

P011 Probing the Q-sites of cytochrome bc_1 complex using Q-site inhibitors and FTIR spectroscopy

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ATR FTIR difference spectroscopy was used to characterise the binding of stigmatellin, antimycin A and funiculosin to cytochrome bc_1 complex in order to identify moieties of importance for Q-binding and enzymatic mechanism.

Inhibitors were perfused at micromolar concentrations over rehydrated protein layers under oxidising conditions. This resulted in accumulation of inhibitor which could be monitored in real time by FTIR spectroscopy to provide IR difference spectra caused by inhibitor binding. Specific binding to the Q_i -/ Q_o -site was confirmed and quantified by simultaneous acquisition of UV/visible double difference spectra and assessment of the spectral shifts of the B haems.

In addition, a novel electrochemical device allowing simultaneous acquisition of FTIR and UV/visible spectra while redox poisoning the rehydrated protein layer has been developed. This was used to obtain difference spectra arising from redox change of single cofactors local to the Q-/inhibitor binding-site. Differences between the FTIR redox difference spectra for control and inhibitor-pre-treated material are interpreted in terms of perturbations of bound inhibitor groups. Also, individual amino acid residues/haem moieties, the environments of which have been affected by inhibitor binding, and redox changes of bound/displaced Q will contribute. Interpretation was assisted by acquiring data at a range of pHs.