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The small iron-sulfur protein from the ORP operon binds a [2Fe-2S] cluster

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ABSTRACT

A linear cluster formulated as $[S_2MoS_2CuS_2MoS_2]^{3-}$, a unique heterometallic cluster found in biological systems, was identified in a small monomeric protein (named as Orange Protein). The gene coding for this protein is part of an operon mainly present in strict anaerobic bacteria, which is composed (in its core) by genes coding for the Orange Protein and two ATPase proposed to contain Fe-S clusters. In *Desulfovibrio desulfuricans* G20, there is an ORF, *Dde_3197* that encodes a small protein containing several cysteine residues in its primary sequence. The heterologously produced Dde_3197 aggregates mostly in inclusion bodies and was isolated by unfolding with a chaotropic agent and refolding by dialysis. The refolded protein contained sub-stoichiometric amounts of iron atoms/protein (0.5 ± 0.2), but after reconstitution with iron and sulfide, high iron load contents were detected (1.8 ± 0.1 or 3.4 ± 0.2) using 2- and 4-fold iron excess. The visible absorption spectral features of the iron-sulfur clusters in refolded and reconstituted Dde_3197 are similar and resemble the ones of [2Fe-2S] cluster containing proteins. The refolded and reconstituted [2Fe-2S] Dde_3197 are EPR silent, but after reduction with dithionite, a rhombic signal is observed with $g_{max} = 2.00$, $g_{med} = 1.95$ and $g_{min} = 1.92$, consistent with a one-electron reduction of a $[2Fe\text{-}2S]^{2+}$ cluster into a $[2Fe\text{-}2S]^{1+}$ state, with an electron spin of $S = ½$. The data suggests that Dde_3197 can harbor one or two [2Fe-2S] clusters, one being stable and the other labile, with quite identical spectroscopic properties, but stable to oxygen.

1. Introduction

An Orange Protein (ORP) was firstly isolated from *Desulfovibrio* (*D.*) *gigas* and shown to harbor a novel heterometallic cluster containing copper and molybdenum of the type $[S_2MoS_2CuS2MoS_2]^{3-}$ [1]. Analysis of its primary sequence revealed that there is no common metal binding motifs, and thus the cluster binding region in the polypeptide chain remained elusive until recently [2].

The cluster is non-covalently bound to the polypeptide chain and recently it was shown that it is bound to the protein by both hydrophobic and electrostatic interactions [2,3], but it does not involve any of the common metal binding residues, such as cysteine, histidine and methionine side chains. Moreover, this protein can be reconstituted in a protein-assisted mode [2,4], a feature that might be related to its function.

With the increasing number of sequenced genomes in the recent years, it is possible to identify that the ORP encoding gene is present in the genome of several anaerobic bacteria, mostly found next to two genes coding for ATPases containing Fe-S cluster binding motifs, with a high homology between each other [4,5]. In the case of *D. vulgaris* Hildenborough (DvH), the ORP gene is part of a group of genes organized in two divergent operons that are under the control of a sigma-54 transcription regulator [5]. An indication of its function was provided by the partially inactivation of this gene, which revealed that it might be implicated directly or indirectly in the cell division of this organism [5].

In addition, this same study showed that the Orange Protein formed a protein complex composed of the proteins encoded by these two divergent operons [5], the ORP Complex. Two of the proteins that composed this ORP complex (encoded by *DVU2105* and *DVU2107*) present, in its primary sequence, several cysteine residues, contrary to the Orange protein, which might indicate that they can bind a metal, such as an Fe-S cluster or Mo atom, though these cysteine residues are not organized in a motif similar to any known Fe-S cluster binding proteins [6].

In *Desulfovibrio desulfuricans* G20, also a sulfate reducer, the gene that codes for the ORP, *Dde_3198*, is organized in a monocistronic unit (Fig. 1) [4], which also includes genes encoding two Fe-S cluster ATPases (*Dde_3200* and *Dde_3201*), a MinD-like protein (*Dde_3202*) and a small putative protein that present several cysteine residues in its primary sequence (*Dde_3197*), and are under the regulation of a transcription regulator (Dde_3196) similar to DVU2106. The gene organization in different bacteria of the ORP operon is shown in Fig. 1.

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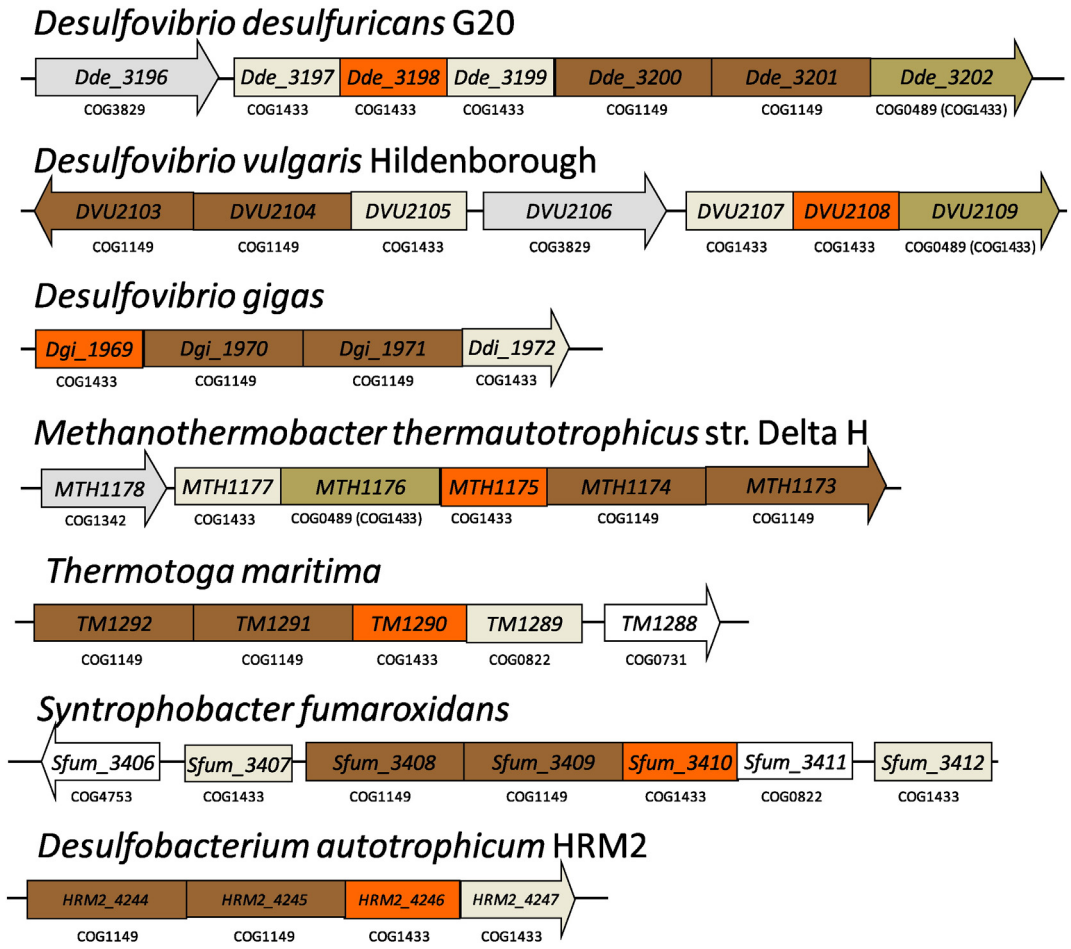

Fig. 1. Genome organization of ORP operons from different organisms, from which the Orange Protein has been isolated. The gene encoding for this protein is colored in orange, the one of the ATPases containing Fe-S clusters are brown, the transcription regulator are in grey, the MRP-like protein with a COG1433 associated domain is in dark green, and the putative Fe-S cluster binding protein encoding gene in light brown. Underneath each gene is indicated the correspondent COG family.

These small proteins containing cysteines, together with ORP, belong to COG1433 (COG - Cluster of Orthologous Groups), which is an uncharacterized conserved domain among anaerobic bacteria [7], to which NifX and NafY (dinitrogenase iron-molybdenum accessory cofactors) also belong to [8,9]. In fact, these genes are often annotated as putative dinitrogenase iron-molybdenum accessory cofactors. Although, these proteins have a low sequence homology between each other, they share a common fold of the ribonuclease H superfamily, a

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Dgi_1972    MSAIRIAVPSALPGGLDAAVGEHFGHCDIYTIITAQDGAVQAVETLENMPHVQGGCMAPV 60
Sfum_3407   MKSTVVAIPSTHPGGLESALGAHFGHCDLYTLVEVADGKVVKVKTLPNVPHQQGGCMAPV 60
HRM2_4247   ---MKFAIPMAMG-----KLTAHFGHCREFCFVTVENNEITGTEVLEPPAHEPG---VLP 49
Dde_3197    ----MIALAVYGP-----RLAALFESAPECALFLPQGDICTPAGMLPLPAQDML---QRA 48
DVU2107     -MIVHACLASYEG-----RLATLLETASALHFVRLDDAGPHTLHVRPFRPTTPM---HLA 51
                  .:.         :   :  .    :.   .            .

Dgi_1972    NHLASNGVQVLIAGGMGMRPLMGFQQVGITVFHGAGAPSVGHAVQALLEGALR------- 113
Sfum_3407   NHLARHGVQVLIAGGMGMRPLMGFTQVGISVLYGADALTVGDAVKAYLNGSLP------- 113
HRM2_4247   KWLHDHGINIVLAGGMGPKAQELFAEAGVKVVTGAPTESPEVLVKQYLDQSM-------- 101
Dde_3197    SILAGADVTHLLCGGICGCHRRQLSQAGIIVVPWLCG-GVQDIIRAWMHDNLGPHIMPGC 107
DVU2107     ATLLEADTHLLVCGGVCGRWLHTLEAQGVEVIPWLSG-TEQEVLAALAKGTVDELVMPGC 110
              *   .   ::.**:       :   *: *.          :    .  :

Dgi_1972    PFAPEAT---CGGGCS----------- 126
Sfum_3407   QFTNDFT---CGGGGGR---------- 127
HRM2_4247   -VTGENV---CGHDPESPCNH------ 118
Dde_3197    PRIQQPR---CRARQGQTKQTVKRSTT 131
DVU2107     MRRLGTTGGVCARARCRTRRERNGCE- 136
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Fig. 2. Primary sequence alignment of the small proteins containing cysteine residues from the ORP complex. Sequences are identified by their ORF name (see Fig. 1). Alignment was prepared using Clustal W [11]. Asterisks, colons or stops below the sequence indicate identity, high conservation or conservation of the amino acids, respectively. In grey are highlighted the cysteine residues.