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Interaction of Cytochrome c and Mn^{2+}-Cytochrome c Peroxidase

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Yeast cytochrome c peroxidase (CcP) was cloned and overexpressed in *E. coli*, and purified by a Ni^{2+}-affinity column. HoloCcP was obtained by reconstituting apoCcP with Mn^{3+}-protoporphyrin IX (MnPP). Electron paramagnetic resonance (EPR) spectra of spin-labeled holoCcP showed a slightly more immobilized signal than spin-labeled apoCcP. Both apoCcP and holoCcP showed further immobilization upon complexation with cytochrome c (Cc), a physiological redox partner of CcP in the mitochondrial intermembrane space. The reconstitution rate and amplitude of apoCcP with MnPP was greater for the apoCcP-Cc complex than for free apoCcP, indicating that binding of Cc induces a conformational change that facilitates reconsitution. The EPR spectra of spin-labeled Cc clearly demonstrated that bound Cc has more than two different conformations although CcP has only one high-affinity binding site. Replacement of Lys72 or Lys87 of Cc by a Glu residue resulted in weaker binding as predicted by the crystal structure of the CcP-Cc complex. ApoCcP, however, did not induce any conformational changes in Cc as evidenced in the EPR spectra.