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Review

The ergothioneine transporter controls and indicates ergothioneine activity — A review

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ABSTRACT

Ergothioneine (ET) is a natural compound which humans and other vertebrates cannot synthesize themselves; it must be absorbed from food in which it is distributed very unevenly. In general, ET is considered an intracellular antioxidant. However, the precise physiological purpose of ET and the consequences of ET deficiency are still unclear. The ergothioneine transporter ETT (old name OCTN1; human gene symbol *SLC22A4*) is a powerful and highly specific transporter for the uptake of ET. Cells lacking ETT do not accumulate ET, since the plasma membrane is virtually impermeable for this hydrophilic zwitterion compound. The existence of an evolutionary conserved ergothioneine transporter implies a beneficial role for ET. ETT is the first and so far only biomarker of ET activity. Only cells with strong expression of ETT can accumulate ET to high levels. In the human body, the ability to absorb, distribute, and retain ET depends entirely on this transporter. Blockade or inactivation of ETT in animal models may be essential to at last understand the function of ET. In this review of ETT, the focus is on substrate specificity, subcellular localization, human expression profile and expression profiles across species.

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Introduction

Ergothioneine (ET) is a natural compound which humans and other vertebrates cannot synthesize themselves; it must be absorbed from food in which it is distributed very unevenly. The best known dietary sources of ET are mushrooms (0.1–1 mg/g dried material) (Melville, 1958) and cyanobacteria (Pfeiffer et al., 2011). After ingestion, ET is rapidly cleared from the circulation and then avidly retained in the body with minimal metabolism. In general, ET is considered as an intracellular antioxidant. However, the precise physiological purpose of ET and the consequences of ET deficiency are still unclear.

Chemically, ET is the betaine of histidine with a sulfur atom attached to the imidazole ring. It should not be considered a thiol compound, but rather a thione, a derivative of thiourea (Hartman, 1990). As a consequence of the prevailing thione tautomer, ET has some properties markedly different from glutathione (GSH); for example, ET does not autoxidize at physiological pH and does not promote the generation of hydroxyl radical from H_2O_2 and Fe^{2+} ions (Fenton reaction) (Chaudiere and Ferrari-Iliou, 1999).

Abbreviations: ET, ergothioneine; ETT, ergothioneine transporter; LC, liquid chromatography; MS, mass spectrometry.

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A few years ago, we have discovered an ET transporter (ETT; human gene symbol *SLC22A4*) (Gründemann et al., 2005). Cells lacking ETT do not accumulate ET, since the plasma membrane is virtually impermeable for this hydrophilic zwitterion compound. By contrast, cells with expression of ETT accumulate ET to high levels. The function of ETT has been confirmed by others in vitro (Nakamura et al., 2008; Paul and Snyder, 2010) and in vivo (Kato et al., 2010).

Much interest in ETT has been generated by case–control studies that suggest an association of polymorphisms in the *SLC22A4* gene with susceptibility to chronic inflammatory diseases such as Crohn's disease (Fisher et al., 2006; Lin et al., 2010; Peltekova et al., 2004), ulcerative colitis (Waller et al., 2006) and Type I diabetes (Santiago et al., 2006). It is presently unknown how the mutation in the transporter gene promotes disease.

The aim of this review was to compile current knowledge about major features of the ergothioneine transporter ETT.

Transporter structure

ETT is a protein that is permanently and thoroughly integrated into the cell membrane. As a member of the *SLC22* family of transport proteins, it is expected both from hydropathy analysis and by analogy to distantly related bacterial transport proteins for whom crystal structures have been resolved to contain a core of 12 alpha-helical transmembrane segments. However, the exact 3D structure is unknown. The amino acid sequences of mammalian ETT orthologues are highly conserved (human and rat: 85.5% identity, 89.1% similarity). Mammalian ETTs are made of 551 or 553 amino acids. Structural features include a large extracellular loop with multiple potential N-glycosylation sites between transmembrane segments 1 and 2, and multiple potential intracellular phosphorylation sites. Both N- and C-termini are probably located in the cytosol.

Substrate specificity

The substrate specificity is key to understanding the physiological and pathophysiological significance of a transporter. In order to discriminate good and poor substrates, one must determine the transport efficiency (TE), which is defined, analogous to catalytic efficiency for enzymes, by k_{cat}/K_m. This ratio takes into account turnover number (k_{cat}) and affinity (K_m) of a carrier for a particular substrate. It is very difficult to determine turnover numbers; we can, however, determine TE from v/S (v, velocity of uptake; S, substrate concentration) or from V_{max}/K_m, which is a measure directly proportional to k_{cat}/K_m (since $V_{max} = k_{cat} * T_{total}$) (Schömig et al., 2006). Note that the TE is completely unrelated to signal-to-noise ratio in uptake assays (Schömig et al., 2006). A comparison of TEs can provide important insights. Our basic and simple assumption is that because of evolutionary benefit, relevant substrates are transported with high efficiency. For physiological substrates, our experience suggests that TE should be on the order of 50 μl min^{-1} mg $protein^{-1}$ or higher in mammalian cell lines with regular transporter overexpression based on strong viral promoters. If the TE is below 1 μl min^{-1} mg $protein^{-1}$, then the substrate is probably irrelevant.

ETT at first was called "novel organic cation transporter" (OCTN1), and tetraethylammonium (TEA) was proposed as substrate (Tamai et al., 1997; Yabuuchi et al., 1999). Because of the high sequence homology with the carnitine transporter CTT (old name OCTN2; gene symbol *SLC22A5*), the carrier was also considered another carnitine transporter (Tamai et al., 1998, 2000). However, in our experiments, using heterologous expression of the transporter cDNA in 293 cells, a human cell line, the TEs were consistently very low for both TEA (0.8 μl min^{-1} mg $protein^{-1}$) and carnitine (0.6 μl min^{-1} mg $protein^{-1}$). This stimulated a search for the physiological substrate. A substrate lead, stachydrine *alias* proline betaine, was discovered by LC–MS Difference Shading (Gründemann et al., 2005). Since the TE of stachydrine (approximately 20 μl min^{-1} mg $protein^{-1}$) still was not as high as expected for a relevant substrate, we screened related compounds until we found ergothioneine.

ET is by far the best known substrate of ETT (TE range 70–195 μl min^{-1} mg $protein^{-1}$). A major contribution to the high TE comes from the small K_m of about 20 μmol/l (Gründemann et al., 2005). This indicates high affinity for a transport protein. Most importantly, ET is transported together with Na^+ ions (Gründemann et al., 2005). The Na^+ gradient across the plasma membrane (there is >10 times more extracellular Na^+ than intracellular) provides a strong driving force for uptake of ET into cells. Indeed, with ETT expressed in 293 cells, at 10 μmol/l extracellular ET we have determined at equilibrium 850 μmol/l intracellular ET. Because of low intracellular Na^+, ETT does not catalyze significant efflux of ET (Gründemann et al., 2005). The close relative of ETT, the carnitine transporter CTT (amino acid sequence homology among human carriers: 77% identity, 82% similarity) also catalyzes cotransport of its substrate with Na^+. Note that both carriers discriminate their substrates precisely, *i.e.* ETT does not transport CT, and CTT does not transport ET (Bacher et al., 2009).

Other groups have reported several compounds unrelated in structure to ET as substrates of ETT, *e.g.* quinidine, verapamil, pyrilamine; however, we could not detect significant uptake via ETT with those (Grigat et al., 2007). Moreover, even the ET biosynthesis precursor hercynine (which lacks only the sulfur of ET) and the widely-used antithyroid drug methimazole (an imidazole-2-thione like ET) were transported with very low efficiency (Grigat et al., 2007). Collectively, ETT is a powerful and highly specific uptake transporter of ergothioneine.

Subcellular localization

On a subcellular level, the principal site of ETT expression is the plasma membrane, *i.e.* the cell envelope. This assignment is directly supported, for example, by immunohistochemistry in mouse kidney, where ETT was detected with a polyclonal antibody at the apical membrane of proximal tubular epithelial cells (Tamai et al., 2004). At this site ETT can salvage ET from urine.

It must be noted that Lamhonwah and Tein have suggested that ETT is located primarily in mitochondrial membranes (Lamhonwah and Tein, 2006). Most of their data were generated with a fusion of eGFP to the N-terminus of ETT from human. Close scrutiny of their cDNA construction methods has revealed that in the fusion construct the transporter lacks 34 original amino acids at the N-terminus (including 13 out of 21 amino acids of transmembrane segment 1); at the C-terminus, it contains 36 non-natural amino acids. Because of these severe aberrations, it is very likely that the reported results are invalid. Indeed, fluorescence of eGFP attached to the C-terminus of the entirely original ETT was observed in the plasma membrane only (Bacher et al., 2009).

Recently we have shown by real-time RT-PCR that ETT mRNA from pig is highly abundant in boar seminal vesicles (Nikodemus et al., 2011). We assume that in these glandular cells ETT is located in the basolateral membrane to catalyze sodium-driven uptake of ET from blood. Apical exit into the seminal fluid probably occurs by non-specific apocrine secretion. Thus, depending on the context, ETT may be located in apical (kidney, intestine) or basolateral (seminal vesicles) plasma membrane compartments.

Human expression profile

All established places of strong ET accumulation (Melville, 1958) like developing red blood cells (all mammalia) and seminal vesicles (pig) express ETT. In reverse, cells with strong expression of ETT will accumulate as much ET as is available via blood from food. In this sense ETT is the first and so far only indicator or biomarker of