

**ABSTRACT****Direct Evidence for Two Active Cytochrome c Binding Sites on a Monomeric Form of Cytochrome c Oxidase.****Anthony Lalla**

Unlike the dimeric forms found in most mammals e.g. cow and man, cytochrome c oxidase from the hearts of hammerhead shark (*Sphyrna lewini*) is reported to be monomeric. In this study, this monomeric form was employed to investigate substrate binding. Before commencing these studies, a partial characterization of the enzyme was performed. Spectral analysis revealed that both the oxidised and reduced spectra were similar to those previously published except that the typical  $\alpha$  and soret ( $\gamma$ ) maxima were 2 nm blue shifted. site was found to be ionic strength. SDS / PAGE revealed that the shark enzyme was approximately 11% heavier and gel filtration chromatography demonstrated that the enzyme was about 25% heavier than the bovine monomer, confirming that the shark enzyme was indeed monomeric. SDS/PAGE also showed that the enzyme has 13 subunits and atomic absorption spectroscopy confirmed the presence of Fe, Cu and Zn ions in an approximate 3:2:1 ratio.

EDC was employed to catalyse the formation of the 1:1 covalent cytochrome c oxidase – cytochrome c enzyme-substrate complex which was used to investigate the number of enzyme catalytic sites. Exclusion chromatography confirmed that the bound cytochrome c was linked at its “high affinity site” and reaction of the complexes with ascorbate illustrated that the attached cytochrome c could be reduced and could relay its electrons to the enzyme. On the other hand steady-state and pre-steady state kinetics revealed that exogenously applied ferrocycytochrome c could still transfer electron to the 1:1 covalent complex inferring the existence of a second independent catalytic site. Interestingly, the Eddie-Hofstee plots generated from the polarographic assay of the 1:1 covalent complex with ferrocycytochrome c were biphasic suggesting that biphasicity is a function of each catalytic site.

The binding of substrate to the second site was found to be ionic strength dependent. However, pH / anionic strength studies and viscosity investigations showed no clear-cut dependency as observed for the free enzyme.