

P1.1.48. ATR-FTIR REDOX DIFFERENCE SPECTROSCOPY OF YARROWIA LIPOLYTICA AND BOVINE COMPLEX I

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Electrochemically-induced redox difference ATR-FTIR spectroscopy was used to investigate *Yarrowia lipolytica* and bovine complex I. The redox spectra show broad similarities with previously published data on *Escherichia coli* complex I (1). Comparisons of amide I/II changes that dominate the redox IR spectra of complex I with redox IR spectra of small model ferredoxins demonstrate that they arise primarily from characteristic structural changes local to the iron-sulfur centers rather than more global alterations. Bands arising from substrate ubiquinone were evident, as was a characteristic 1405 cm⁻¹ band of the reduced form of the FMN cofactor. Other signals are likely to arise from perturbations or protonation changes of a carboxylic amino acid, histidine and, possibly, several other specific amino acids. Redox difference spectra of center N2, together with substrate ubiquinone, were isolated from those of the other iron-sulfur centers by selective redox potentiometry. Its redox-linked amide I/II changes were typical of other 4Fe-4S iron sulfur proteins. Features of the substrate ubiquinone associated with the center N2 spectrum were particularly clear, with firm assignments possible for bands from both oxidized and reduced forms and the data could be used to estimate stoichiometry and midpoint potential.

Comparable redox difference spectra could also be obtained by perfusion with NADH/NAD⁺ and these will be compared with electrochemically-induced redox difference spectra in order to address concerns of whether substrate reduction may induce additional physiologically-important changes.

1. Hellwig, P., Scheide, D., Bungert, S., Mäntele, W., and Friedrich, T. (2000) FT-IR spectroscopic characterization of NADH:ubiquinone oxidoreductase (complex I) from *Escherichia coli*: oxidation of FeS cluster N2 is coupled with the protonation of an aspartate or glutamate side chain, *Biochemistry* 39, 10884-10891.