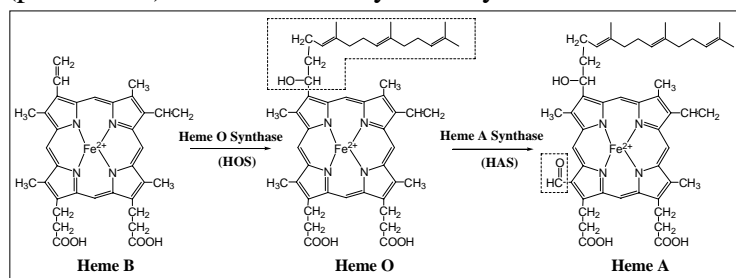


Assembling the Heme Cofactors in Cytochrome *c* Oxidase

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Heme A is an obligatory cofactor in eukaryotic cytochrome *c* oxidase (CcO), present at both an electron transfer site and the site of oxygen reduction. Heme A differs from heme B (protoheme) in that a farnesyl moiety has been added to one of the vinyl groups, and a methyl substituent has been oxidized to an aldehyde. These two reactions are catalyzed by heme O synthase and heme A synthase, respectively.



Surprisingly, despite the obvious importance of heme A to both CcO and the energy transduction pathway, very little is known about how heme A

is synthesized, how it is transported, or how it is inserted into cytochrome *c* oxidase. The focus of our research is to elucidate the biosynthesis of heme A and its method of transport.

We have determined that heme A biosynthesis is strictly dependent on molecular oxygen. Surprisingly, however, despite the fact that O₂ is absolutely required for heme A synthase activity, the oxygen atom incorporated into the product is derived *not* from O₂, but rather from H₂O. This result was completely unexpected and suggests that the current paradigm in which heme O is oxidized to heme A via successive monooxygenase reactions needs to be revisited. We propose instead that HAS oxidizes heme O to heme A via a series of electron-transfer steps. Whether this reaction utilizes the heme B cofactor to generate compound I or whether heme O is oxidized via autoxidation remains to be elucidated.

We are also interested in elucidating the regulation of heme A biosynthesis and understanding how heme A is transported/inserted in CcO. We recently determined that, contrary to previous suggestions, the heme A biosynthetic pathway is not regulated by copper. In addition, we demonstrated that heme O synthase and heme A synthase form a stable and physiologically relevant complex and that the heme is transferred directly from HOS to HAS without being freely released. Screens are currently underway to identify additional proteins that might be involved with heme regulation, transport, or insertion into cytochrome *c* oxidase.