

***Pseudomonas stutzeri* cytochrome c peroxidase – the role of calcium**

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Paracoccus (Pa.) denitrificans cytochrome c peroxidase (CCP) is known to have an activity dependence on calcium. The protein contains two distinct calcium binding sites: site I, thought to be located between the two domains of the monomer, and site II, which is thought to promote the low-spin to high-spin switch of the peroxidatic heme and dimerization of the enzyme. Recent studies proved that *Ps. stutzeri* CCP also needs Ca^{2+} ions to be active, but unlike *Pa. denitrificans* CCP, this CCP was isolated in a dimeric form with one tightly-bound Ca^{2+} ion. This form is readily active upon reduction by sodium ascorbate. The mixed valence CCP exhibits a very high affinity for Ca^{2+} ions (higher than EGTA's). Further addition of Ca^{2+} does affect both the kinetics of oxidation of horse heart cytochrome and produces higher aggregation states of the enzyme. These suggest the presence of a Ca^{2+} -binding site with low affinity at the protein surface. The protein was successfully crystallized in space group $P2_1$ and diffraction data were collected at the ESRF to beyond 1.66 Å resolution. The crystal structure was solved by molecular replacement, which revealed that a dimer of dimers is present in the asymmetric unit. The characteristic calcium-binding site at the interface of the two domains is present in all monomers, but no further Ca^{2+} ions were found. Preliminary fluorescence studies reveal that at least one tryptophan residue is affected upon calcium addition to oxidized CCP. This is in accordance with *Ps. nautica* CCP 3D structures published recently. In the only known structure of an inactive calcium free form of a bacterial CCP there is a displacement of the tryptophan-94 from the position occupied in all other structures, which is thought to lay in the electron transfer pathway.

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