

**P045** Purification and characterisation of cytochrome *c'*  
from *Neisseria meningitidis*  
Wilhelmina M. Huston, Elisabeth C. Lowe, Clive S. Butler  
and James W.B. Moir  
*University of York*

Cytochrome *c'*, a *c*-type cytochrome with unique spectroscopic and magnetic properties, has been characterised in a variety of denitrifying and photosynthetic bacteria. Cytochrome *c'* has a role in defence and/or removal of nitric oxide (NO) but the mechanism of action is unclear. In order to examine the function of *c'* from *Neisseria meningitidis* the protein was purified after heterologous overexpression in *Escherichia coli*. The electronic spectra of the oxidised *c'* demonstrated a pH dependent transition (over the pH range of 6-10) in the Soret and high spin region, typical of a high-spin *c*-type cytochrome. NO-*c'* complexes were generated by incubation of *c'* in the presence of either a NO donor (DEA-NONOate) or NO gas from a cylinder and examined using electronic and EPR spectroscopy. Ferric *c'* when incubated with DEA-NONOate formed ferrous *c'*-NO 5 co-ordinate or 6 co-ordinate complexes, dependent on the concentration of donor. This presumably occurs via reductive nitrosylation. Reduced *c'* formed a 5 co-ordinate NO-*c'* complex when NO was supplied from DEA-NONOate. However, both ferric and ferrous *c'* resulted in ferric *c'*-NO complexes when NO was supplied in the form of NO gas. That NO may cause oxidation or reduction of cytochrome *c'*, as shown here, may have implications for the mechanism of NO detoxification by cytochrome *c'*