

P029 Physico-chemical Studies of Cytochrome c Nitrite Reductase (NrfA) from *Escherichia coli*

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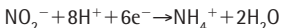
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The cytochrome c nitrite reductase (NrfA) is a deca-heme homodimer involved in respiratory denitrification in bacteria. This enzyme catalyses the reduction of nitrite into ammonia, following the reaction:



It has been shown that nitric oxide, as well as hydroxylamine, are metabolised by NrfA suggesting that these compounds are intermediates in the catalytic reduction of nitrite.

Each monomer contains four low spin ($S=1/2$) *bis*-histidine ligated hemes and an unusual high spin ($S=5/2$) heme which has lysine and water as axial ligands in the oxidised state. The substrate nitrite binds distal to the lysine in the reduced enzyme.

The catalytic mechanism of NrfA is being investigated using several complementary techniques:- structural information is obtained from Electron Paramagnetic Resonance (EPR) and Magnetic Circular Dichroism (MCD) spectroscopies; kinetic and thermodynamic properties from Protein Film Voltammetry (PFV). We present results concerning (i) the interaction of each substrate with the oxidised enzyme and (ii) the factors which influence the NO reductase activity of NrfA.