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Review

Molecular mechanisms of reduced glutathione transport: role of the MRP/CFTR/ABCC and OATP/SLC21A families of membrane proteins

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Abstract

The initial step in reduced glutathione (GSH) turnover in all mammalian cells is its transport across the plasma membrane into the extracellular space; however, the mechanisms of GSH transport are not clearly defined. GSH export is required for the delivery of its constituent amino acids to other tissues, detoxification of drugs, metals, and other reactive compounds of both endogenous and exogenous origin, protection against oxidant stress, and secretion of hepatic bile. Recent studies indicate that some members of the multidrug resistance-associated protein (MRP/CFTR or ABCC) family of ATP-binding cassette (ABC) proteins, as well as some members of the organic anion transporting polypeptide (OATP or SLC21A) family of transporters contribute to this process. In particular, five of the 12 members of the MRP/CFTR family appear to mediate GSH export from cells namely, MRP1, MRP2, MRP4, MRP5, and CFTR. Additionally, two members of the OATP family, rat Oatp1 and Oatp2, have been identified as GSH transporters. For the Oatp1 transporter, efflux of GSH may provide the driving force for the uptake of extracellular substrates. In humans, OATP-B and OATP8 do not appear to transport GSH; however, other members of this family have yet to be characterized in regards to GSH transport. In yeast, the ABC proteins Ycf1p and Bpt1p transport GSH from the cytosol into the vacuole, whereas Hgt1p mediates GSH uptake across the plasma membrane. Because transport is a key step in GSH homeostasis and is intimately linked to its biological functions, GSH export proteins are likely to modulate essential cellular functions.

Keywords: Glutathione; Transport; MRP; CFTR; ABCC; OATP; SLC21A; Membrane transporters

Contents

Abbreviations: ABC, ATP-binding cassette; Bpt1p, bile pigment transporter-1; BSO, L-buthionine (*S,R*)-sulfoximine; CFTR, cystic fibrosis transmembrane conductance regulator; DTY167, yeast strain lacking functional Ycf1p; EHBR, Sprague–Dawley Eisai hyperbilirubinuric rat, mutant rat strain that lacks Mrp2 activity; GY, Groningen Yellow rat, Wistar rat strain that lacks Mrp2 activity; GSH, reduced glutathione; GSSG, glutathione disulfide; Hgt1p, high affinity glutathione transporter in yeast; MDR1, multidrug resistance protein 1; MRP and Mrp, multidrug resistance-associated protein; MSD, membrane spanning domain; NBD, nucleotide binding domain; OATP and Oatp, organic anion transporting polypeptide; Pgt, prostaglandin transporter; SUR, sulfonylurea receptor; TR-, transport deficient rat, Wistar rat strain that lacks Mrp2 activity; Ycf1p, yeast cadmium factor-1.

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Introduction

Reduced glutathione (GSH) plays a central role in a multitude of biochemical processes, and disturbances in its homeostasis are implicated in the etiology and progression of a number of diseases. GSH is required for proper protein and DNA synthesis, cell cycle regulation, thermotolerance, exocrine secretion, maintenance, and regulation of the thiol-redox status of the cell, protection against oxidative damage, detoxification of endogenous and exogenous reactive metals and electrophiles, biosynthesis of mercapturic acids (*S*-substituted *N*-acetyl-L-cysteines), and storage and transport of cysteine (DeLeve and Kaplowitz, 1990; Meister, 1984; Meister and Anderson, 1983; Wang and Ballatori, 1998). Additional important roles for this tripeptide include the regulation of gene expression, apoptosis, and membrane transport of both endogenous and exogenous molecules (Hammond et al., 2001).

The synthesis and catabolism of GSH and its adducts occurs by a regulated series of enzymatic and plasma membrane transport steps that are collectively referred to as the γ-glutamyl cycle (Meister and Anderson, 1983; Meister and Tate, 1976). GSH is synthesized in every cell of the body, but the liver is quantitatively the major site of synthesis (DeLeve and Kaplowitz, 1990; Hahn et al., 1978; Lauterburg et al., 1984). GSH is synthesized intracellularly from its precursor amino acids by the ATP-requiring cytosolic enzymes γ-glutamylcysteine synthetase and GSH synthetase. Within the cell, it exists mainly (>98%) in the thiol-reduced form (GSH), but some are also present in the thiol-oxidized (GSSG), thioether, mercaptide, or other thioester forms (glutathione *S*-conjugates). After its synthesis, GSH is delivered to other intracellular compartments, including mitochondria and endoplasmic reticulum, and to the extracellular space (e.g., blood plasma and bile) for utilization by other cells and tissues.

In contrast to GSH synthesis which occurs intracellularly, GSH degradation occurs exclusively in the extracellular space, and only on the surface of cells that express the ectoenzyme γ-glutamyl transpeptidase (also called γ-glutamyl transferase or γGT). γ-Glutamyl transpeptidase, which is abundant on the apical surface of most transporting epithelia, including liver canalicular and bile ductular membranes, is the only enzyme that can initiate catabolism of GSH, glutathione *S*-conjugates, and glutathione-complexes under physiological conditions. Because γ-glutamyl transpeptidase is a plasma membrane-bound enzyme with its active site on the extracellular surface of the membrane, export of GSH and its adducts into the extracellular space is the initial, and presumably regulated step in their turnover in all mammalian cells.

Despite the importance of this transport step, relatively little is known at the molecular level about GSH transporters. The paucity of information is explained in large part by a number of practical and theoretical limitations that have hampered the functional and molecular characterization of GSH transporters. The first factor is the relative difficulty of the study of efflux transporters when compared to the study of uptake transporters. To date, we know relatively little about the proteins that mediate export out of cells (other than the ABC proteins), whereas we know a great deal about uptake transporters (Borst and Elferink, 2002; Gao and Meier, 2001; Meier and Stieger, 2002; Sekine et al., 2000; Suzuki and Sugiyama, 2000). The major limitations of efflux studies are that it is often impossible to determine intracellular concentrations (or more precisely, chemical activities) of substrates and inhibitors, and that it is difficult to load or alter intracellular substrate or inhibitor concentrations without affecting other cellular functions. These problems are particularly acute in the characterization of GSH transport, given the many roles that this tripeptide plays in cellular functions. Thus, it is difficult to manipulate GSH levels without indirectly affecting a myriad of other biochemical pathways and physiological functions that are regulated by this tripeptide. The use of isolated plasma membrane vesicles overcomes some of the limitations; however, directionality of transport is lost for all secondary-active transporters when using vesicle systems. Moreover, membrane vesicle preparations usually contain both inside-out and right side-out vesicles, further confounding interpretation of results.

Second, GSH transporters exhibit low catalytic efficiency (Ballatori and Dutczak, 1994; Paulusma et al., 1999; Rebbeor et al., 1998a, 1998b, 2000, 2002). That is, the apparent affinity values (K_m values) for GSH exporters are relatively high and the transport velocities (V_{max} values) are only moderate, leading to a low V_{max}/K_m ratio, a measure of catalytic efficiency. The high K_m values are not unexpected given that GSH is present in high concentrations within cells (1–10 mM), but they pose a severe problem in measuring transport rates in intact cells or in membrane vesicles.