ABSTRACT

A Kinetic And Structural Characterization Of The Interactions Between The Enzyme Cytochrome c Oxidase And Its Substrate Cytochrome c

Valerie Bernadette Sampson

Two covalent enzyme-substrate complexes: (i) the 'traditional' complex, formed between oxidised cytochrome c and the fully oxidised enzyme and (ii) a 'steady state' complex formed in the presence of ascorbate were prepared. For the former, cytochrome c was bound exclusively to subunit II while for the latter, it was bound to subunits II, VIa and VIb. This result suggests that during turnover, conformational changes either modified an existing site or formed a second binding site.

Spectral analysis (400-630 nm) and stopped-flow studies confirmed that for both complexes the bound cytochrome c mediated the transfer of electrons from ascorbate to the enzyme. The half-life of the pre-steady state reduction of the bound cytochrome c and the concomitant reduction of heme a were both <5 ms. In contrast, the reduced covalent complexes were totally unreactive towards oxidised cytochrome c oxidase, confirming that the ascorbate mediated reduction of heme a proceeded via an intracomplex and not intercomplex mechanism.

Polarographic and stopped-flow analyses showed that cytochrome c oxidase within the two complexes catalyzed the oxidation of exogenous cytochrome c (k for the latter: ~0.7 x 10 $M^{-1}s^{-1}$). Taken together, these results suggest the existence of two independent binding sites on cytochrome c oxidase.

Three computer simulated enzyme-substrate complexes were generated: (i) an initial [ES] complex, (ii) a 'pulsed' intermediate and (iii) a complex indicative of substrate dissociation. The complexes exhibited 3-8, 7 and 4 salt bridges and 6-24, 64 and 3 hydrogen bonds respectively. As low accessibility was detected for lysine 13 and several other residues reported to be vital to enzyme-substrate binding, an 'induced fit' mechanism was proposed.

For complexes (i) and (ii) the respective heme c to Cu_A distances were 21 Å and 10 Å. Of several possible electron transfer pathways examined, one in which His 161 mediates the transfer directly from heme c to the Cu_A ligand Glu 198, seems particularly attractive. Finally, on the basis of estimates of intermolecular Coulombic forces and accessibility data, it is suggested that conformational changes and possibly intermolecular proton transfer, may modulate substrate binding and dissociation.