

P010 Reactivity of pseudomonas aeruginosa nitrite reductase
Centola Fabio, Rinaldo Serena, Maurizio Brunori
and Francesca Cutruzzolà

*Department of Biochemistry, University of Rome La Sapienza,
Rome, Italy*

Cd1 nitrite reductase (NIR), which reduces nitrite to NO in the denitrification chain of *Pseudomonas aeruginosa*, bears 2 redox centres per monomer, the c-heme, the electron entry site and the d1-heme, the catalytic site. NIR is able to reduce both nitrite and oxygen; both reactions are relevant to understand the interplay between denitrification and aerobic respiration in the same organism and in evolution. We have shown that the conserved His present in the distal d1-heme pocket are needed during nitrite reduction to stabilize the negative charge of the substrate bound to the reduced iron and to displace NO, the reaction product. The role of these residues in the reduction of oxygen has been characterized by stopped-flow kinetics. The time course of the reaction show 3 kinetic phases; the first depends upon oxygen concentration, whereas the other two are monomolecular. A major difference seen between the wt and the mutants is that the oxygen dependence of the first phase is less pronounced in the mutants. Our data show that these histidines are crucial not only for nitrite but also for oxygen reduction: in the latter reaction they are mainly involved after the binding of substrate, probably in the stabilization of a negatively charged transient intermediate.