Mutagenic Studies Of Heme A Synthase

George Chaus, Behzad Khodaverdian, Eric L. Hegg
Department of Chemistry

Cytochrome c oxidase (CcO) is the terminal enzyme in the electron transport chain in all plants, animals, aerobic yeast, and many bacteria. This enzyme contains a heme A cofactor at the active site that binds molecular oxygen for reduction to water. The heme A cofactor is absolutely required for cytochrome c oxidase function.

Our research focuses on the biosynthesis of heme A and its transport and insertion into CcO. Heme A is produced from heme B via two enzymes, heme O synthase (HOS) and heme A synthase (HAS). HOS converts the C2 ethyl group into a 17-hydroxy-ethyl farnesyl moiety, and HAS oxidizes the C8 methyl group to an aldehyde.

Heme A synthase is an integral membrane protein and previous spectroscopic studies on inactive HAS suggest the presence of two hemes, postulated to be a heme B cofactor and unreleased heme A product. Each iron is axially coordinated to two histidines. Primary sequence alignment of HAS from different organisms shows that there are five conserved histidines, three of which are conserved in both bacteria and eukaryotes. Conserved residues of HAS from Bacillus subtilis have been mutated to assess their mechanistic importance. To date, five histidines, four cysteines, and two arginines have been mutated to alanine, cysteine, or methionine. In vivo assays are currently underway to assess the activity of these mutants via reverse phase HPLC and western analysis.

Preliminary results suggest that with the exception of one of the mutant derivatives, all have very low or no activity and therefore are important for function. In addition, the western blot analyses of the impaired mutants show that the protein levels are wild type.

We predict that these histidines are important for binding the heme cofactor and heme substrate in HAS and/or the activation of the oxidizing species required for catalysis.