NMR characterization of the PioA cytochrome, a putative Fe oxidoreductase in a phototrophic iron oxidizing bacterium

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Fe(II) phototrophic oxidation is considered one of the most ancient forms of metabolism. However a molecular characterization of this process is lacking. Recently, two operons encoding proteins required for phototrophic iron oxidation in *Rhodopseudomonas palustris* (*pio* operon) and *Rhodobacter* strain SW2 (*fox* operon), were discovered. Both operons contain three genes, the first of which encodes a *c*-type cytochrome (*pioA* and *foxE*, respectively). The gene *pioA* encodes a 60 kDa decaheme cytochrome predicted to be the Fe(II) oxidoreductase.

Using the expression vector pUX19, the gene coding for this cytochrome was cloned in *E. coli* JCB7123 strain containing the plasmid pEC86 that constitutively expresses the heme maturation operon, *ccm*. The *E. coli* periplasmic signal sequence *pelB* was used to localize PioA in the periplasm. A purification protocol, based on periplasmic extraction, was established for PioA.

NMR studies of a 60 kDa paramagnetic protein are challenging but the spectroscopic characterization of the four-heme 64 kDa flavocytochrome c_3 from *Shewanella* sp. was achieved. Using standard NMR techniques in a 500 MHz spectrometer, it was possible to assign the heme signals of this protein and to determine their order of oxidation.

A preliminary spectroscopic characterization of PioA will be presented, setting the stage for a detailed study of the structure and function of this protein.