protein (TIM barrel openings and closings) coupled with smaller rearrangements can be invoked to explain the 10^{12±2}-fold rate acceleration of C-Co bond homolysis in the presence of substrate.

Case 2: Glutamate mutase

With no structures available without substrate or substrate mimics (such as the buffer tartrate), we do not know if this enzyme undergoes the same kind of large conformational changes that have been observed for the other AdoCbl enzymes described in this review. However, a crystal structure of MutES co-crystallized with AdoCbl and *S*-glutamate allows us to describe the small conformational changes of the cofactor itself that are associated with controlling the generation of the highly reactive Ado• species. First, this co-crystal structure shows the effective sequestering of the substrate within the active site due to the positioning of the AdoCbl by the Rossmann domain onto the C-terminus of the substrate-binding TIM barrel (55). The resulting interactions, which consist of a highly ordered hydrogen-bonding network, are believed to be responsible for this enzyme's superb substrate specificity (28). Very few alternate substrates have been identified for MutES, and those found thus far display greatly decreased

rate (e.g. (*S*)-2-hydroxyglutarate) (58) or only tritium exchange between substrate and tritiated AdoCbl (e.g. 2-ketoglutarate) (57).

Second, in the structure solved by Kratky and colleagues (25), the Ado moiety of AdoCbl is observed to be in two conformations above the corrin ring, representing two snapshots of the MutES reaction sequence with a mixture of *S*-glutamate and the product (2*S*,3*S*)-3-methylaspartate bound at the active site. The two Ado orientations, referred to as A and B, vary by the ribose pucker (*C2'*-endo in A versus *C3'*-endo in B) and an approximate 25° rotation around the glycosidic bond, positioning *C5'* of conformer A towards the AdoCbl (C-Co bond distance of 3.2 Å) and of conformer B towards the substrate molecule (C-Co bond distance of 4.5 Å) (Figure 9). The hydroxyl-groups of the ribose in both conformers are engaged in hydrogen-bonding interactions with Glu330 and Lys326 of the MutE protein and one or two buried water molecules (25). The Glu330 interaction has been implicated in catalytic enhancement through electrostatic interactions with the ribose ring (56, 60). Positioning of the *C5'* of conformer A 3.2 Å from the Co resembles an 'en route' position; too far from the Co for a covalent attachment but not yet close enough to the substrate for hydrogen-atom abstraction (4.7 – 4.9 Å from the substrate), while the *C5'* of conformer B is within van der Waals distance of the substrate (3 – 3.3 Å); ideally positioned for hydrogen-atom abstraction (25). Theoretical calculations support the conformer B Ado orientation to be involved in catalysis (20).

Some additional rearrangement will be necessary to complete the catalytic cycle in MutES, allowing for product release and substrate re-entry. While subtle movement of the Cbl acetamide/propionamide groups and surrounding protein residues may suffice, it is tempting to consider the example of 5,6-LAM discussed above, in which a large movement of the Cbl-binding Rossmann domain opens the active site to the "resting" state, allowing for the exchange of product and substrate. Like 5,6-LAM, the Rossmann domains and TIM barrel domains of MutES are on different polypeptide

chains, however no PLP is present to covalently attach the separate chains in the absence of substrate. Instead, these chains have low affinities for each other in MutES (30, 83), and it is interesting to consider whether this weak binding is actually part of the catalytic mechanism, permitting both substrate binding and product release.

SUMMARY FOR ADENOSYLCOBALAMIN DEPENDENT REACTIONS

Here we provide three examples for how AdoCbl enzymes use the same protein folds in different ways to catalyze radical-based chemistry. In every case, conformational changes are key to reactivity. Interestingly, the older literature on AdoCbl suggested mechanisms for the $10^{12\pm 2}$ -fold homolysis rate enhancement based on properties of the cofactor itself (pucker of corrin ring for example). However, when the structures of AdoCbl-dependent enzymes started to become available, it was clear that the protein fold held many of the secrets to AdoCbl's amazing reactivity. In each case, the protein-fold must be able to respond to substrate binding in such a way as to trigger C-Co bond homolysis. With substrates so different in shape and size (from molecules with 4 non-hydrogen atoms to molecules with 55 non-hydrogen atoms as shown in Figure 2), this challenge is immense. However, instead of adopting completely different protein folds, designed based on the nature of the substrate, we find the use of the *same* protein-folds, and these folds are not unique to AdoCbl chemistry. They are in fact two of the *most* common folds: a TIM barrel and a Rossmann domain. How then can AdoCbl enzymes modify these folds to respond to their substrate? For MCM, which uses a relatively large substrate (methylmalonyl-CoA), substrate binding is coupled to a conformational change in the TIM barrel, from 'open' to 'closed' (Figure 10a). Known for their use as rigid scaffolds and typically lined with hydrophobic residues, this type of conformational change was completely unexpected, and required a re-design of the barrel interior. In the case of 5,6-LAM and 4,5-OAM, the substrates are much smaller (lysine and

ornithine), whose binding to a TIM barrel could not possibly be expected to trigger barrel closing. In fact, the TIM barrels in the AdoCbl-aminomutases are rigid. Instead, the Cbl-binding Rossmann domain has been modified by the disruption of a helix, resulting in the formation of a covalent linkage between a lysine of the Rossmann domain and the PLP bound to the TIM barrel. Here, substrate binding initiates the cleavage of that covalent bond, allowing rotation of the Cbl-binding Rossmann domain to bring the cofactor into position for catalysis (Figure 10a). Thus, one enzyme uses a modified TIM barrel and the other uses a modified Rossmann domain -- identical folds with adaptations for different substrates.

Finally, following the cleavage of the C-Co bond of AdoCbl, the Ado• species must reposition to abstract a hydrogen atom from the substrate. We now have structural data from two AdoCbl mutases to help us understand the molecular basis for this reaction. Both the structures of MCM and glutamate mutase show rearrangement of the position of the Ado moiety following C-Co bond homolysis. The conformational changes currently observed are similar in nature but not identical. The rearrangement in glutamate mutase involves a change in the pucker of the ribose to bring the C5' of Ado• within van der Waals distance of the substrate. The other in MCM is a 90° tilt of the Ado moiety, which brings C5' of Ado• close to the substrate (Figure 10b). These relatively simple movements compared to the swinging-domains and closing-barrels described above are less spectacular but no less important. While dramatic conformational changes can help to drive the formation of a powerful catalyst such as an Ado• species, once formed that radical must be tightly controlled. The structure of human MCM shows the elegant hydrogen-bonding network that is created upon C-Co bond homolysis, wielding the radical toward its substrate. These structures show the beauty and the power of the protein fold in harnessing radical-based chemistry.

SUMMARY POINTS

1. Radical-based chemistry allows for amazing reactivity that comes at a price, and with protection from radical damage afforded by the protein scaffold, the study of AdoCbl-dependent mutases allows one to discern the elegant design principles involved.
2. All structurally characterized AdoCbl-dependent mutases use the same protein folds, TIM barrels and Rossmann domains, which are also two of the most common protein motifs, to harness their radical-based chemistry.
3. Although the basic architectural units are the same for the AdoCbl-dependent mutases, connectivity between these units can differ, even in closely related enzymes.
4. While all AdoCbl-dependent mutases use TIM barrels and Rossmann folds to modulate and control their radical-based chemistry, these folds are custom tailored such that the binding of a particular substrate can trigger radical generation with high specificity.

FUTURE ISSUES

1. To fully understand how MCM harnesses its radical-based reactivity, more structural and biochemical data are needed on MCM's metallochaperones. It will be important to understand the molecular basis for their protective functions.
2. To better understand how MutES allows substrate and product to bind and escape, a structure of a truly substrate-free enzyme state is needed. It will be interesting to discover if MutES is the only member of the AdoCbl-dependent mutase family that does not engage large conformational changes in its enzymatic mechanism.
3. For the aminomutases, crystal structures are limited to resting state conformations. A structure of a catalytic state would confirm the proposed catalytic structural models and would allow for the evaluation of the interactions between AdoCbl, substrate, and PLP.

4. For all AdoCbl-dependent mutases, information of the rates of these large conformational changes is lacking. In general, much more is known about rates of enzymatic chemical steps, than about rates of protein conformational steps.

DISCLOSURE STATEMENT

The authors are not aware of affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Annotated References

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Important Acronyms

AdoCbl: 5'-deoxyadenosylcobalamin

AdoH: 5'-deoxyadenosine

Ado•: 5'-deoxyadenosyl radical

“Base-on” configuration: Form of cobalamin in which the dimethylbenzimidazole moiety is coordinated to the cobalt of the vitamin.

“Base-off/His-on” configuration: Form of cobalamin in which a histidine residue from the protein replaces the dimethylbenzimidazole as the ligand to the cobalt of the vitamin.

Cbl: cobalamin

CoA: Coenzyme A

MCM: methylmaloyl coenzyme A mutase

MutES: glutamate mutase

PLP: pyridoxal phosphate

Rossmann fold: a (β/α) fold with five or six parallel β -strands in a central sheet with helices on either side, named after Michael Rossmann who solved the structure of the second protein to use this fold (lactate dehydrogenase). The first structure, that of flavodoxin, was solved by Martha Ludwig.

TIM barrel fold: a (β/α)₈ fold named for the first enzyme for which it was seen, triosephosphate isomerase

5,6-LAM: lysine 5,6-aminomutase

4,5-OAM: ornithine 4,5-aminomutase

Figure Legends

Figure 1. Schematic drawing of coenzyme B₁₂ or adenosylcobalamin (R=Ado).

Figure 2. Examples of AdoCbl-dependent enzyme catalyzed reactions.

Figure 3. Schemes of proposed mechanisms for (a) methylmalonyl-CoA mutase, (b) glutamate mutase, and (c) lysine 5,6-aminomutase.

Figure 4. Quaternary structures of AdoCbl-dependent enzymes that utilize Rossmann and TIM-barrel domains for radical generation: *P. shermanii* MCM heterodimer (bMCM) in complex with Cbl and desulfo-CoA, PDB 1REQ (42); *C. cochlearium* MutES heterotetramer complexed with AdoCbl and a mixture of *S*-glutamate and (2*S*,3*S*)-3-methylaspartic acid, PDB 1I9C (25); *C. sticklandii* 5,6-LAM heterotetramer with AdoCbl and PLP, PDB 1XRS (7); and *C. sticklandii* 4,5-OAM heterotetramer with AdoCbl and PLP, PDB 3KP1 (81), colored as follows: TIM-barrel domains, blue and violet; Rossmann domains, orange and yellow; protein extensions, shades of gray; Cbl, PLP, and desulfo-CoA atoms are colored red.

Figure 5. TIM barrel and Rossmann domains of AdoCbl-dependent enzymes. (a,b) Topology diagrams for the Rossmann domain, with AdoCbl shown in yellow and pink, and the TIM barrel domain, respectively. (c) The Rossmann domain of MCM, depicting the Cbl-binding “DXHXXG SXL GG” motif. (d) The TIM barrel domain of 5,6-LAM, depicting the internal lysine aldimine formed with Lys144. (e, f) TIM barrel (blue) and Rossmann (orange) domains of MCM and 5,6-LAM, respectively, PDB

accession codes 1REQ (42) and 1XRS (7). Cbl, the partial substrate molecule desulfo-CoA and PLP are colored as follows: carbon, black; oxygen, red; nitrogen, blue; phosphorus, orange; cobalt, pink. Protein residues of the Cbl-binding motif have carbon atoms in cyan. Note the Ado moiety of AdoCbl is only shown in (f) where the C-Co bond is cleaved but the Ado moiety has remained bound to protein.

Figure 6. Variation in quaternary structure between closely related AdoCbl enzymes. *P. shermanii* MCM (bMCM) and human MCM (hMCM) exist as a heterodimer with one active chain and a homodimer of active subunits, respectively. *C. sticklandii* 5,6-LAM and 4,5-OAM are heterotetramers, depicted with each protein chain in a different color.

Figure 7. Two large conformational changes observed in AdoCbl-dependent enzymes. (a) Wall-eyed stereo view of the TIM barrel domain of *P. shermanii* MCM from structures with and without a substrate molecule, colored in blue (closed barrel) and yellow (open barrel), respectively, PDB accession codes 3REQ and 1REQ (42). Helices are shown as tubes, substrate desulfo-CoA is colored as in Figure 5. (b) Surface and cartoon representation of the resting state of 5,6-LAM in which protein Lys144 is covalently linked to the bound PLP cofactor as an internal aldimine, PDB 1XRS (7). The Rossmann containing subunit, β , is colored orange and the TIM barrel containing subunit, α , is colored blue. Residues of the accessory clamp are colored gray. (c) Model of the expected active conformation for 5,6-LAM in which the Rossmann domain has swung towards the active site and AdoCbl is positioned over the active site. This structure was obtained by superposition of the Rossmann domains of 5,6-LAM and MCM, PDB 1REQ (42). Cbl, Ado, and the PLP-lysine aldimine are colored red, violet, and yellow, respectively. A putative channel for substrate binding in 5,6-LAM is indicated.

Figure 8. Wall-eyed stereo views of AdoCbl and Ado binding within human MCM, depicting a rotation of the Ado moiety towards substrate, “en route” for catalysis. (a) Intact AdoCbl-bound MCM. Red dashed lines indicate hydrogen-bonding interactions with position of malonyl-CoA modeled from the substrate bound structure (yellow carbons). (b) Malonyl-CoA/Cbl bound MCM. The cleaved Ado moiety is observed in two conformations, with carbons colored pink and violet. Red/pink dashed lines indicate hydrogen-bonding interactions, and gray/black dashed lines indicate distances (3.4, 3.9 Å) between C5' of Ado moieties and C-2 of malonyl-CoA (yellow); PDB codes 2XIJ and 2XIQ (23). (c) Close-up view of intact AdoCbl from (a) with position of malonyl-CoA modeled in (yellow carbons). (d) Close-up view of cleaved AdoCbl and malonyl-CoA (yellow) from (b).

Figure 9. Wall-eyed stereo view of Ado binding within glutamate mutase, depicting conformations “en route” to hydrogen-atom abstraction. Two conformations of cleaved Ado moieties are observed: A, representing an activated C-Co species with a bond length of 3.2 Å (carbon colored orange), and B, a conformation within van der Waals distance to the bound substrate, poised for catalysis (carbon colored cyan). Additional atoms are colored as follows: Cbl carbon, green; protein carbon, gray; *S*-glutamate carbon, purple; (2*S*,3*S*)-3-methylaspartate carbon, yellow; oxygen, red; and nitrogen, blue; PDB 1I9C (25).

Figure 10. Cartoon depictions of (a) large conformational changes that occur in the regulation of C-Co bond cleavage in MCM and putatively in 5,6-LAM, and (b) smaller regulated changes in the Ado group after C-Co bond cleavage in MCM and glutamate mutase (MutES). The Rossmann domain is shown as an orange rectangle, and a blue cylinder represents the TIM barrel. See Text for description.

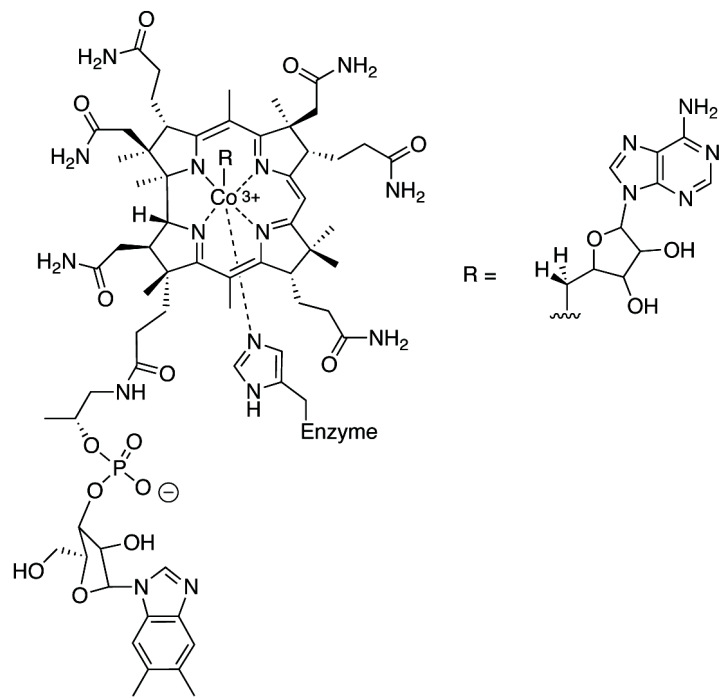
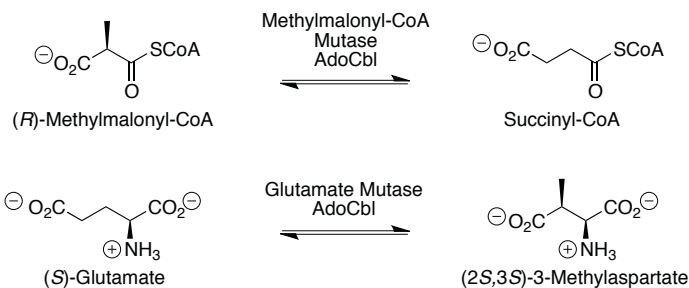
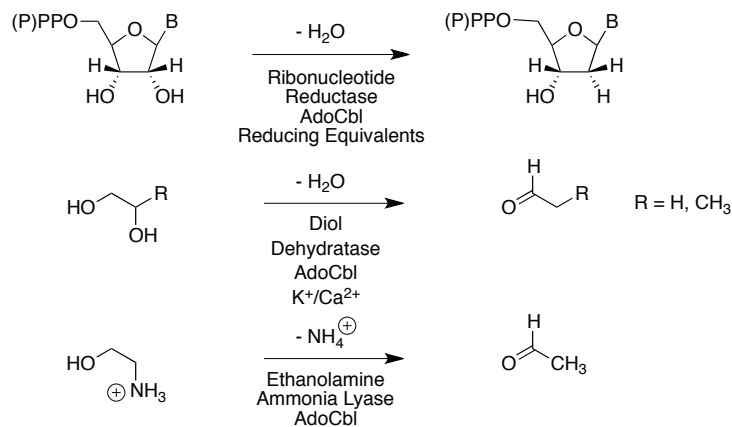


Figure 1

Class I: Carbon Skeleton Mutases



Class II: Eliminases



Class III: Aminomutases

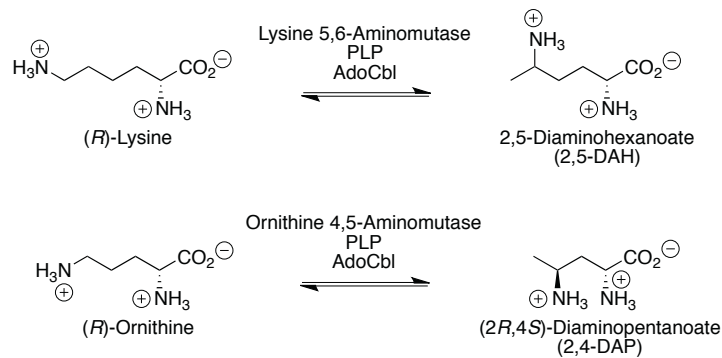


Figure 2

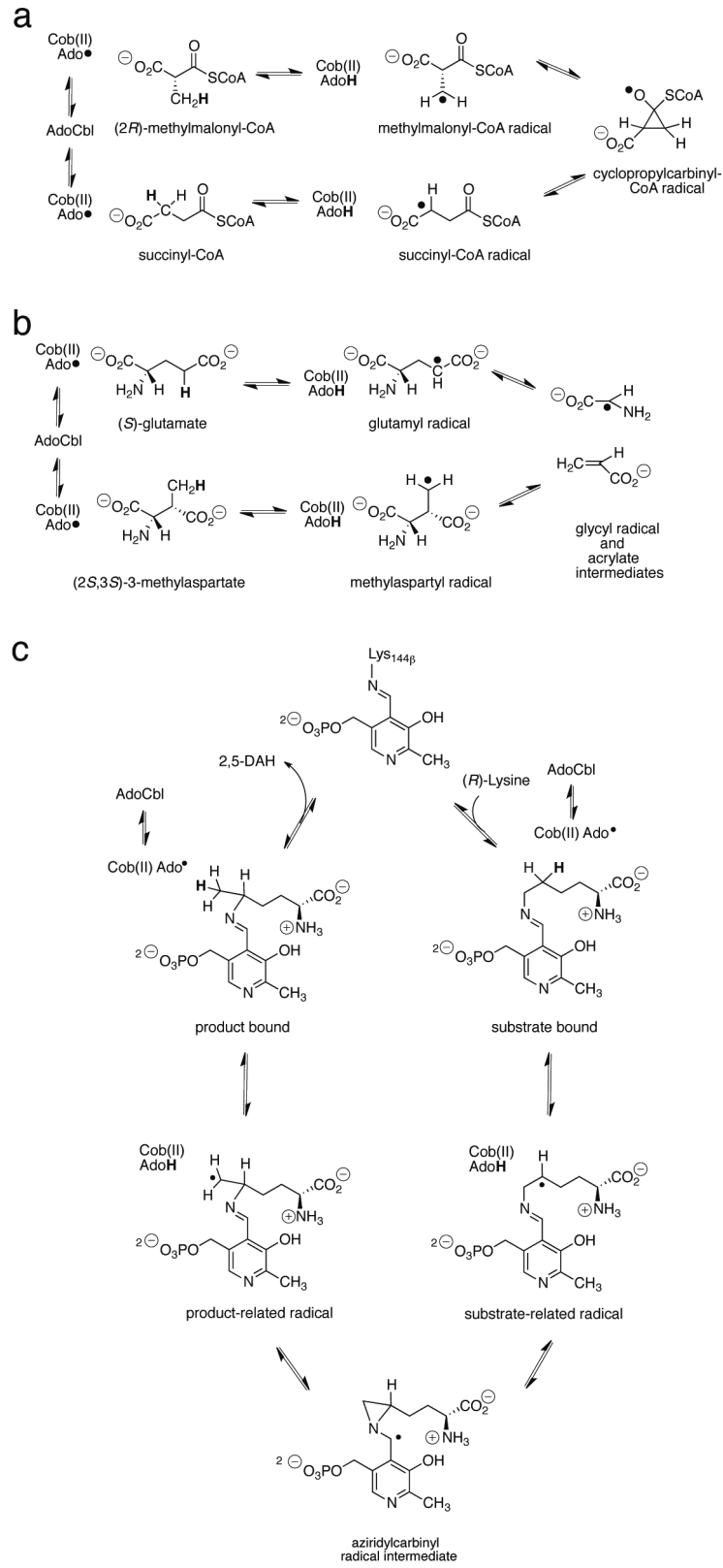


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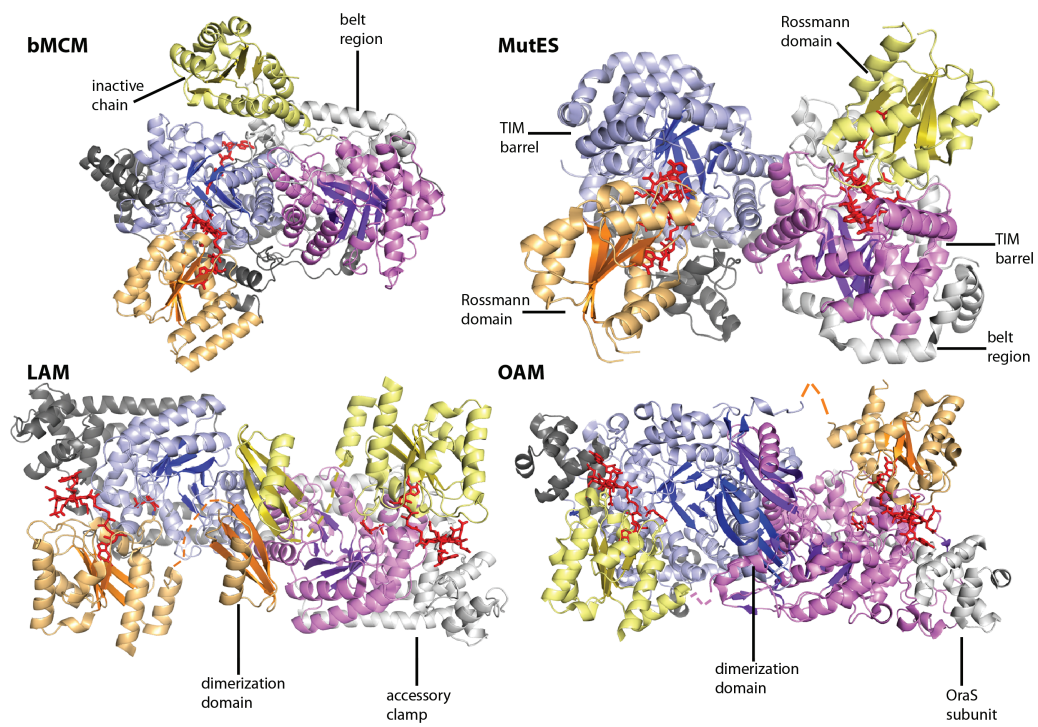


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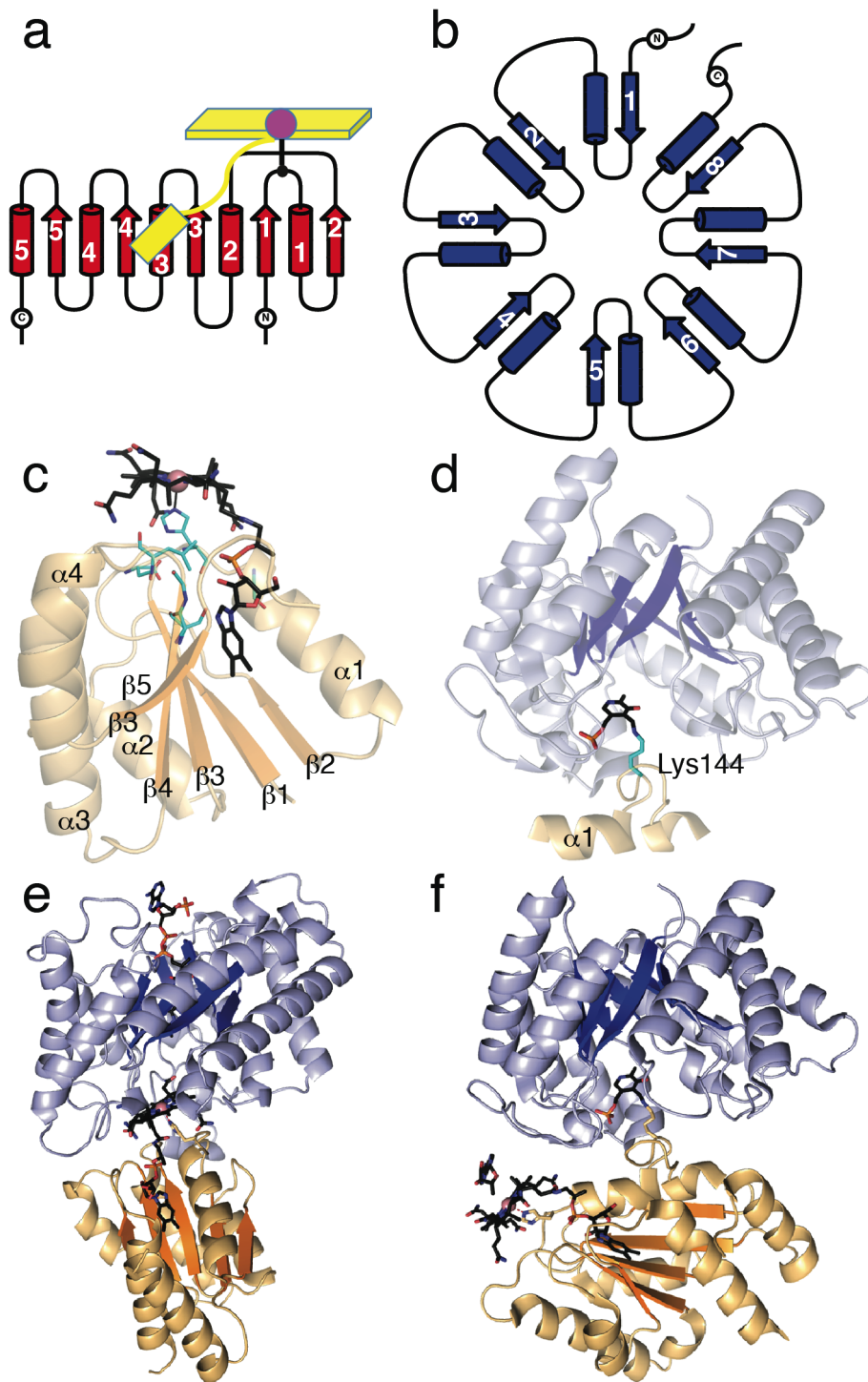


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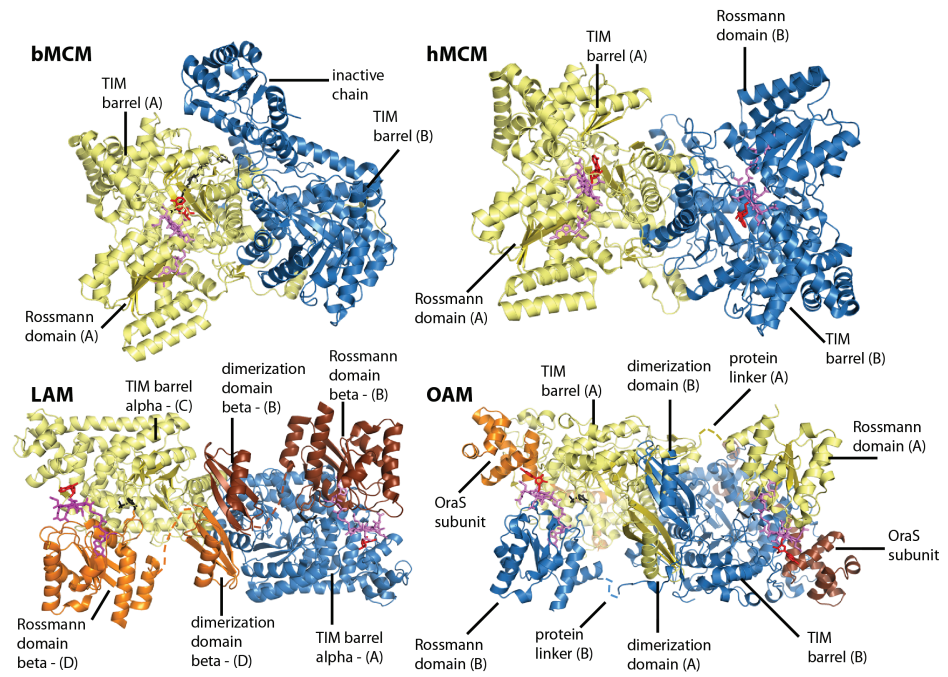


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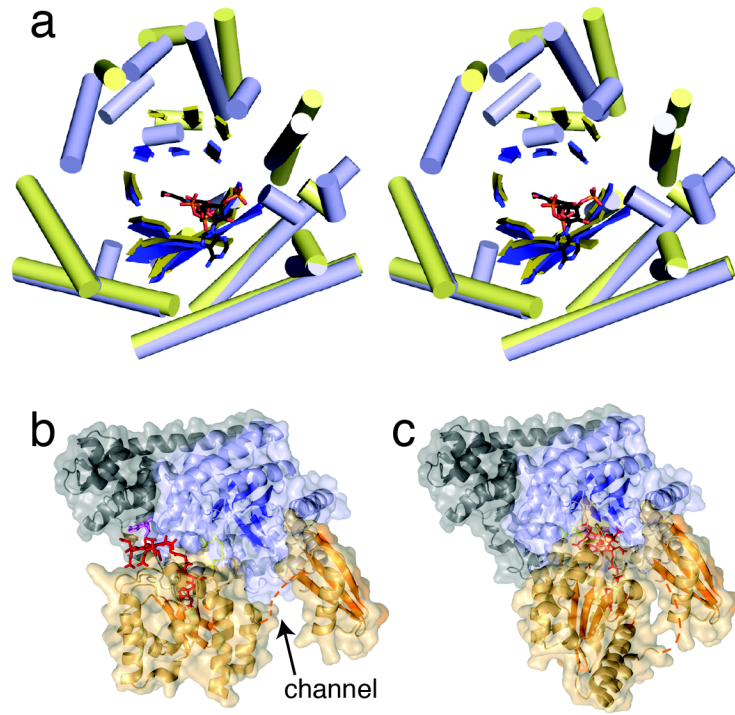


Figure 7

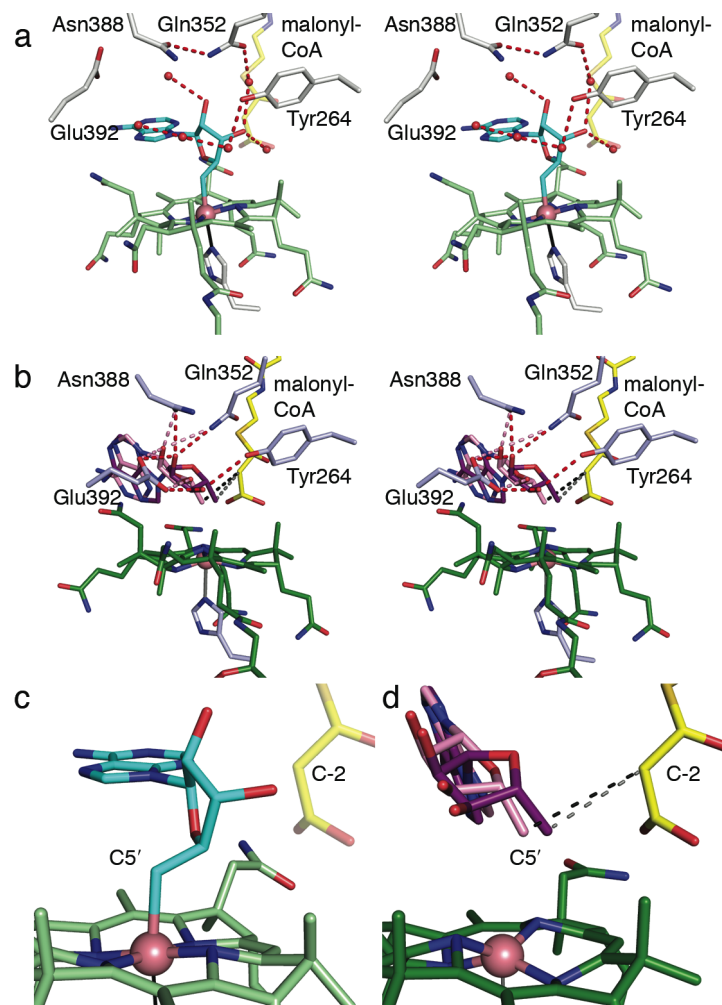


Figure 8

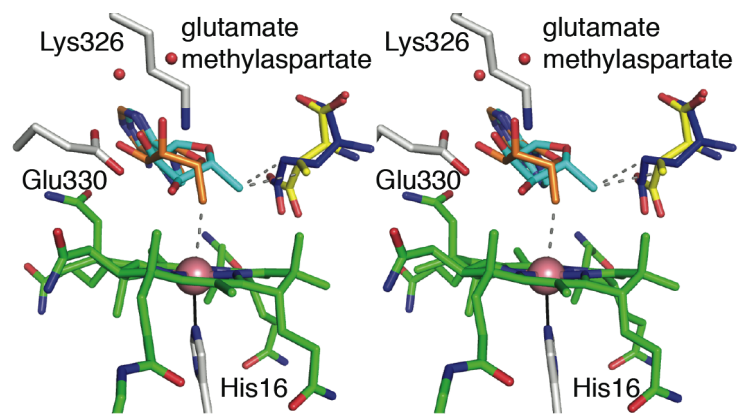


Figure 9

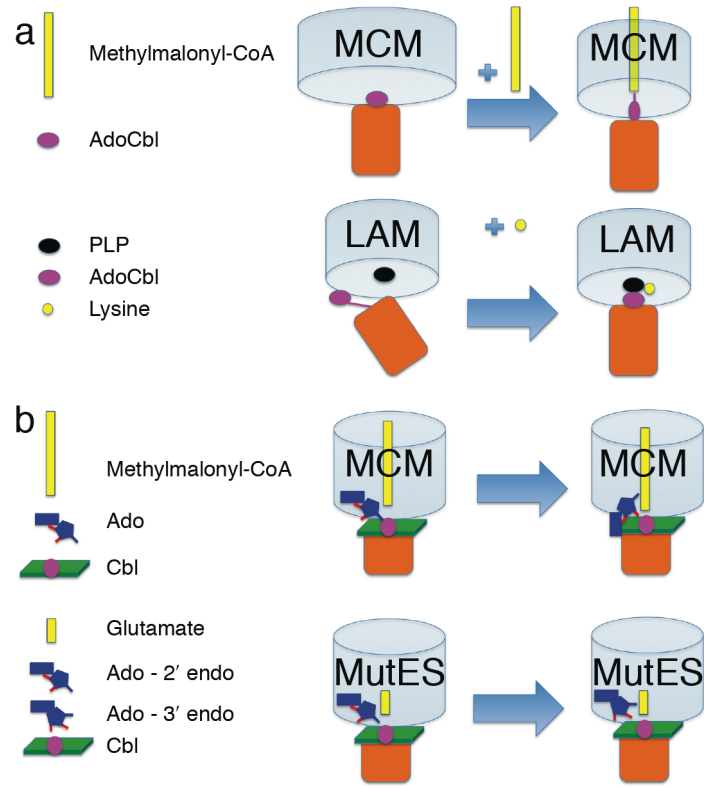


Figure 10

Supplementary Table 1. Characterized enzymes that utilize coenzyme AdoCbl to generate a reactive free radical.

Enzyme Name	Enzyme class	Domains	Cobalt lower axial ligand	PDB ID	Organism	Ligands bound / Notes
Methylmalonyl-CoA mutase	Carbon-skeleton mutase	TIM barrel Rossmann-like	Histidine	1REQ (11) 2REQ, 3REQ(10) 4REQ, 5REQ (Y89F) (25) 6REQ, 7REQ (12) 1E1C (H244A) (24) 2YXB (To be published) 3BIC, 2XIJ, 2XIQ (3)	<i>Propionibacterium freudenreichii</i> <i>subsp. Shermanii</i> <i>Aeropyrum pernix</i> <i>Homo Sapiens</i>	AdoCbl, Cob(II), AdoH, CoA analogues, succinyl-CoA, methylmalonyl-CoA, malonyl-CoA
Glutamate mutase	Carbon-skeleton mutase	TIM barrel Rossmann-like	Histidine	1CB7, 1CCW (15) 1I9C (4, 15, 16) NMR: 1BE1 (27), 1ID8 (26) NMR: 1B1A (5), 1FMF(6)	<i>Clostridium cochlearium</i> <i>Clostridium tetanomorphum</i>	Cob(II), AdoH, CNCbl, MeCbl, tartrate, <i>S</i> -glutamate, (2 <i>S</i> ,3 <i>S</i>)-3-methylaspartate
Ethylmalonyl-CoA mutase	Carbon-skeleton mutase			No structure	<i>Rhodobacter sphaeroides</i>	Reference (2)
Isobutryl-CoA mutase	Carbon-skeleton mutase	TIM barrel Rossmann-like	Histidine	No structure	<i>Streptomyces cinnamonensis</i>	Homology model (14)
2-Methylene glutarate mutase	Carbon-skeleton mutase			No structure	<i>Clostridium barkeri</i>	Reference (7)
Diol dehydratase	Eliminase	TIM barrel α/β domain	AdoCbl-DMB	1DIO (19) 1EEX, 1EGM, 1EGV (13) 1IWB (18) 1UC4, 1UC5 (20)	<i>Klebsiella oxytoca</i>	K ⁺ , Cob(II), AdePeCbl, CNCbl, (<i>S</i>)- and (<i>R</i>)-1,2-propanediol
Glycerol dehydratase	Eliminase	TIM barrel α/β domain	AdoCbl-DMB	1IWP (29) 1MMF (9)	<i>Klebsiella pneumoniae</i>	K ⁺ , Cob(II), (<i>S</i>)-1,2-propanediol
Ethanolamine ammonia-lyase	Eliminase	TIM barrel α/β domain	AdoCbl-DMB	2QEZ (To be published) 3ABO, 3ABQ, 3ABR, 3ABS (21) 3ANY, 3AO0 (17)	<i>Listeria monocytogenes serotype 4b</i> <i>Escherichia coli</i> <i>Salmonella typhimurium</i>	Na ⁺ , Cob(II), CNCbl, AdePeCbl, ethanolamine, (2 <i>S</i>)- and (2 <i>R</i>)-2-amino-1-propanol Homology model (23)
RNR Class II	Eliminase and reductase	(β/α) ₁₀ barrel	AdoCbl-DMB	1L1L (22) 3O0N, 3O0O (8)	<i>Lactobacillus leichmannii</i> <i>Thermotoga maritima</i>	AdoCbl, Mg ²⁺ , dTTP, GDP
Lysine 5,6-aminomutase	Aminomutase	TIM barrel Rossmann	Histidine	1XRS (1)	<i>Clostridium sticklandii</i>	AdoCbl, PLP
Ornithine 4,5-aminomutase	Aminomutase	TIM barrel Rossmann	Histidine	3KOW, 3KOX, 3KOY, 3KOZ, 3KP0, 3KP1 (28)	<i>Clostridium sticklandii</i>	AdoCbl, Cob(II), PLP, <i>R</i> -ornithine, 2,4-diaminobutyrate

Abbreviations:

AdoCbl, adenosylcobalamin

AdoH, deoxyadenosine

AdePeCbl, adeninylpentylcobalamin

CNCbl, cyanocob(III)alamin

Cob(II), cob(II)alamin

DMB, dimethylbenzimidazole

MeCbl, methylcob(III)alamin

PLP, pyridoxal phosphate

TIM, triosephosphate isomerase

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