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The Increasingly Complex Mechanism of HMG-CoA Reductase

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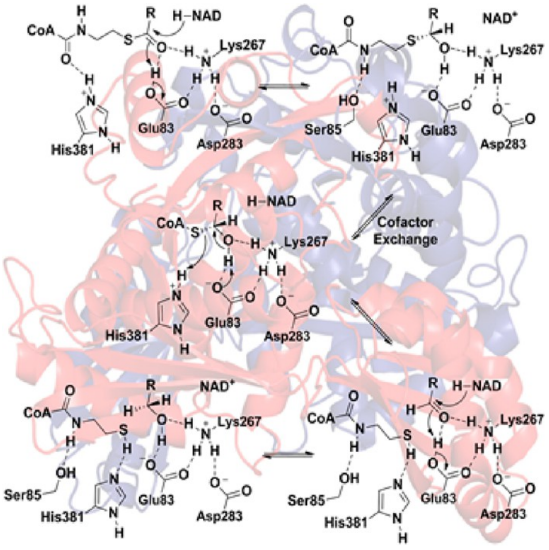
CONSPECTUS

HMG-CoA reductase (HMGR) is the target of statins, cholesterol-lowering drugs prescribed to millions of patients worldwide. More recent research indicates that HMGR could be a useful target in the development of antimicrobial agents. Over the last seven decades, researchers have proposed a series of increasingly complex reaction mechanisms for this biomedically important enzyme.

The maturation of the mechanistic proposals for HMGR have paralleled advances in a diverse set of research areas, such as molecular biology and computational chemistry. Thus, the development of the HMGR mechanism provides a useful case study for following the advances in state-of-the-art methods in enzyme mechanism research. Similarly, the questions raised by these mechanism proposals reflect the limitations of the methods used to develop them.



The mechanism of HMGR, a four-electron oxidoreductase, is unique and far more complex than originally thought. The reaction contains multiple chemical steps, coupled to large-scale domain motions of the homodimeric enzyme. The first proposals for the HMGR mechanism were based on kinetic and labeling experiments, drawing analogies to the mechanism of known dehydrogenases. Advances in molecular biology and bioinformatics enabled researchers to use site-directed mutagenesis experiments and protein sequencing to identify catalytically important glutamate, aspartate, and histidine residues. These studies, in turn, have generated new and more complicated mechanistic proposals.

With the development of protein crystallography, researchers solved HMGR crystal structures to reveal an unexpected lysine residue at the center of the active site. The many crystal structures of HMGR led to increasingly complex mechanistic proposals, but the inherent limitations of the protein crystallography left a number of questions unresolved. For example, the protonation state of the glutamate residue within the active site cannot be clearly determined from the crystal structure. The differing protonation state of this residue leads to different proposed mechanisms for the enzyme.

As computational analysis of large biomolecules has become more feasible, the application of methods such as hybrid quantum mechanics/molecular mechanics (QM/MM) calculations to the HMGR mechanism have led to the most detailed mechanistic proposal yet. As these methodologies continue to improve, they prove to be very powerful for the study of enzyme mechanisms in conjunction with protein crystallography. Nevertheless, even the most current mechanistic proposal for HMGR remains incomplete due to limitations of the current computational methodologies. Thus, HMGR serves as a model for how the combination of increasingly sophisticated experimental and computational methods can elucidate very complex enzyme mechanisms.

Introduction

Methods for the study of complex enzyme mechanisms, and with them the ever-increasing level of detail accessible, are constantly evolving. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGR) is an excellent example of the constant revisitation, revision, and refinement of a complex mechanism which has taken place over seven decades of studies and employed numerous methods.

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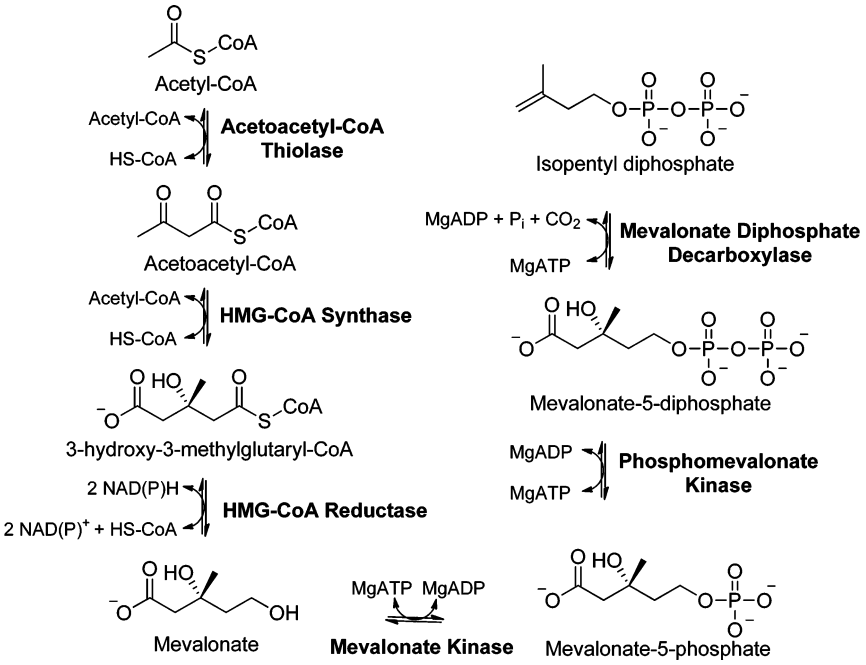

FIGURE 1. The Mevalonate Pathway.[1,6]

HMGR is arguably the most well-known enzyme of the eukaryotic mevalonate pathway for isoprenoid biosynthesis because of its importance in the biomedical fields as a target for cholesterol-lowering drugs called statins.[1−3] In effect, the extraordinary health impact of the statins has somewhat overshadowed the fascinating mechanism of HMGR. This account will closely examine the mechanistic proposals for HMGR over time by considering how their increasing complexity is connected to the developing methodologies of the time. The culmination of the discussion will arrive at the present time with a demonstration of how powerful the combination of X-ray crystallography and computational methods can be in enzymology.

The mevalonate pathway is found predominantly in eukaryotes but also in a few prokaryotes. Most eubacteria depend on a different pathway for isoprenoid biosynthesis called the nonmevalonate pathway or deoxyxylulose 5-phosphate pathway, which is also found in plants.[4,5] In eukaryotes, the mevalonate pathway produces the precursor molecule isopentenyl 5-diphosphate from acetyl-CoA, which feeds into the production of important biomolecules and natural products such as farnesyl phosphate, dolicohol, and cholesterol.[5,6] The complete mevalonate pathway and its enzymes are indexed in Figure 1.[6] More recently, the essentiality of the mevalonate pathway has been demonstrated in a subset of pathogenic bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and *Streptococcus pneumonae* (SPn),[7−9] inspiring efforts to find small molecule inhibitors of the mevalonate pathway enzymes for treatment against infection.[10−12]

As a result of its central position in the metabolic network, HMGR is highly regulated and serves as the point of feedback control for the mevalonate pathway.[13] Expression of low-density lipoprotein (LDL) receptors is increased upon competitive inhibition of the human HMGR leading to increased clearance of cholesterol-LDL.[14,15] Statins bind tightly to the human HMGR, with inhibition constants in the nanomolar range,[16] and are, therefore, commonly prescribed to treat hypercholesterolemia and reduce the risk of cardiovascular disease.[1,2] However, the drugs bind less tightly to the HMGRs of pathogenic bacteria with inhibition constants in the micromolar range.[17,18] This observation is explained in part by protein sequence alignments of the known HMGR enzymes. Two evolutionarily divergent classes of HMGRs that split roughly by eukaryotes (class I) and prokaryotes (class II) were discovered.[19,20] The class I HMGRs are generally membrane-bound with a transmembrane domain and a catalytic domain. Initially, all of the known HMGR enzymes belonged to class I and were difficult to express and purify because of their membrane-bound nature. In contrast, the class II enzymes are cytostolic and lack the transmembrane domain of the class I enzymes.[22] The first class II HMGR was discovered from a eubacterium, *Pseudomonas mevalonii* (*Pm*HMGR), although the classes had not yet been defined at the time. It remained the only known

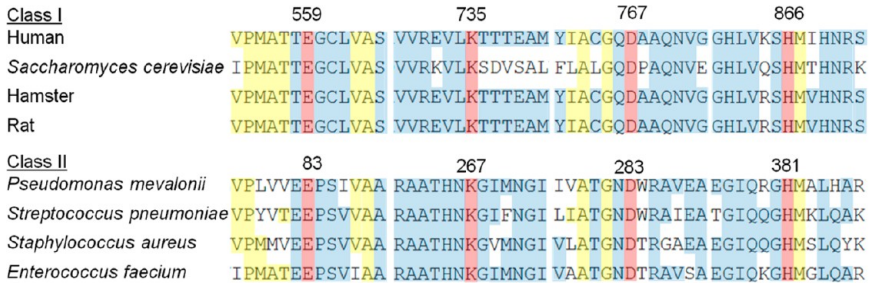

FIGURE 2. Sequence alignment of various HMGRs showing the catalytic residues highlighted in red. Conserved residues across classes are highlighted in yellow, and conserved residues within classes are highlighted in blue. Human HMGR numbering is shown for the class I enzymes and *Pm*HMGR numbering is shown for the class II enzymes.

class II enzyme until the modern sequencing efforts uncovered additional examples. *Pm*HMGR was found to possess qualities desirable for mechanistic and structural studies in that it is soluble, can be induced in large quantities, and forms relatively robust crystals.[22,23] Thus, *Pm*HMGR took center stage as a model HMGR for studying the enzymatic reaction mechanism.

The structures of the catalytic domains within the classes are well-conserved. Sequence identities within class I HMGRs are ~60%, while sequence identities within class II enzymes are ~50%.[19] Structurally, HMGRs across classes possess similar folds and conserved active site residues, as shown in Figure 2. However, their sequences are with ~14−20% only weakly conserved, leading to distinct structural features.[19,21] The most interesting of these structural features is the conserved "cis-loop" motif found only in the class I enzymes, which contains a cis-peptide and forms part of the HMG-CoA binding pocket.[24,25] Other differences between the classes include the stereochemistry of the chemical reaction[25−28] and, in some cases, cofactor specificity.[14,29] The structural differences between classes are sufficient to explain the preference of statins for class I enzymes. It has been suggested that modified statins would be a logical starting point to find class II selective inhibitors.[16,18]

The overall reaction mechanism of HMGR presents a case that is arguably as remarkable in enzymology as the enzyme is biomedically relevant. It is a rare example of a four-electron oxidoreductase that uses two molecules of the cofactor nicotinamide adenine dinucleotide [NAD(P)] in the reversible conversion of (*S*)-HMG-CoA and (*R*)-mevalonate.[30] The reaction is thought to proceed through two intermediates, mevaldyl-CoA and mevaldehyde, and consist of two hydride transfer steps, a cofactor exchange step and a hemithioacetal decomposition step, as shown in Figure 3. The enigmatic aldehyde intermediate has never been detected experimentally,

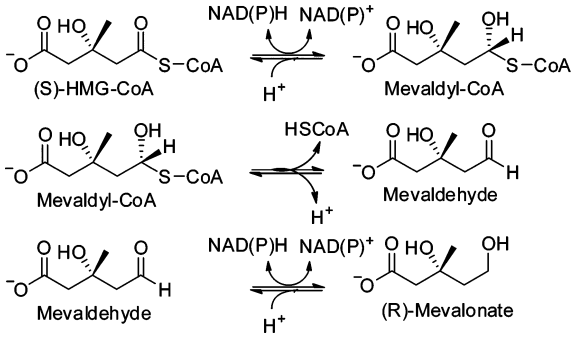

FIGURE 3. General reaction pathway of HMGR.

leading to the hypothesis that the hemithioacetal is the prominent reaction intermediate.[31−35] This theory is further supported by a crystal structure with electron density for a tetrahedral hemithioacetal analogue in the active site and free energy calculations performed on the ternary complexes of a proposed reaction pathway.[36,37] However, this poses the following question. At what point is the aldehyde formed, since the hemithioacetal is presumably unreactive toward NAD(P)H?

The reaction mechanism is further complicated by the cofactor exchange step. In *Pm*HMGR crystal structures with one or no ligands present, there is no electron density for the C-terminal 50 residues of the enzyme, which is known as the flap domain.[23] When both ligands are present, electron density for the flap domain is found positioned over the active site in a three α-helix bundle, making direct contacts with both substrate and cofactor, as shown in Figure 4. This suggests that the movements of the flap domain are somehow involved in the process of substrate and cofactor binding.[28] It stands to reason then that some degree of flap domain motion must occur between the hydride transfer reactions in order to exchange the expended cofactor. This process must have an impact on the enzyme intermediate states and may contribute to the elusiveness of the aldehyde intermediate. Thus, the reaction mechanism of HMGR

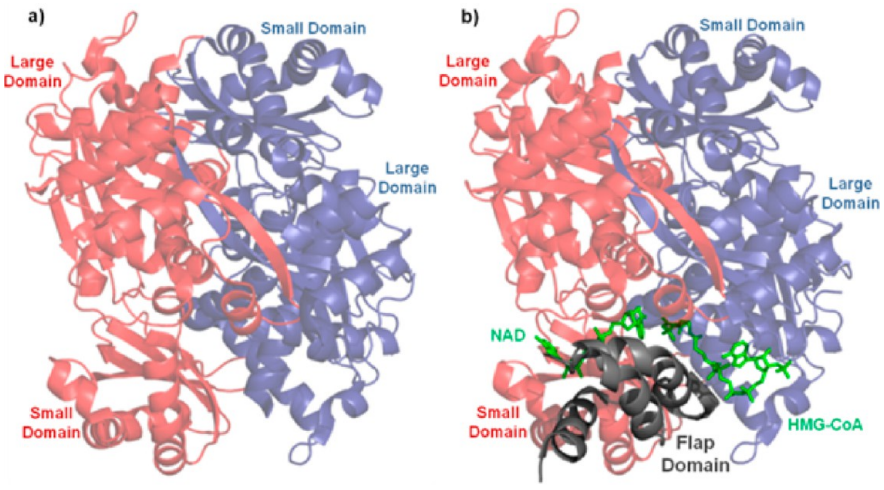

FIGURE 4. The flap domain of *Pm*HMGR is disordered in crystal structures with one or no ligands.[38] However, it is ordered over the active site in the presence of both substrate and cofactor.[28] (a) *Pm*HMGR crystal structure with no ligands and a disordered flap domain (pdb code 1R7I). (b) Nonproductive HMG-CoA/NAD+ ternary complex with an ordered flap domain over the active site (pdb code 1QAX).

has revealed itself as a complex and delicately controlled reaction, which can only be appropriately described in a dynamic context that considers both structural and chemical elements.

Thus, we trace the efforts to elucidate the increasingly complex mechanism of HMGR by highlighting the connections between the mechanistic proposals and the research tools available at the time they were proposed. This connection begins when early kinetic studies, heavy-atom labeling studies, and site-directed mutagenesis studies revealed a set of catalytically important residues and information on the reaction intermediates.[21,31,39−41] Then, crystal structures have provided complete spatial resolution of the active site, a full set of catalytic residues, and evidence for the impact of the flap domain on the reaction.[28,37,38] As the mechanism was being formulated, structures of the class I human HMGR suggested a conflicting mechanism and brought the potential effects of the class differences to light.[14,24,25] Lastly, computational methods have provided a partial energetic description of the reaction and visualization of intermediate states that cannot be obtained by experiment.[36]

Early Mechanistic Proposals for HMGR and the Discovery of *Pm*HMGR

Before the discovery of *Pm*HMGR, only Class I enzymes were known, but they were difficult to study because of their membrane-bound nature. Most research was performed on the yeast HMGR to explore the possibility of a hemithioacetal intermediate. The curious absence of the expected aldehyde intermediate during the reaction was inconsistent with the proposed reaction pathway.[31,34,35,42] Qureshi et al. described the stereoselective chemical mechanism of hydride transfer and hemithioacetal breakdown, implicating unspecified acidic and basic residues.[34] Shortly afterward, Veloso et al. expanded the mechanism using pH-dependent kinetic analyses to specify an acidic residue and a cationic histidine as the catalytic residues. The reduction steps were likened to the mechanism of the classic dehydrogenases and were presented as two separate reactions, as shown in Figure 5.[42] However, complicated kinetics led to uncertainty around the ligand binding mechanism and identification of the rate-determining step. It was implied that the protonation state of the catalytic histidine had an effect on which the redox form of the cofactor would bind.[42]

P. mevalonii was found among a group of organisms capable of living on mevalonate as their sole source of carbon.[43,44] Growth of the organism on mevalonate was found to produce large quantities of *Pm*HMGR for the initiation of mevalonate catabolism through a reversal of the mevalonate pathway.[44] As the only known class II enzyme and therefore the only available soluble enzyme, *Pm*HMGR was identified as a good model HMGR for mechanistic studies.[22] It had been shown previously that several class I HMGRs are inactivated through cysteine modification by sulfhydryl reagents such as *N*-ethylmaleimide (NEM).[32,33,45] *Pm*HMGR is also susceptible to sulfhydryl reagents, and it was shown that NEM prevents the enzyme from catalyzing all reactions in either the forward or the backward directions.[22] It was speculated that a cysteine could be a catalytically important residue in the active site or important for substrate binding. To test this hypothesis either or both of

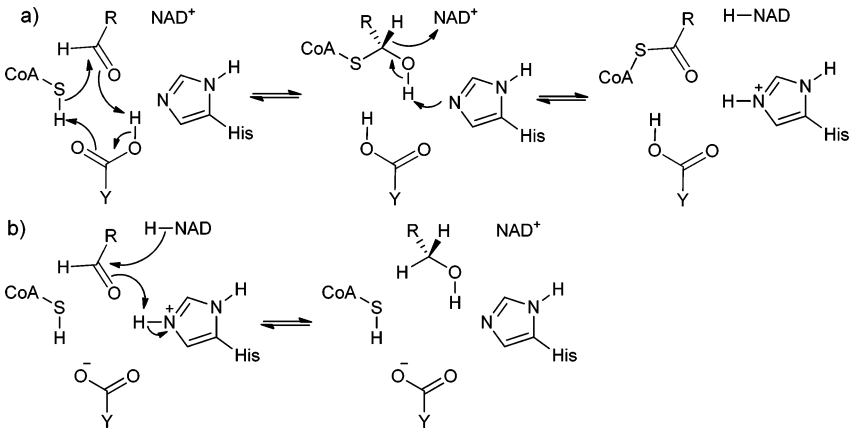

FIGURE 5. Separated dehydrogenase reactions proposed for the HMGR mechanism. (a) Aldehyde reduction to HMG-CoA and (b) aldehyde reduction to mevalonate.[42]

the two cysteines of *Pm*HMGR were mutated to alanine residues in site-directed mutagenesis (SDM) experiments.[40] The mutants catalyzed the reactions at the same level of efficiency as the wild-type enzyme, and the binding of the substrate was not affected. It was therefore concluded that the cysteine residue responsible for the inactivation must be causing an irreversible conformational change upon modification and is not an essential active site residue.[40]

On the basis of these results, the mechanism shown in Figure 5 was revisited. Protein sequence alignment of the known HMGRs identified conserved acidic and histidine residues. The residues that were found to be essential for the *Pm*HMGR activity through SDM experiments were Glu83 and His381.[39,41] An attempt to confirm the identity of these catalytically important residues in a mammalian enzyme revealed an additional conserved acidic residue, an aspartate (Asp766 of the Syrian hamster HMGR), that inactivated the enzyme upon mutation.[46] From the investigation of the effects of mutating these catalytically important residues on the individual half reactions of the enzyme, a new mechanism, shown in Figure 6, was proposed.[47] Mutation of the catalytic histidine to glutamine turned coenzyme A into an inhibitor of the mevaldehyde reduction step, while replacing CoA with desithio-CoA in the mutant enzyme returns mevaldehyde reduction activity to wild-type levels. Here, the catalytic histidine was assigned the role of protonating the CoA anion upon decomposition of the hemithioacetal intermediate.[47] Additionally, the glutamate and histidine mutants are capable of catalyzing one or more of the half reactions, while the aspartate mutant had no activity in catalyzing either of the half reactions.[47] Therefore, the authors proposed that the catalytic acidic residue was the aspartate despite inconsistencies with the studies on the bacterial HMGR, which declared the catalytic acidic residue to be Glu83.

The Crystal Structures of *Pm*HMGR

The next step in the evolution of our understanding of the HMGR mechanism was the determination of the first crystal structure, *Pm*HMGR. It revealed the enzyme as an obligate dimer with the active site at its interface.[23,38,46] Each monomer consists of three domains: the large domain, the small domain, and the C-terminal flap domain. The large domain (1−108 and 220−375) is centered on a 24 residue hydrophobic α-helix surrounded by a triangle of three mixed α/β walls. The binding site for HMG-CoA has a well-defined pocket for the HMG moiety, whereas the CoA portion is positioned in a shallow surface groove, making few direct hydrogen bonds with the enzyme. The adenine ring of CoA is tacked down by a surface arginine residue, while the CoA phosphate groups contact the enzyme through water-mediated hydrogen bonds.[37] The small domain (110−215) binds NADH with a nonclassical dinucleotide fold, comprised of a four-strand antiparallel β sheet with two crossover helices on one side. A conserved loop containing the DAMG sequence connects the third strand and the second helix, which is analogous to the G-rich loop in the classic dinucleotide-binding domain.[28] The cofactor is bound such that it is stretched out on the enzyme with the nicotinamide ring inserted into the active site pocket parallel to the HMG moiety of the substrate. Typically, the cofactor is positioned over or under the substrate, so its position in the HMGR active site is unique and may be necessary for its exchange.

Initial attempts to solve the crystal structure of the ternary enzyme−substrate complex were foiled by the discovery

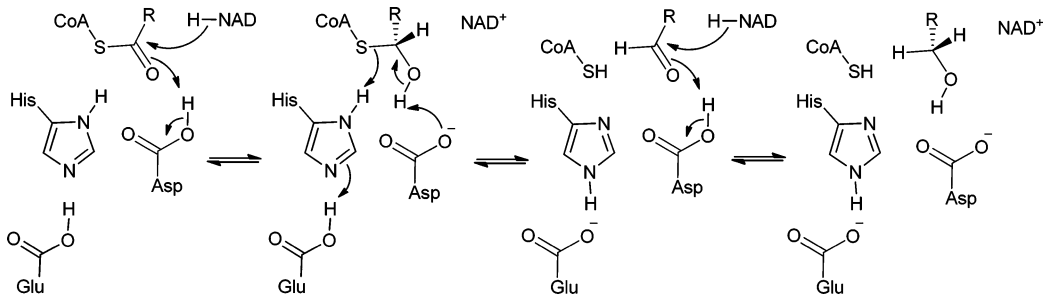

FIGURE 6. The mechanism proposed by Frimpong et al. after the catalytic histidine was assigned the role of protonating the CoA thiolate anion after hemithioacetal decomposition.[47]

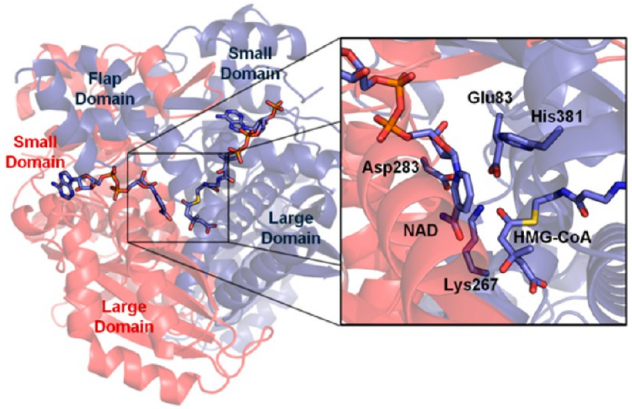

FIGURE 7. Crystal structure of the nonproductive HMGR ternary complex with HMG-CoA and NAD^+ (pdb code 1QAX).[28]

that HMGR had enzyme activity in the crystal form. However, nonproductive ternary complexes with the oxidized form of the cofactor produced a model complex for this state. This structure contains electron density for the flap domain (377−428), which was not previously seen in the apo enzyme, ordered over the active site in three α helices connected by short loops.[28] The first helix is connected to the central α helix of the large domain by a hinge region (375−377) that likely plays a role in substrate binding.[28] The flap domain contacts occur between this first helix and the small domain of the second monomer, in addition to other direct contacts of flap domain residues with the bound substrate and cofactor. Ordering of the flap domain aligns hydrophobic residues to form a pocket at the inner face of helix 1 that has been suggested to provide protection of the reaction center from the solvent.[28,38] The closing of the flap domain also has implications for catalytic activity as it brings His381 into the active site within reach of the sulfur atom of HMG-CoA consistent with its proposed role in the HMGR reaction.[28,47] The various domains, ligands bound at the dimer interface, and active site residues are shown in the crystal structure in Figure 7.

In addition to the essential residue Glu83, the *Pm*HMGR crystal structures also revealed the positions of the conserved residues Asp283, Lys267, and Asn271 in the active site. The discovery of Lys267 centrally located in the active site near Glu83 and Asp283 was surprising because it had not been previously implicated in catalysis. SDM experiments subsequently confirmed that it is indeed important for enzyme activity.[28] The crystal structures show the presumably protonated Lys267 hydrogen bonding with the thioester carbonyl of the substrate, which led to the proposal of a revised mechanism shown in Figure 8. Here, Lys267 polarizes the substrate carbonyl for each hydride transfer and acts as the general acid to protonate mevalonate at the end of the reaction. A proton relay is suggested where Glu83 is close enough to assist in mevaldyl-CoA decomposition and subsequently passes this proton to Lys267. An anionic Asp283 and multiple other residues support the position of Lys267 through secondary hydrogen bonds. Consistent with the previously proposed mechanisms, His381 is positioned to protonate the CoA thiolate anion after the first hydride transfer.[28]

Crystal Structures of the Human HMGR and the Protonation State of the Catalytic Acidic Residue

In the next set of studies, Istvan et al. solved the crystal structure of the human HMGR catalytic domain and observed a similar set of conserved catalytic residues in the active site.[25] The human HMGR crystal forms a tetramer with obligate dimers that assemble similarly to those of *Pm*HMGR, despite their lack of sequence identity.[14] However, the active site is formed from very different secondary structure elements, in particular, the cis-loop of the human HMGR that runs along one side of the active site positioning the catalytically important lysine residue, Lys735.[14,24] Differences in the positions of the catalytic residues between the human HMGR and the nonproductive *Pm*HMGR complexes led to further modification of the proposed mechanism shown in Figure 9. In this mechanism, the acidic residue, Glu559, which is analogous to Glu83, is protonated. The

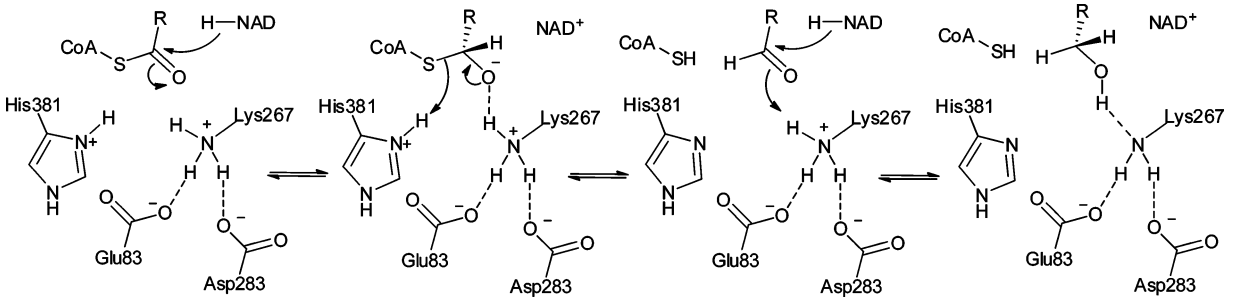

FIGURE 8. The mechanism proposed for *Pm*HMGR based on crystal structures and discovery of Lys267 in the active site.[28]

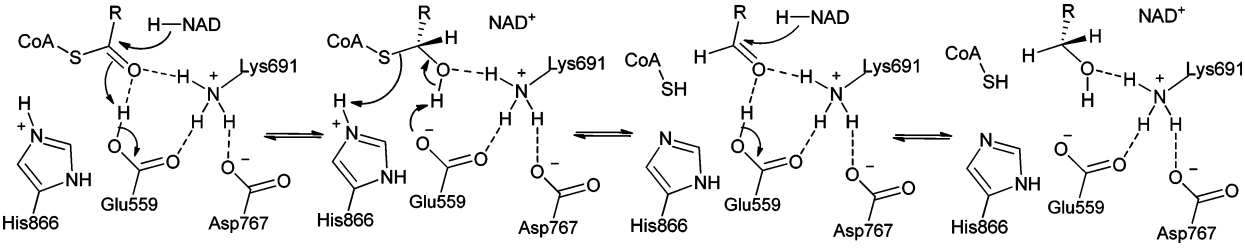

FIGURE 9. The mechanism described by Istvan et al. for the human HMGR.[24]

authors speculate that the negatively charged aspartate residue, Asp767, which is analogous to Asp283 in *Pm*HMGR, is close enough to Glu559 to affect its pK_a value.[24] This change results in the proposal of a different chemical mechanism in which Glu559 and Lys735 form an oxyanion hole to stabilize the reaction intermediates after hydride transfer, and Glu559 is identified as the general acid/base for the reaction. Asp767 supports the position of Lys691 and exerts some control over the protonation state of Glu559, while the catalytic histidine, H866, performs the same function as previously discussed.[24] The difference between the mechanisms shown in Figures 8 and 9 thus hinges on the protonation state of Glu83/Glu559, which of course cannot be unequivocally assigned based on the crystal structures.

Istvan et al. proposed that the different mechanisms for the enzymes could arise from class-related differences.[24] However, the structural differences in the active site residues are subtle, and further studies were needed to test this hypothesis. Recently, computational methods were employed to investigate the effect of the protonation state of Glu83 on the *Pm*HMGR mechanism.[36] Direct calculation of the pK_a of a given residue in a given environment is difficult and most likely not accurate enough to unambiguously assign the protonation state, but quantum mechanical calculations allow for the investigation of the effect of such a variable on a given chemical reaction. Reaction barriers and energies calculated for both protonation states suggest that the first hydride transfer proceeds along a lower energy pathway when Glu83 is protonated.[36] When Glu83 is deprotonated, both the reaction barrier and the reaction energy are more than 10 kcal/mol higher than when Glu83 is protonated. The structures show that Glu83 easily acts as an acid/base during the reaction, but Lys267 cannot perform this function in either case. In fact, the computational models predict that the *Pm*HMGR active site environment with Glu83 deprotonated cannot stabilize the experimentally observed hemithioacetal intermediate. In these calculations, the carbon–sulfur bond of the model hemithioacetal lengthens and it dissociates, which is inconsistent with the available experimental results.[35–37] These calculations were performed on a class II enzyme and affirm the mechanism that was proposed for a class I enzyme, therefore, it is likely that a general mechanism exists for all HMGRs.

Reaction Intermediates and Cofactor Exchange

Mevaldyl-CoA generated in situ is an active substrate of the enzyme and, in the presence of cofactor, is quickly converted to products in the crystal.[22,35] The HMG-CoA analogue, dithio-HMG-CoA, was initially thought to be a competitive inhibitor of HMGR.[48] In the complex of *Pm*HMGR with dithio-HMG-CoA and NADH, designed to capture the enzyme–substrate complex, this inhibitor was found to be a slow substrate.[37] This structure, shown in Figure 10, has electron density for a tetrahedral dithiohemiacetal species in the active site, which is analogous to the hemithioacetal

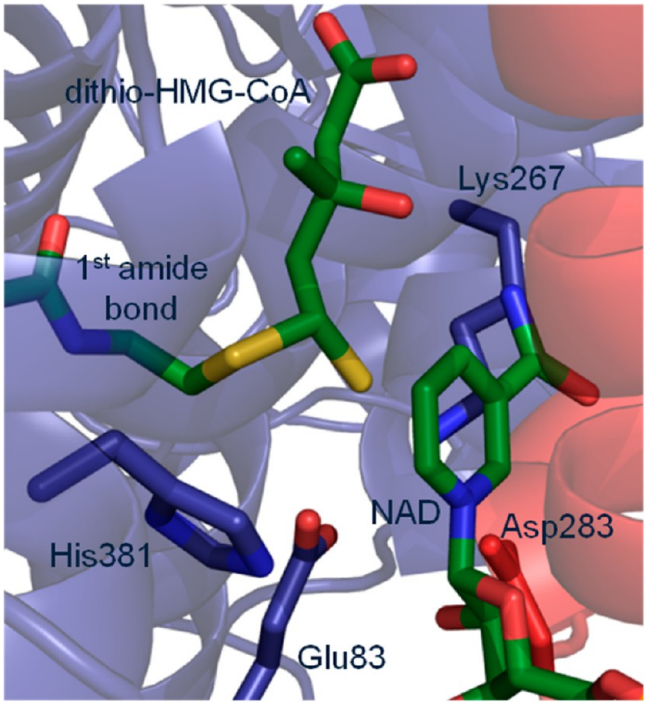

FIGURE 10. The tetrahedral dithiohemiacetal species in the active site of a *Pm*HMGR crystal structure.[37]

intermediate postulated to form from the natural substrate.[37] It not only reveals specific contacts between the enzyme and intermediate but also provides clues about the structural changes that occur along the reaction coordinate. A hydrogen-bonding network was identified that includes the residues of the flap domain, a class II conserved serine residue (Ser85), and the amide group closest to the sulfur of HMG-CoA. Ser85 was found to be critical for enzyme activity through SDM experiments, confirming the involvement of yet another remote residue in the reaction.[37] The structure shows how changes at the reaction center propagate to changes in the hydrogen-bonding patterns of the aforementioned groups, which may potentially act to govern processes such as the flap domain opening and closing. From a comparison of this intermediate structure to the initial HMG-CoA binary complex, nonproductive HMG-CoA/NAD+ ternary complex as well as molecular dynamics simulations of the HMG-CoA/NADH ternary complex indicates that a major distortion of the substrate, a 180° flip of its first amide bond through interactions with Ser85 and His381, is structurally important to the reaction pathway.

The fact that mevaldehyde is not detected during the reaction still adds to the remaining mechanistic ambiguity. The aldehyde has not been captured in a crystal structure and was not observed in isotope dilution and semicarbazide trapping experiments.[31−33,49] In solution, equilibration of CoA thiols, aldehydes, and their corresponding hemithioacetals is known to be rapid,[50] so one might expect the formation of an appreciable amount of aldehyde with the accumulation of the enzyme−hemithioacetal complex. It appears unlikely that the aldehyde stays bound to the enzyme during the reaction because K_m values for mevaldehyde are in the micromolar to millimolar range, which should lead to dissociation upon solvent exposure of the active site. It might be beneficial for the efficiency of the enzyme to develop a mechanism to protect the aldehyde from the solvent because mevaldehyde is significantly hydrated in H_2O and D_2O.[42]

Inclusion of the cofactor exchange step provides the conceptual framework to expand the mechanism and explain these experimental observations. On the basis of the available data, there are two steps along the reaction pathway for cofactor exchange to reasonably occur, either before or after hemithioacetal decomposition. If cofactor exchange occurs after hemithioacetal breakdown, then presumably the aldehyde would dissociate and be detected. But if cofactor exchange occurs before hemithioacetal breakdown, the aldehyde would never be exposed to a solvent, which is consistent with the observations above. This would indicate that the hemithioacetal breakdown is slower than the cofactor exchange. Free-energy calculations offer some support for this notion, indicating that the enzyme thermodynamically prefers mevaldyl-CoA to mevaldehyde.[36] QM/MM structures along the hydride transfer coordinate suggest a mechanism by which this could occur. An electrostatic interaction between the oxidized NAD cofactor and deprotonated Glu83 may contribute to slowing the base-catalyzed decomposition of the hemithioacetal.[36,42]

Revised Mechanism

The latest proposal for the mechanism of HMGR and the structural changes involved is shown in Figure 11. HMG-CoA and NADH bind to the active site, and the flap domain becomes ordered. The thioester of HMG-CoA is reduced and the oxyanion is stabilized by proton donor Glu83 and hydrogen bond donor Lys267. The hemithioacetal persists, while the flap domain opens and the oxidized NAD^+ cofactor dissociates. Once another molecule of reduced NADH cofactor binds to the active site, the flap domain reorders. Then, Glu83 assists in the base-catalyzed decomposition of the hemithioacetal to produce mevaldehyde and the CoA thiolate anion, which is then protonated by the cationic His381. CoASH is held in place by Ser85 and His381, while

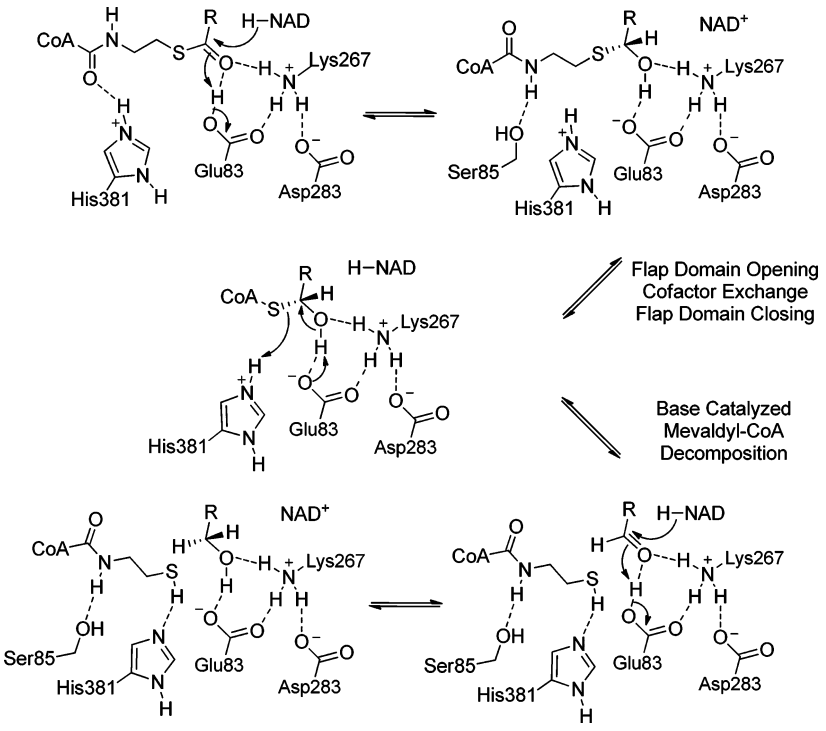

FIGURE 11. The revised HMGR mechanism.

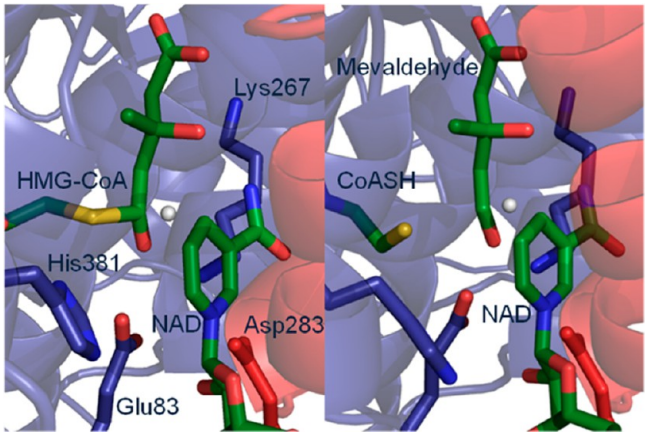

FIGURE 12. The QM/MM calculated hydride transfer transition structures for the first and second reduction steps of *Pm*HMGR.[36]

mevaldehyde is reduced and the oxyanion is again stabilized by proton donor Glu83 and hydrogen bond donor Lys267. The flap domain becomes disordered, and the product mevalonate, CoASH, and oxidized cofactor are released.

Computational methods have afforded a structural and energetic description of the two hydride transfer steps. The transition structures generated with QM/MM methods are shown in Figure 12. The reaction energies for both conversions are estimated to be approximately thermoneutral, which is consistent with the calculated transition structures for hydride transfer. The rate-determining step of the reaction has not been experimentally determined, however. The calculations rule out the mevaldehyde reduction step because its barrier is lower than the thioester reduction step by almost 3 kcal/mol. The energy barrier calculated for the first hydride transfer step, 21.8 kcal/mol, is consistent with the experimentally determined rate constant, which places the reaction on the order of 1/s to 1/min.

Conclusions and Open Questions

The combined use of kinetic studies, X-ray crystallographic studies, site-directed mutagenesis and, more recently, computational methods have provided iterative refinement of our understanding of the mechanism of HMGR, as shown in the progressively more complex mechanistic proposals summarized in Figures 3, 5, 6, 8, 9, and 12. The most recent proposal rationalized the available experimental results, including the importance of remote residues. However, even this mechanistic proposal does not completely describe the exceptionally complex reaction mechanism of HMGR. For example, there is no structural or energetic description of the cofactor exchange step, so the rate-determining step is still unknown. The cofactor exchange step includes the structural mechanism for opening and closing of the flap domain and an energetic description of substrate and cofactor binding. Hinge residues have been identified, but the structure of the open flap could not be resolved in the apo-structure of

HMGR. Although a dependence on substrate and cofactor binding is implicated, the forces that govern the flap movement are also unknown.

Further experimental and computational studies along the entire reaction coordinate will be necessary to determine the identity of the rate-determining step and to confirm or refute the hypotheses developed here about the relationship between the intermediate states, including the role of the observed interactions between Ser85, His381, and coenzyme A. The possibility of an alternate chemical mechanism must also be considered. For example, it is possible that the aldehyde intermediate does not actually form and the reaction proceeds through a substitution-type mechanism. Nonetheless, the combination of structural biology and computational chemistry has thus far developed the HMGR mechanism to a new level of detail. This combination of methods has the capacity to answer the questions outlined above concerning the HMGR mechanism and to also uncover new questions to challenge the present knowledge of the mechanism. The HMGR mechanism demonstrates that a model for how increasingly sophisticated methods can, in combination, elucidate even very complex mechanisms.

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Supporting Information. Structures associated with Figures 11 and 13 in .pdb format. This material is available free of charge via the Internet at http://pubs.acs.org.

BIOGRAPHICAL INFORMATION

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FOOTNOTES

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