

Characterization of the Cu_A-cytochrome *c* domain from *Bacillus subtilis* cytochrome *c* oxidase.

Danielle E. Arnold, Diann Andrews and Bruce C. Hill

Department of Biochemistry, Queen's University, Kingston, ON K7L 3N6

Cytochrome *c* oxidase is the terminal enzyme in the mitochondrial respiratory chain, catalyzing the reduction of O₂ to H₂O. In mitochondrial respiration cytochrome *c* and cytochrome *c* oxidase are separate proteins, whereas *Bacillus subtilis* cytochrome *c* oxidase contains an additional sequence as part of subunit II that encodes a cytochrome *c* domain. The *Bacillus subtilis* cytochrome *caa*₃ complex thereby serves as a model for the transient complex formed between cytochrome *c* oxidase and cytochrome *c* in the process of mitochondrial electron transfer. Recombinant Cu_A-cytochrome *c* containing a 6 amino acid His tag was expressed in *E. coli* transfected with two plasmids, pEC86 and the pET22b+/CtaC-Th-His construct and purified from the periplasmic fraction by affinity chromatography. While the spectral properties of the haem C in the cytochrome *c* sub-domain are normal, no copper is found in the Cu_A center, with the two thiols presumably forming a disulfide bond. Redox titrations were carried out on horse heart cytochrome *c*, the *caa*₃ complex, and the Cu_A-cytochrome *c* domain. The redox potential of the former was found to be 286mV, while the latter two constructs each possessed a redox potential considerably lower at approximately 195mV. The Cu_A-cytochrome *c* domain was subject to denaturation at various concentrations of Gdn-HCl to determine its unfolding behavior. By observing the shift in the Soret band, it was found that the cytochrome *c* sub-domain exhibits the same behavior as horse heart cytochrome *c*. However, by looking at the fluorescence spectra, it is apparent that another portion of the protein is unfolding at much lower concentrations. It is proposed that the Cu_A domain is unstable due to the absence of Cu. The results of these experiments show that the cytochrome *c* sub-domain is stable in the Cu_A-cytochrome *c* protein and that the Cu_A and cytochrome *c* sub-domains are acting independently of each other.