

Multi-heme Cytochromes: New Folds, New Ligation, New Chemistry

Stephen K. Chapman¹, Graeme A. Reid², Malcolm D. Walkinshaw², Christopher G. Mowat¹,
Caroline S. Miles², Emma Rothery¹ and Sally Atkinson¹

¹*EaStCHEM, School of Chemistry, University of Edinburgh,*

²*Institute of Structural and Molecular Biology, University of Edinburgh.*

The bacteria *Shewanella* and *Geobacter* exhibit remarkable flexibility in their ability to use a diverse range of electron acceptors for anaerobic respiration. The genome sequences of both *Shewanella oneidensis* and *Geobacter sulfurreducens* have been determined and are particularly rich in *c*-type cytochromes possessing around 40 and 100 of these cytochromes respectively. Although these proteins contain the CXXCH motif characteristic of *c*-type cytochromes, there are many that appear to have no homologs of known function. We have identified a number of multi-heme *c*-type cytochromes from these bacteria and we are analyzing their structure and function. One such protein from *Shewanella*, known as, flavocytochrome *c*₃, is a tetraheme *c*-type cytochrome that also contains flavin. We have determined the structure of this protein and shown it to be a fumarate reductase. More recently we have determined the structure of an octaheme cytochrome, OTR (Fig. 1.), that exhibits tetrathionate reductase activity. We discovered that for one of the heme groups the CXXCH motif does not provide a ligand to the heme iron with this role being taken by a lysine residue. Previously such ligation has only been observed in the pentaheme nitrite reductase but in this case the lysine is part of a CXXCK motif. Interestingly, there are two homologs of the *Shewanella* OTR in *Geobacter* and we are now in the process of characterizing these proteins. In addition to the OTR type protein, *Geobacter* also contains an octaheme that has nitrite reductase activity. We have cloned the gene encoding this protein and have now expressed and purified it prior to characterization. We have also recently crystallized and determined the structure of one of two dodecaheme cytochromes from *Geobacter*.

