Published in final edited form as:

Biochem Soc Trans. 2012 December 01; 40(6): 1268–1273. doi:10.1042/BST20120106.

Mind the gap: diversity and reactivity relationships among multihaem cytochromes of the MtrA/DmsE family

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Abstract

Shewanella oneidensis MR-1 has the ability to use many external terminal electron acceptors during anaerobic respiration, such as DMSO. The pathway that facilitates this electron transfer includes the decahaem cytochrome DmsE, a paralogue of the MtrA family of decahaem cytochromes. Although both DmsE and MtrA are decahaem cytochromes implicated in the long-range electron transfer across a ~300 Å (1 Å = 0.1 nm) wide periplasmic ‘gap’, MtrA has been shown to be only 105 Å in maximal length. In the present paper, DmsE is further characterized via protein film voltammetry, revealing that the electrochemistry of the DmsE haem cofactors display macroscopic potentials lower than those of MtrA by 100 mV. It is possible this tuning of the redox potential of DmsE is required to shuttle electrons to the outer-membrane proteins specific to DMSO reduction. Other decahaem cytochromes found in *S. oneidensis*, such as the outer-membrane proteins MtrC, MtrF and OmcA, have been shown to have electrochemical properties similar to those of MtrA, yet possess a different evolutionary relationship.

Keywords

anaerobic respiration; cytochrome; electron transfer; haem; *Shewanella oneidensis*

Introduction

A periplasmic ‘gap’

Shewanella oneidensis MR-1 is a facultative anaerobe that is being studied intensely because of its ability to utilize a diverse set of terminal electron acceptors [1,2], such as iron and manganese oxides, chromate, uranium, nitrate/nitrite and DMSO [3–9]. This versatility stems from the various internal electron-transfer pathways that are able to shuttle electrons from the inner membrane through the periplasm and, as needed, across the outer membrane

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where the terminal electron acceptor is located (Figure 1). It is believed that these pathways share a common mechanism to acquire electrons: the quinol pool and the tetrahaem cytochrome CymA [10–12]. From CymA, the pathways branch as there are numerous proteins that can serve as its redox partner [13–17], including members of the MtrA family, a class of decahaem *c*-type cytochromes. MtrA in particular has been shown to receive electrons from CymA [15,17], and subsequently pass electrons to an outer-membrane decahaem cytochrome (MtrC), when docked in a MtrCAB complex [18], where MtrB is an outer-membrane porin protein. Additionally, MtrC has been demonstrated to interact with yet another outer-membrane decahaem cytochrome, OmcA [19,20].

Recently, measurements of the size of the periplasmic space of *S. oneidensis* MR-1 using microscopy have shown that the distance needed to be traversed by trans-periplasmic electron-transfer events is approximately 240 Å (1 Å = 0.1 nm) [21], whereas the outer membrane is typically estimated to have a thickness of 40–70 Å as well [22]. Although previous models suggested that MtrA might be able to span the periplasmic space [1,23,24], recently we have shown that SAXS (small-angle X-ray scattering) of purified MtrA indicates that at most, MtrA has a 2:1 aspect ratio with the maximal length dimension of 105 Å [25]. The SAXS analysis, in excellent agreement with supporting studies using analytical ultracentrifugation, shows that spanning a range of high nanomolar to micromolar concentration regimes, MtrA is a monomeric protein consistent with dimensions of 25–50 Å × 100 Å, meaning that it is insufficiently long to span both the periplasmic and outer-membrane compartments [25]. How extracellular electron transfer is achieved is still an open question, even when considering just the periplasmic compartment. As the majority of studies of *Shewanella* electron-transfer pathways have examined MtrA only with a single report of another MtrA orthologue in the literature (MtoA, from metal-oxidizing *Sideroxydans lithotrophicus* [26]), we review the current understanding of the biochemical and biophysical properties of the MtrA family, highlighting DmsE, which is associated with outer-membrane DMSO reduction, in order to identify the commonalities associated with extracellular electron-transfer chemistry.

Extracellular DMSO reduction

The current model for extracellular DMSO reduction in *Shewanella* posits that DmsE transfers electrons to the DmsAB protein complex via the integral outer-membrane β -barrel protein DmsF [9]. DmsAB is proposed to be similar to the DMSO reductase heterodimer of *Escherichia coli*, with molybdopterin and iron–sulfur cluster cofactors [9,27]. Although, on the surface, MtrA and DmsE may appear to be highly similar, *in vivo* studies have examined the modularity of the different electron-transfer pathways available in *Shewanella*, to probe the interchangeability of the different components [28–30]. Although MtrA and DmsE are both periplasmic decahaem cytochromes with similar amino acid sequences, they need not behave identically. During reduction of ferric citrate, DmsE is able to partially function in the place of an MtrA/MtrD knockout, suggesting some modularity; however, the reverse is not true [28]. It has been shown MtrA is not able to recover DMSO reduction activity in DmsE-knockout strains, suggesting that these proteins do have unique reactivity. A caveat is that, although DmsE is required for robust DMSO reduction activity, in its absence the CctA protein may aid in electron transfer across the periplasm, recovering some activity [28].

Redox characteristics of the MtrA family

As predicted by the appearance of CXXCH motifs in the primary amino acid sequence, recombinant DmsE prepared as described previously [15] is found to have ten α -type haem groups. The oxidized and reduced electronic absorbance spectra (Supplementary Figure S1 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) and the as-isolated EPR spectrum (Supplementary Figure S2 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) are nearly identical with MtrA [14]. The CD spectra of DmsE and MtrA are also strongly similar (Supplementary Figure S3 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>). As described below, the electrochemical analysis of DmsE, however, differs from that of MtrA [31].

Purified recombinant DmsE was characterized electrochemically using PFV (protein film voltammetry). DmsE was deposited on a PGE (pyrolytic graphite edge) electrode and non-turnover voltammograms were collected at a span of pH values. These reversible and symmetric non-turnover voltammograms show that DmsE has a wide window of potential (Figure 2). Using the ‘potential window’ metric describing the overall width of the electrochemical response [31], DmsE electrochemistry can be compared with other members of the DMSO reduction and DMR pathways (Supplementary Table S1 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>). The window of potential for DmsE spans 270 mV at pH 6 (Figure 2A). This width is in good agreement with other decahaem cytochromes (300 mV for MtrA and 275 mV for MtrC) [18,23,31]. DmsE has a window of potential between – 90 and – 360 mV compared with the SHE (standard hydrogen electrode), which is shifted ~100 mV lower than MtrA (Figure 2B). There is overlap between the redox potential windows of DmsE and CymA (Figure 2C), indicating that it is feasible for electron transfer to occur between these cytochromes. The voltammograms of DmsE show that there is little change in potential at neutral and high pH; however, there is a ~150 mV shift to higher potential at low pH (Supplementary Figure S4 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>). This behaviour is not seen with MtrA [31]. MtrA has a potential window that is dependent upon pH at both high and low pH values. The electron-transfer rate for DmsE is $\sim 122 \text{ mV} \cdot \text{s}^{-1}$ (Supplementary Figure S5 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) which is equivalent to that of MtrA [18,31]. In comparison, for MtoA, the MtrA orthologue implicated in iron oxidation approximately 100 mV more positive than MtrA, the reduction potential window was approximately + 50 to – 300 mV at pH 7.1 [26]. Thus the overall shift in macroscopic redox potentials appears to be a major difference between members of the MtrA family.

This difference in thermodynamics may be important for the next electron-transfer step in the respective pathways. Although it has been established through visible assays of the MtrCAB complex, and electrochemically detected protein–protein interactions that MtrC can clearly receive electrons from MtrA [15,16,18], DmsAB from *S. oneidensis* has not been characterized at all to date. Yet, presuming similarity with DMSO reductase from *E. coli* (DmsABC heterotrimer) [9,32], it is likely that *S. oneidensis* DmsE is responsible for passage of electrons into an iron–sulfur cluster-binding DmsB subunit, which in turn supplies electrons to DmsA, which houses the molybdopterin active site. Notably, *EcDmsB* contains four [4Fe–4S] clusters with midpoint potentials ranging from – 330 to – 50 mV

[33], and the sequence of *SoDmsB* contains 16 conserved cysteine residues, which are predicted to be the ligands of the iron–sulfur clusters. Thus we hypothesize that DmsE may possess a lower range of haem reduction potentials in order to facilitate electron transfer into the low-potential clusters of DmsB, and could equally well reduce the haem cofactors of MtrC, providing the partial recovery observed for substituting DmsE into the *mtrA* mutant reported by Coursolle and Gralnick [28]. In such a model, MtrA itself would not be able to serve as a DmsE substitute, as its potentials are too high to support efficient electron transfer into DmsAB.

Protein family relationships

Previous analysis of the collection of sequences of multihaem cytochromes found in prokaryotes has revealed that there are two distinct groups of decahaem proteins [34]. The first group contains the periplasmic proteins DmsE and MtrA (*S. oneidensis*), whereas the second group contains the outer-membrane proteins OmcA and MtrF [35] (*S. oneidensis*). The pentahaem class of proteins related to NrfB has been proposed to be evolutionarily related to MtrA/DmsE proteins [36], and it has been suggested that MtrA has evolved from a dimer (or gene duplication) of NrfB, although we have illustrated that the homology between NrfB and MtrA only holds for the N-terminal portion of MtrA itself (Supplementary Figure S6 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>). Protein similarity networks provides a tool for visually assessing questions of relatedness using criteria of either sequence or structural similarity, which can then be depicted with the freely available program Cytoscape [37]. The deployment of Cytoscape in this case allows us to pose the question how similar is DmsE to other decahaem cytochromes *c* and NrfB families?

Using DmsE from *S. oneidensis* (Swiss-Prot accession number NP_717047.1) as the basis for queries using protein sequence-based BLAST searches [38], a list of protein sequences with an *E* value less than 10^{-7} and having more than five CXXCH motifs was generated. The resulting list contained 154 sequences (Supplementary Table S2 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) used for generating and visualizing a protein sequence similarity network in Cytoscape, where each node represents a unique sequence and each edge represents a level of similarity that is equal to the *E* value cut-off.

Figure 3(A) shows the sequence-similarity relationships between the three families of multihaem cytochromes. As expected, NrfB and MtrA/DmsE cluster together and are more closely related to each other than the two decahaem protein families. In fact, the relationship of OmcA and DmsE is not much greater than functionally unrelated proteins [34]. By a cut-off *E* value of 10^{-12} , the OmcA family (Supplementary Table S3 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) is completely separate, and the NrfB family (Supplementary Table S4 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>) is beginning to separate entirely from the MtrA/DmsE cluster. Figure 3(B) follows the MtrA/DmsE family, visualized by Cytoscape and labelled by haem content. Notably, the cluster contains a wider range of haem-containing protein than might be predicted, and is not limited to decahaem proteins from gammaproteobacteria (Supplementary Figure S7 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>).

The decahaem cytochromes of this family, however, cluster tightly together and it takes an extremely strict cutoff value to separate DmsE (orange triangle) and MtrA (yellow hexagon). The list of proteins that cluster with each are found in Supplementary Tables S5 and S6 at <http://www.biochemsoctrans.org/bst/040/bst0401268add.htm>.

An observation that has been mentioned before [9], but highlighted in the present paper, is that not all species of *Shewanella* have a *dms* operon. *Shewanella loihica*, for example, has been shown to lack the ability to respire on DMSO [39] and does not have a DMSO reductase, and, indeed, Cytoscape-based visualization of the similarity of MtrA family members shows that, although *S. loihica* contains two decahaem proteins, both protein sequences cluster with MtrA and not with DmsE. In contrast, it has been shown previously that *Shewanella baltica* does not respire on DMSO [9], yet one subspecies (*S. baltica* OS195) does contain a gene product that clusters with DmsE. The reasoning behind some species containing the components to enable DSMO reduction whereas others are lacking is unknown. In this way, protein sequence similarity networks may be a useful tool for elucidating multihaem protein function: the *S. oneidensis* gene product SO_4360 (Swiss-Prot accession number NP_719884.1) has been proposed to be a potential homologue of DmsE [9,28]. This gene product is found in the DmsE family and clusters with the other decahaem proteins initially, but, at a cut-off value of 10^{-125} , when DmsE and MtrA still cluster, it is no longer connected. Thus we hypothesize that, whereas SO_4360 is encoded by a gene that exists within an operon bearing other *dms* genes, it does not cluster with DmsE, and therefore will probably have distinguishing biochemical/biophysical characteristics.

The deployment of protein sequence-similarity networks simultaneously allows for the delineation of known functionality, as well as suggesting regions of sequence space that may correlate with novel chemistries. For example, considering MtrA paralogues implicated in iron oxidation (compared with extracellular iron reduction), our analysis identified MtrA homologues including PioA (Swiss-Prot accession number YP_001989850.1), which participates in phototrophic iron oxidation in *Rhodospseudomonas palustris* TIE-1 [40], and MtoA (Swiss-Prot accession number YP_00352109.1) a decahaem cytochrome from *S. lithotrophicus*, which is presumed to oxidize extracellular iron through MtoAB and CymA (MtrAB and CymA homologues) [26]. PioA and MtoA are both found to be initially clustered with MtrA and DmsE, yet both diverge from the iron-reducing paralogues at more stringent *E* values. MtoA, which possesses a similar molecular mass to that of MtrA and similar (although more positive) reduction potentials, diverges from MtrA/DmsE around an *E* value of 10^{-80} . PioA, which includes an N-terminal extension of unknown function, diverges from the main MtrA/DmsE cluster at a similar *E* value, yet forms its own small cluster with related proteins from other alphaproteobacteria at an *E* value of 10^{-125} . Thus we hypothesize that MtrA paralogues that participate in iron oxidation will similarly diverge, and that bioinformatics methods will be useful tools in the future in the identification of novel functionalities for MtrA family members involved in extracellular electron transfer.

Conclusions

Although it is clear that periplasmic decahaem electron-transfer proteins such as MtrA and DmsE are vital components to the dissimilatory metal reduction and DMSO reduction

pathways in *S. oneidensis*, a truly molecular view of extracellular electron transfer remains elusive. Whereas MtrA has been an object of study for over a decade, only recent efforts have demonstrated that MtrA itself cannot span the periplasmic space as a static molecular 'wire', although certainly the disposition of its ten haem moieties is critical to successful long-range electron transfer. As demonstrated by the measurements made by ourselves and others, there is clearly a gap in our understanding of what periplasmic events must occur in order to move electrons nearly 300 Å. We propose that the comparative study of MtrA orthologues will elucidate the function and electron-transfer properties of these proteins, the most 'haem-dense' proteins that have been identified to date [34]. We have shown that, biochemically, MtrA and DmsE share most properties (number of haems, electronic absorption, EPR and electron-transfer rate), yet they differ in terms of overall thermodynamics of electron transfer, consistent with the observation of Gralnick and co-workers that MtrA and DmsE are not completely interchangeable *in vivo* [28]. Whether or not structural differences in these two proteins contribute to the differences in redox potential is unknown. Undoubtedly, through the systematic structural and biophysical interrogation of newly elucidated MtrA orthologues, coupled to additional biological and bioinformatic approaches, the gap in our understanding may finally become closed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding

This work was generously supported by the National Science Foundation [grant numbers MCB 0546323 and CHE 0840418], the Boston University UROP Office, a Scialog[®] Award from the Research Corporation for Science Advancement, and the National Institutes of Health [grant number F32GM904862 (to N.A.)]. C.L.D. is a Howard Hughes Medical Institute Investigator.

Abbreviations used

PGE	pyrolytic graphite edge
SAXS	small-angle X-ray scattering

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Figure 1. A model for DMSO reduction by DmsEFAB and iron reduction by MtrABC(DEF)
 The arrows indicate the flow of electrons. DmsE and MtrA(D) are proposed to accept electrons from the menaquinone pool via CymA. Haem groups in CymA, MtrACDF and DmsE are shown. IM, inner membrane; OM, outer membrane.

Figure 2. Electrochemical comparison of MtrA and DmsE

(A) A cyclic voltammogram of DmsE on PGE, raw and baseline subtracted data, pH 6, 4°C. (B) Normalized voltammograms showing the difference in potential window of DmsE (continuous trace) and MtrA (broken trace), pH 6, 4°C. (C) Histogram comparison of DmsE, MtrA and CymA. Even though DmsE is lower in potential than MtrA, its potential window still overlaps with CymA. SHE, standard hydrogen electrode.

Figure 3. Protein sequence similarity network analysis

(A) Cytoscape analysis of MtrA (burgundy), OmcA (blue) and NrfB (yellow) families of multihaem cytochromes. MtrA and NrfB families are more closely related and still cluster together at an E value of 10^{-12} . (B) Cytoscape analysis of the MtrA family of proteins. Each node is labelled with the number of CXXCH motifs contained in each protein. Several proteins of interest are highlighted: DmsE (orange triangle), MtrA (yellow hexagon), MtrD (green circle), PioA (black diamond) and MtoA (blue square).