Probing the structure and function of CymA, a key protein of *Shewanella oneidensis* by NMR and EPR

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Shewanella (*S.*) *oneidensis* MR1 is a facultative anaerobe organism with a remarkable respiratory versatility, capable of reducing insoluble metallic compounds, such as Fe(III) and Mn(IV) [1]. The genome of this organism is fully sequenced showing an exquisitely complex respiratory network that is believed to set the basis for this respiratory versatility [2]. The details of the mechanism of metal oxide reduction remain unknown, but the large number of cytochromes in the periplasm and outer membrane confers to *S. oneidensis* MR-1 the ability to reduce the insoluble metal oxides, radionuclides and to perform electron transfer to electrodes in microbial fuel cells [3, 4]. One of the most important cytochromes in anaerobic conditions is CymA. This is a globular protein containing four hemes that is tethered to the cytoplasmic membrane by a single α-helix [5]. It was shown that CymA mediates electron transfer between the quinone pool of the inner membrane and periplasmic proteins [5]. These proteins may be terminal reductases (such as flavocytochrome c_3) [6], or proteins that shuttle electrons for the outer membrane proteins for the reduction of insoluble metal oxides (such as MtrA). CymA has 21 kDa and all the hemes are *c*-type hemes with two histidine residues as axial ligands . The moderate size of CymA is well within the range of application of standard NMR procedures to obtain structural and functional information [7, 8]. The paramagnetic shifts generated by the low spin hemes contain geometric information that enables the determination of the relative spatial arrangement of the hemes and the relative orientation of their axial ligands. Also, due to the spectral discrimination provided by NMR, the thermodynamic order in which the specific hemes become oxidised can also be determined. EPR is also a well established method for structural characterization of low spin hemes with bis-histidine axial coordination [9]. The g-tensors obtained for each heme in CymA allows establishing the orientation of the magnetic axes relative to each heme.

The functional and structural information obtained through NMR or EPR will be an essential step for understanding how this protein feed electrons into the periplasm respiratory network of *Shewanella*, a fundamental task to sustain its highly versatile anaerobic respiratory capability.

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