**Structure Studies of CATPO (Catalase-phenol oxidase)**

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**Introduction**

- Catalases [EC 1.11.1.6] decompose two molecules of hydrogen peroxide to water and oxygen [1].
- Ubiquitous class of enzymes in all aerobic prokaryotes and eukaryotic organisms [2].
- Primary function is the removal of small peroxides, particularly hydrogen peroxide [3].
- Three well established groups of catalases:
  - monomeric heme catalases,
  - catalase-peroxidases and
  - peroxisomal catalases
- A fourth group of catalases, (CATPO), can degrade H2O2 (catalase activity) and also oxidize α-diphenolic compounds (phenol oxidase activity) in the absence of hydrogen peroxide [4,5].
- The crystal structures of eleven monomeric heme-containing catalases are known from prokaryotic [6-8], lower eukaryotic [9-11] and mammalian [12] sources.

**Scytophily thermophilum catalase-phenol oxidase (CATPO)**

- **Homotetramer (4 x 80 kDa = 320 kDa)** [13]
- **Similar to Penicillium vitale catalase structure** [13]
- **1α per monomer**

**Charactersisation of native CATPO**

- **Four peaks :** 280, 406, (soret band), 592, 691
- **Heme content :** \( R_l (406/280) = 0.5 \)

**UV-Vis spectra of CATPO**

- Molecular weight of CATPO as monomer : 80 kDa
- UV-Vis spectra of WT, H101N and V142F. 590 nm peak characteristic of Heme a in WT and V142F, but presence of Heme b in H101N.

**Conclusions**

- Catalase-phenol oxidase is the first reported example of a fungal enzyme displaying bifunctional catalase-phenol oxidase activity
- Spectroscopic and mutagenesis studies show CATPO contain a heme d centre
- Both catalase and phenol oxidase activities seem to occur at the heme
- Bifunctionality may provide some advantages for industrial applications

**Future Work**

- Analysis of three dimensional structure of native CATPO
- Complete structure determination of recombinant CATPO
- Studies to determine metal content of CATPO
- Optimization of conditions for kinetic constants determination
- EPR to identify reaction intermediates in catalytic cycle(s) of CATPO
- Further mutagenesis studies to dissect reaction mechanisms

**References**

