Exploring the redox properties of flavocytochrome c from Shewanella sp. DSM9451 – Comparison between pathogenic and non-pathogenic Shewanella species

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Biotechnological applications are considered a viable solution for several environmental challenges. *Shewanella* are ubiquitous Gram-negative bacteria, that are capable of reducing several inorganic and organic compounds by a process designated extracellular electron transfer (EET). This capacity allows the development of several biotechnological applications, such as microbial fuel cells. The most studied organism for this purpose is *Shewanella oneidensis* MR-1. However, there are *Shewanella* species that were isolated from clinical samples and their EET wasn't explored. Flavocytochrome c (FccA) is a tetraheme cytochrome c involved in the EET process in *Shewanella* spp., with a key role in controlling the flux of electron transfer within the periplasmatic space in anaerobic conditions towards EET or fumarate reduction. The FccA from *S. oneidensis* MR-1 and *Shewanella frigidimarina* NCIMB400, two environmental species, were already characterized, showing distinct properties.

To compare the redox properties of FccA from environmental and clinical isolates, the FccA from *Shewanella* sp. DSM9451, isolated from the cerebrospinal fluid of a 1-year-old child, was purified from the native organism grown aerobically. The structure was determined by X-ray crystallography and showed the same overall fold. Paramagnetic ¹H-NOESY and ¹H-¹³C HMQC NMR spectra were used to discriminate spectral fingerprints of the individual hemes and determine their order of reduction. Protein film voltammetry was used to characterize the electrochemical properties of the protein. The results revealed that the order of oxidation changes in all the three proteins studied, but, in contrast to the other FccA, the hemes of FccA from *Shewanella* sp. DSM9451 revealed a redox-Bohr effect and a different orientation of the heme axial ligands. These differences will be discussed in the context of the different ecosystems where the host organisms were isolated.