Functional characterization of multiheme cytochromes by NMR

R.O. Louro[1],

[1]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal
e-mail: louro@itqb.unl.pt

Anaerobic microorganisms isolated from sediments and stratified bodies of water, such as *Shewanella oneidensis* have revealed a remarkably versatile respiratory metabolism, in numerous cases associated with a paradigm shift whereby respiration is based on the transfer of electrons to the outside of the cell. This extracellular respiratory capability allows these organisms to utilize substrates that are insoluble in the aqueous phase such as metal ores, and is the basis for the considerable interest surrounding applications of these organisms in bioremediation of metal and radionuclide contaminated sites, as well as applications in the assembly of microbial fuel cells. It is still not clear how the ATP generation associated with the cytoplasmic membrane is coupled to the extracellular reduction of substrates but this process relies on a complex network of soluble cytochromes that mediate electron transfer across the periplasm. This lecture will focus on one of the most abundant periplasmic cytochromes of *Shewanella* when this organism is grown in anaerobic conditions, which is a small tetraheme cytochrome of 12 kDa. NMR Data collected for the protein isolated from *Shewanella oneidensis* MR-1 was used to perform a detailed thermodynamic characterization in the physiological pH range. These data allow the parsing of the redox- and redox-protonation interactions that occur during the titration of the hemes in the physiological pH range. The results show that electrostatic effects dominate the heme-heme interactions, and in the physiological pH range protonation has a considerable influence on the redox properties of the hemes. Theoretical calculations using the oxidised and reduced structures of this protein revealed that the bulk redox-Bohr effect arises from several of the heme propionates, which have a fractional contribution to this process. This detailed characterization of the thermodynamic properties of the cytochrome reveals that, of the multiple microscopic redox states that the protein can access only a few show dominant populations. On this basis a functional pathway for the redox activity of the small tetraheme cytochrome from *Shewanella oneidensis* MR-1 is proposed, where reduction and protonation are thermodynamically coupled in the physiological range.

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