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Functional and Structural Role of Amino Acid Residues in the Odd-numbered Transmembrane α-Helices of the Bovine Mitochondrial Oxoglutarate Carrier

Anna R. Cappello[1,2], Daniela V. Miniero[1], Rosita Curcio[1] Anna Ludovico[1], Lucia Daddabbo[1], Italo Stipani[1,3], Alan J. Robinson[4] Edmund R. S. Kunji[4]* and Ferdinando Palmieri[1,3]*

[1]*Department of Pharmaco-Biology, Laboratory of Biochemistry and Molecular Biology, University of Bari Via E. Orabona 4, 70125 Bari Italy*

[2]*Department of Pharmaco-Biology, University of Calabria Via P. Bucci, 87036 Cosenza Italy*

[3]*CNR Institute of Biomembranes and Bioenergetics, Via E. Orabona 4, 70125 Bari, Italy*

[4]*The Medical Research Council Dunn Human Nutrition Unit Hills Road, CB2 2XY Cambridge, UK*

**Corresponding authors*

The mitochondrial oxoglutarate carrier (OGC) plays an important role in the malate-aspartate shuttle, the oxoglutarate-isocitrate shuttle and gluconeogenesis. To establish amino acid residues that are important for function, each residue in the transmembrane α-helices H1, H3 and H5 was replaced systematically by a cysteine in a fully functional mutant carrier that was devoid of cysteine residues. The transport activity of the mutant carriers was measured in the presence and absence of sulfhydryl reagents. The observed effects were rationalized by using a comparative structural model of the OGC. Most of the residues that are critical for function are found at the bottom of the cavity and they belong to the signature motifs P-X-[DE]-X-X-[KR] that form a network of three inter-helical salt bridges that close the carrier at the matrix side. The OGC deviates from most other carriers, because it has a conserved leucine (L144) rather than a positively charged residue in the signature motif of the second repeat and thus the salt bridge network is lacking one salt bridge. Incomplete salt-bridge networks due to hydrophobic, aromatic or polar substitutions are observed in other dicarboxylate, phosphate and adenine nucleotide transporters. The interaction between the carrier and the substrate has to provide the activation energy to trigger the re-arrangement of the salt-bridge network and other structural changes required for substrate translocation. For substrates such as malate, which has only two carboxylic and one hydroxyl group, a reduction in the number of salt bridges in the network may be required to lower the energy barrier for translocation. Another group of key residues, consisting of T36, A134, and T233, is close to the putative substrate binding site and substitutions or modifications of these residues may interfere with substrate binding and ion coupling. Residues G32, A35, Q40, G130, G133, A134, G230, and S237 are potentially engaged in inter-helical interactions and they may be involved in the movements of the α-helices during translocation.

Keywords: cysteine scanning mutagenesis; membrane transport proteins; mitochondria; oxoglutarate carrier; structural modeling

Introduction

The mitochondrial oxoglutarate carrier (OGC) exchanges cytosolic malate for 2-oxoglutarate from the mitochondrial matrix. The OGC belongs to a large family of related transport proteins called the mitochondrial transporter or carrier family.[1–8] The main structural fold is a six α-helical bundle with 3-fold pseudo-symmetry,[9,10] which is reflected in the

Abbreviations used: MTSES, sodium(2-sulfonatoethyl)-methanethiosulfonate; MTSET, [2-(trimethylammonium)-ethyl]methanethiosulfonate bromide; OGC, oxoglutarate carrier; C-less OGC, cysteine-less OGC.

E-mail addresses of the corresponding authors: ek@mrc-dunn.cam.ac.uk; fpalm@farmbiol.uniba.it

three homologous amino acid sequence repeats[11] identified in the first amino acid sequence of the ADP/ATP carrier.[12] A characteristic amino acid signature motif, typically P-X-[DE]-X-X-[RK], is usually present in all three repeats and in all members of the family (PROSITE PS50920 and PFAM PF00153). The proline residues of the signature motif induce sharp kinks in the odd-numbered α-helices, closing the central pore of the α-helical bundle at the matrix side, whereas the charged residues form a salt bridge network at the bottom of the cavity.[10] Some residues of the signature motif have been shown to be important for function in the ADP/ATP carrier,[13–15] the phosphate carrier[16,17] and the citrate carrier.[18]

The structure of the mitochondrial ADP/ATP carrier in the cytoplasmic state is known,[10] but the structural changes required for the translocation of the substrate are not. The significant sequence conservation in the mitochondrial carrier family suggests that the main structural fold is similar for all carriers and that the specific recognition of substrates is coupled to a common structural mechanism of transport. By using comparative models of carriers with known substrate specificity, a common substrate binding site was identified by correlating the chemical properties of a substrate to a site with the correct chemistry and geometry to bind the substrate.[19] The substrate binding site is at the midpoint of the membrane and close to the salt bridge network at the bottom of the cavity. The translocation could be triggered by substrate-induced rearrangement of the salt bridge network from inter to intra-domain interactions.[15,20] In our earlier study of the even-numbered transmembrane α-helices of OGC, residues R90, R190 and R288 that are in the proposed substrate binding site were found to be critical for function.[21] Another set of important residues in the even-numbered α-helices were found in the conserved [YWLF]-[KR]-G-X-X-P sequence motif, which may fulfill a structural role as a helix breaker or a dynamic role as a hinge region for conformational changes during substrate translocation.

Here, cysteine-scanning mutagenesis was applied to identify residues of the transmembrane α-helices H1, H3 and H5 of OGC that are important for function. This is the first time that a systematic study into the functional importance of the network and its environment has been undertaken. The results are interpreted by using a comparative model of OGC[21] based on the structure of the bovine AAC1.[10] Labeling studies with the cysteine-reactive reagents sodium(2-sulfonatoethyl)-methanethiosulfonate (MTSES) and [2-(trimethylammonium)ethyl]methanethiosulfonate bromide (MTSET) were carried out to establish residue accessibility and the effect of the sulfhydryl modifications on transport activity. Residues in the direct neighborhood of the putative substrate binding site cannot be replaced by cysteine residues or be modified subsequently without loss of carrier activity, indicating that the structural and geometric context is important for function. Residues that form the bottom of the cavity in the cytoplasmic state of the carrier are virtually all conserved and important for transport, highlighting the importance of this area for function. The majority of these residues belong to the signature motifs that form the salt-bridge network. The strictly conserved hydrophobic residue L144 in the signature motif of OGC H3 cannot be replaced by a C, K, R, I or V without loss of carrier activity, indicating a possible relationship between the number of salt-bridges involved in substrate binding and the number of salt bridges in the network. This observation supports the hypothesis that substrate binding and rearrangement of the salt bridge network are related events.

Results

Transport activity of reconstituted single-cysteine mutant OGC

The amino acid sequence conservation of the odd-numbered transmembrane α-helices of OGC was lower than that of the even-numbered transmembrane α-helices in the subfamily of mitochondrial oxoglutarate carriers. The percentage of strictly conserved amino acid residues was 50%, 32% and 42% for transmembrane α-helices H1, H3 and H5 compared to 52%, 63% and 79% for transmembrane α-helices H2, H4 and H6, respectively. The strictly conserved amino acid residues of the odd-numbered transmembrane α-helices were largely confined to those lining the cavity as was the case for the even-numbered α-helices.[21] The overall identities are low because the strictly conserved residues are mainly on one face of the helix, which is less than one quarter of the total surface. Conservation in the odd-numbered α-helices is biased towards the bottom of the cavity where the salt bridge network is situated, whereas the even-numbered α-helices are conserved throughout. Similarly, most conserved residues of the odd-numbered α-helices that interact with the lipid bilayer belong to the signature motif with few exceptions (Figure 1).

Figure 1. Conservation of amino acid residues in the odd-numbered α-helices of oxoglutarate carriers. The structural model of bovine OGC is shown in cartoon (a) and surface representation (b) with a view into the cavity from the cytoplasmic side (first panel) and lateral views from opposite sides (second and third panel). Amino acid positions that are strictly conserved within the set of oxoglutarate carriers are colored orange, those that have two or three amino acid substitutions yellow and those with four and more substitutions green. The loop regions, matrix α-helices and the even-numbered transmembrane α-helices are colored in wheat. The strictly conserved residues are also labeled with their one-letter amino acid code and their residue number. The nomenclature for the α-helices was taken from Pebay-Peyroula *et al.*[10]