Investigating the Role of the HMG-CoA Reductase C-Terminal Domain

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Introduction: The Mevalonate Pathway

The mevalonate pathway produces the precursors to isoprenoids, the most diverse class of natural products.[1] 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), a four-electron oxidoreductase, catalyzes the rate limiting step of this pathway (Fig. 1).

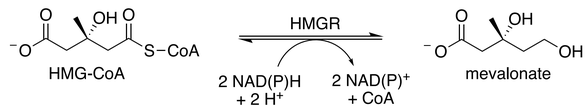

Fig. 1 The HMGR-catalyzed reaction in the mevalonate pathway.

Bacterial class II HMGR has a C-terminal domain (CTD) consisting of three α-helices. This region is disordered in most crystal structures of the enzyme. Some structures have captured the CTD occupying different conformations, suggesting that the CTD is more than a flap that covers the active site; however, the role of this region remains unknown.[1] Determining the function of this domain could provide valuable information for metabolic engineering and using the mevalonate pathway for the production of isoprenoid drugs.

Background

Little is known about the purpose of the CTD other than its role in supplying a catalytic histidine that is moved into the active site when both the substrate and the cofactor are bound.[2] While the earliest studies of HMGR's structure relied on site-directed mutagenesis to study important residues, HMGR has also been studied more recently with X-ray crystallography.

HMGR has evolved into two classes: class I enzymes are NADPH-dependent and are found in eukaryotes and some archaea and bacteria, while class II enzymes are found in archaea and bacteria and vary in cofactor preference. Crystal structures of class I HMGR all have disordered CTDs, thus the function of the CTD in these HMGRs is unknown.

Some class II structures have been solved that depict the CTD, such as two ternary complexes (HMG-CoA/NAD^+ and mevalonate/NADH) of *Pseudomonas mevalonii* HMGR (PmHMGR), which show the CTD over the active site in a "closed" conformation (Fig. 2).[3] Several interactions between the CTD and the NADH binding region led to early hypotheses that the CTD acts as a flap and facilitates hydride transfer.[3]

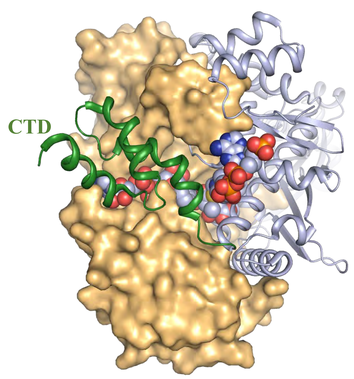

Fig. 2. Structure of the PmHMGR homodimer (orange surface and blue ribbons) with the CTD in green and HMG-CoA and NAD^+ in blue spheres.[3]

Novel CTD conformations

Several new conformations of the CTD have been solved by the Kung Lab, indicating that the CTD is not a two-way flap.[1] Structures of HMGR from *Streptococcus pneumoniae* (SpHMGR) and *Delftia acidovorans* (DaHMGR) with cofactor bound indicate that the CTD is highly flexible and does not take on the "closed" conformation upon cofactor binding (Fig. 3A-B).[4] A "partially closed" conformation of SpHMGR with HMG-CoA bound has also been observed; the CTD covers the HMG-CoA binding site while exposing the cofactor binding site (Fig. 3C).[1] We hypothesize that the CTD may bind CoA of the intermediate while the cofactor is exchanged, explaining the lack of observed mevaldyl-CoA and mevaldehyde intermediate release during the reaction.

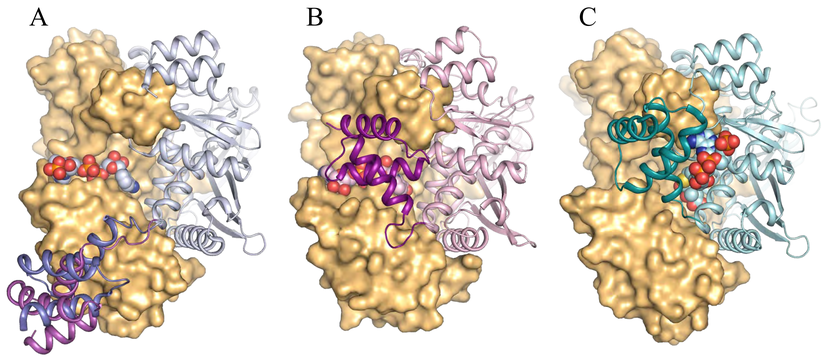

Fig. 3. Novel conformations of the CTD. **A.** The "open" conformation of SpHMGR with NADPH bound. **B.** The "flipped" conformation of DaHMGR with NADH bound. **C.** The "partially closed" conformation of SpHMGR with HMG-CoA bound.

Research Questions

- HGMR does not release intermediates but must exchange the cofactor during the reaction. *How is the CTD involved in substrate and cofactor binding? Does it prevent the release of intermediates?*
- The CTD is flexible and the "closed" position is not induced by the cofactor binding. *Why does the CTD occupy several conformations throughout the reaction?*
- The conserved residues of the CTD do not seem to be involved in binding CoA. *Why might the CTD be highly conserved?*

Future Directions

To investigate the role of the CTD, future studies will truncate the CTD of class II HMGR, helix by helix. In addition, CTDs of various HMGRs will be swapped. After modified enzymes have been obtained with truncated and swapped CTDs, kinetics studies will be performed to determine the effect of the CTD on the activity of the enzyme. This study could identify which parts of the CTD are most important to activity and shed light onto the role of the CTD.

In addition, structures of modified enzymes may be determined by X-ray crystallography to determine whether alterations in the enzyme's structure are responsible for the observed changes in activity.

Finally, to determine whether the CTD is involved in the prevention of mevaldyl-CoA and mevaldehyde intermediate release, the presence of free intermediates in the reaction mixtures will be determined using LC-MS.

Methods

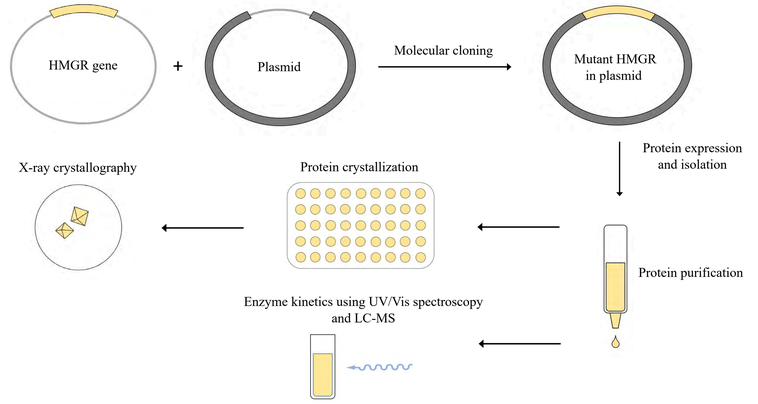

Fig. 4. Class II HMGR gene constructs will be modified and inserted into a plasmid. *Escherichia coli* will then be transformed with the plasmid DNA. The encoded protein will be overexpressed, purified, and studied using X-ray crystallography and enzyme kinetics experiments.

Acknowledgements

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