The Purification of Cytochrome c Peroxidase (CCP) as a GST-fusion Protein, and the Mass Spectrometric Detection and Characterization of its Protein-Based Radicals on Reaction with H_2O_2

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Cytochrome c peroxidase (CCP) from *Saccharomyces cerevisiae* removes H_2O_2 by using cytochrome c as a terminal electron acceptor for mitochondrial respiration. However, in the absence of exogenous donors, CCP can turn over up to 10 equivalents of H_2O_2 by oxidation of endogenous donors on the polypeptide, thereby generating as many as 20 protein-based radicals. The detection and characterization of the sites of radical formation is of interest since recent results suggest that under elevated H_2O_2 conditions, the transcription factor *Pos9* (*Skn7*) is induced by CCP, which leads to the production of antioxidant genes such as thioredoxin. Here, previously constructed GST-CCP, in which mature CCP gene was cloned into pGEX 2T vector, was expressed in *E. coli*. Expression of the construct produced, based on small-scale trials, was approximately 16 mg of CCP per L of culture.

The stable nitroxide radical 2,2,6,6-tetramethylpiperidinyl-1-oxy ($TEMPO^{\cdot}$) was used to scavenge the CCP-based radicals produced upon incubation with 10 fold excess of H_2O_2. The resulting adducts were analyzed by mass spectrometry (MS) and the specific amino acid site of radical formation was determined by MS/MS.