Cytochrome P460 of *Nitrosomonas europaea*

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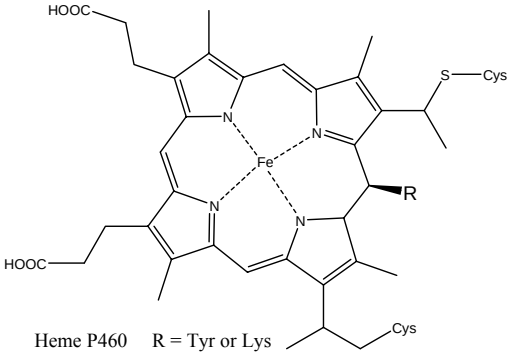
Nitrosomonas europaea, a β-proteobacteria, is an aerobic, obligate chemolithoautotroph that derives all the reductant required for biosynthesis and energy transduction from the oxidation of ammonia to hydroxylamine and sunsequently to nitrite. The second step in this process is catalyzed by the soluble periplasmic enzyme hydroxylamine oxidoreductase (HAO), a crystallographically characterized, covalently-linked homotrimer containing 21 *c*-type hemes and 3 catalytic hemes P460 (~204 kDa). Another hemoprotein with hydroxylamine oxidation activity is found in the periplasm of *Nitrosomonas*. This enzyme, cytochrome P460 is a mono-heme of 19 kDa, making it an especially amenable system to investigate the properties of this unusual heme cofactor. Sequence analysis leads us to the propose that cyt P460 and a closely related cytochrome comprise a new group of cytochromes.

The catalytic heme P460 is remarkable in that it contains three covalent linkages to the protein scaffold; two thioether linkages to cysteinyl residues, typical for cyts *c*, and a remarkable carbon-carbon bond between a carbon of the porphyrin ring and amino acid side chain (Tyr in HAO and Lys in cyt P460). Heme P460 is unique in biology by virtue of being the only heme cofactor known to *withdraw* electrons from its substrate.The visible absorption spectrum of the high-spin ferric enzyme has a broad Soret band at 435 nm and two low intensity bands at 510 and 540 nm. Reaction with the substrate, hydroxylamine, results in a 5 nm redshift of the Soret and the loss of the 510 and 540 nm features. Similar spectral changes are seen in the presence of CN^-. Addition of an appropriate electron acceptor then completes turnover. Chemical reduction or NO addition also eliminates the 510 and 540 peaks, gives the characteristic ferrous Soret at 460 nm, and is accompanied by additional weak features at 610 and 690 nm. Intermediate trapping, characterization and reaction kinetics will be presented.



Heme P460 R = Tyr or Lys