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AUTHOR'S VIEW

Structure and function of a monocarboxylate transporter homolog specific for *L*-lactate

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ABSTRACT

Monocarboxylate transporters play important roles in certain cancers. We have reported structures of an *L*-lactate-transporting solute carrier family 16 homolog with bound substrate and inhibitor. The structures show the transporter in the pharmacologically relevant outward-open conformation. Structure–function analysis provides insights into the molecular working mechanisms of ligand binding and *L*-lactate transport.

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The monocarboxylate *L*-lactate is generated during anaerobic carbohydrate breakdown and serves as fuel or building block in different cell types. Certain monocarboxylate transporters (MCTs) of the solute carrier 16 (SLC16) family mediate stereospecific *L*-lactate transport across cell membranes.[1] Among the 14 mammalian SLC16 family members, MCT1-4 were identified as *L*-lactate:proton symporters, which are also able to transport other monocarboxylic metabolites such as pyruvate and ketone bodies.[1] Other members of the SLC16 solute carrier family encode high-affinity thyroid hormone (MCT8, *SLC16A2*) or aromatic amino acid (MCT10, *SLC16A10*) transporters.[2] The *L*-lactate transporters MCT1 and MCT4 form heterodimers with the glycoproteins embigin (also known as GP70) or basigin (also known as CD147) that are essential for correct surface plasma membrane expression.[3,4]

MCT1 (*SLC16A1*) is expressed in almost all tissues and transports *L*-lactate into cells with a Michaelis constant (K_M) of 3–5 mM.[1] MCT2 (*SLC16A7*) is mainly located in the brain, kidney, liver, and testis, where it mediates inward-directed *L*-lactate transport with a K_M of ~0.7 mM.[1] In the retinal pigment epithelium and in the choroid plexus epithelia, *L*-lactate is exported by MCT3 (*SLC16A8*, K_M of ~6 mM[5]). MCT4 (*SLC16A3*) is overexpressed in highly glycolytic and anaerobic tissues, where the transcription of its gene is regulated by the hypoxia-inducible factor 1α.[1] These cells produce large amounts of *L*-lactate, which must be efficiently exported by MCT4 (K_M of 20–35 mM).[1]

MCTs 1 and 4 play important and complementary roles in cancer metabolism. Due to metabolic disorder, certain tumor cells cover their demand for ATP by increasing the rate of glycolysis even under aerobic conditions ("Warburg effect"[6]). The glycolytic phenotype is maintained if pyruvate is reduced to *L*-lactate, thereby regenerating NAD^+, which is utilized during glycolysis. MCT4 exports the produced *L*-lactate together with a proton and prevents its accumulation and cellular acidification. In tumors, the acidification of the extracellular microenvironment resulting from co-transported protons promotes angiogenesis, invasion, metastasis, and tumor cell proliferation.[6] Exported *L*-lactate can enter other cancer cells via MCT1 to serve as fuel for proliferating cells.[7] Consequently, MCT1 and MCT4 are promising drug targets for treating certain tumors because their inhibition compromises important metabolic processes. This fact is highlighted by clinical trials involving an MCT1 inhibitor (see AZD3965 at http://www.clinicaltrials.gov) and a recent study that identified an approved antihypertension drug (syrosingopine) as MCT4 inhibitor.[8]

The structure of an *L*-lactate transporter of the SLC16 family would enable us to understand the working mechanism of this class of membrane proteins at a molecular level. Furthermore, high-quality experimental structures are essential to build reliable homology models, which can be used for structure-based design of specific *L*-lactate transport inhibitors and to perform meaningful *in silico* ligand docking. For this reason, an ambitious structural biology project was launched in the Fotiadis laboratory (http://www.fotiadislab.com) in 2012 with the goal to identify an *L*-lactate-transporting member of the SLC16 family that can be expressed in large quantities, homogenously purified, and crystallized for structure solution by X-ray crystallography. As a result, we have recently published the first high-resolution structures of an *L*-lactate-transporting SLC16 family homolog from *Syntrophobacter fumaroxidans* (SfMCT) with bound substrate (i.e. *L*-lactate) and inhibitor (i.e. thiosalicylate) (Figure 1).[9] SfMCT contains 12 transmembrane helices (TMs), and it adopts the characteristic fold of membrane proteins belonging to the major facilitator

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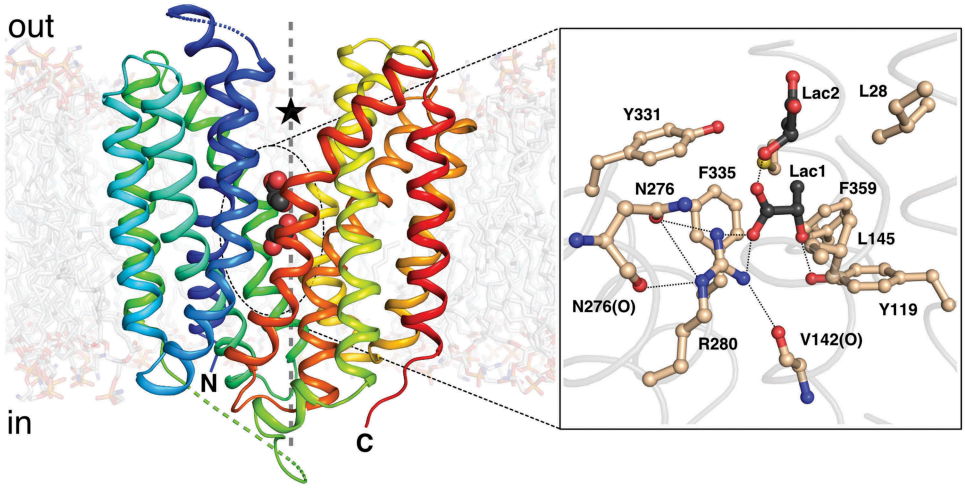

Figure 1. Structure of the *L*-lactate-transporting solute carrier 16 family homolog SfMCT (*Syntrophobacter fumaroxidans* monocarboxylate transporter). The outward-open conformation of the transporter is apparent when viewed in the plane of the membrane (*left*). The lipid membrane was computed using CHARMM (http://www.charmm-gui.org). The two *L*-lactate molecules bound to SfMCT are displayed in black as space-filling models. The central, conical cavity that is open to the periplasmic side (out) is highlighted by the asterisk, and the dark grey, vertical broken line represents the pseudo-twofold symmetry axis of SfMCT. Amino- and carboxy-termini are labeled by "N" and "C". Parts of the loops connecting transmembrane helices (TM) 1 and TM2, TM5 and TM6 as well as TM6 and TM7 could not be fully traced and are therefore indicated by broken lines. The model of SfMCT is colored based on the rainbow coloring scheme from blue (amino-terminus) to red (carboxy-terminus). Residues that are involved in the binding of *L*-lactate to SfMCT are shown in beige and are labeled by numbered one-letter codes (*right*). Interactions with the main-chain carbonyl atoms are additionally labeled with (O). Two *L*-lactate molecules (Lac1 and Lac2) are found in the binding pocket and are colored in black. Dotted lines represent hydrogen bonds.

superfamily. The helices are arranged in 2 six-helix bundles that are related to each other by a pseudo-twofold symmetry axis (Figure 1, *left*, broken gray line), which is perpendicular to the membrane plane. Our structures show SfMCT in an outward-open conformation where the substrate-binding site is accessible from the extracellular side (Figure 1, *left*, highlighted by an asterisk). Residues that are involved in substrate and inhibitor binding (Figure 1, *right*) were identified based on our structural data and extensive functional characterization. A conserved arginine residue in transmembrane helix 8 (R280) is essential for the binding of the carboxylate group of substrates or inhibitors. Removal of the positively charged guanidinium group leads to an inactive SfMCT variant. The same transport deficiency has been observed if the corresponding arginine was mutated in human MCT1, which is associated with ketoacidosis.[10] We have also identified residues in the binding pocket that seem to be involved in regulating the substrate specificity and transport efficiency in SfMCT. Furthermore, a hypothesis was provided on how protons that are co-transported with lactate are captured by proton binding site residues. From a pharmacological perspective, the observed outward-open conformation is relevant for the future design of transport inhibitors using SfMCT-based homology models of human MCT1 and MCT4.

In summary, we have identified a proton-coupled and *L*-lactate-specific homolog of the SLC16 family, which is an appropriate model for studying human MCT1-4. The structural and functional data have provided important insights into the molecular working mechanism of *L*-lactate transporters.

Disclosure of potential conflicts of interest

There are no potential conflicts of interest to disclose.

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