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Characterization of sulfate transport in the hepatic endoplasmic reticulum

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Abstract

The transport of sulfate ion across the endoplasmic reticulum membrane was investigated using rapid filtration and light scattering assays. We found a protein-mediated, bi-directional, low-affinity, and high-capacity, facilitative sulfate transport in rat liver microsomes, which could be inhibited by the prototypical anion transport inhibitor, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid. It was resistant to various phosphate transport inhibitors and was not influenced by high concentration of phosphate or pyrophosphate, which is contradictory to involvement of phosphate transporters. It was sensitive to S3483 that has been reported to inhibit the glucose 6-phosphate transporter (G6PT), but the weak competition between sulfate and glucose 6-phosphate did not confirm the participation of this transporter. Moreover, the comparison of the activity and S3483 sensitivity of sulfate transport in microsomes prepared from G6PT-overexpressing or wild type COS-7 cells did not show any significant difference. Our results indicate that sulfate fluxes in the endoplasmic reticulum are mediated by a novel, S3483-sensitive transport pathway(s).

Keywords: Sulfate transport; Endoplasmic reticulum; Liver; Steroid sulfatase; Facilitated diffusion; Glucose 6-phosphate transporter; Microsome; Phosphate transport; Rapid filtration; Light scattering

Sulfate is one of the major anions in the human body. Its activated form 3′-phosphoadenosine 5′-phosphosulfate (PAPS)[1] is utilized by sulfotransferase isoenzymes (SULTs) to synthesize various organic sulfate esters that represent a remarkable intracellular pool of sulfate [1]. Accordingly, hydrolysis of these esters by sulfatase enzymes is one of the major sulfate-producing reactions in the cells. Steroid sulfatase (STS)—also called arylsulfatase C (ARSC) or estrone sulfatase (ES)—a member of the aryl sulfatase enzyme superfamily, is an integral membrane protein of the endoplasmic reticulum (ER) [2]. It has a mushroom-like tertiary structure with a transmembrane stem and a luminal cap that contains the catalytic site near the internal surface of the ER membrane [3]. The hydrophobic deconjugated products of STS (e.g., steroid hormones) and even the sulfated substrates can probably cross the membrane through a hydrophobic tunnel formed by the two transmembrane helices of the enzyme. However, this route is impassable for the charged and hydrophilic sulfate anion, which hence needs

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[1] *Abbreviations used:* PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SULT, sulfotransferase; STS, steroid sulfatase; ARSC, arylsulfatase C; ES, estrone sulfatase; ER, endoplasmic reticulum; DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; PhPA, phenyl-phosphonate; PFA, phosphono-formate; HNBA, 2-hydroxy-5-nitro-benzaldehyde; MOPS, 4-morpholinepropanesulfonic acid; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid); PIPES, piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); G6PT, glucose 6-phosphate transporter.

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to leave the active site through the "swing doors" also depicted in the model. Consequently, sulfate is liberated by STS in the lumen of the ER and the means by which it exits to the cytosol remains to be clarified.

Due to its hydrophilic nature, sulfate anion cannot diffuse through lipid bilayers; thus, its transmembrane fluxes need to be facilitated by anion transporter proteins. The sulfate transporters in the plasma membrane of various cell types have been extensively studied and many of them are characterized at molecular level [4]. Among the intracellular organelles, transmembrane sulfate traffic has only been evidenced in mitochondria [5] and lysosomes [6]. However, the recent elucidation of the topology of STS suggests a luminal sulfate production that highlighted the importance of the yet unexplored sulfate transport across the ER membrane.

The aim of the present study was to investigate sulfate transport pathways in ER-derived microsomal vesicles. The structural similarities between sulfate and phosphate suggested that they might share a common transporter; therefore, we hypothesized the involvement of the previously reported phosphate or glucose 6-phosphate transporters [7]. However, our investigation revealed a sulfate transport activity with kinetic characteristics, and inhibitor profile distinct from the known sulfate transporters and the known ER anion transporters.

Materials and methods

Materials

Sodium sulfate, alamethicin, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), phenyl-phosphonate (PhPA), phosphono-formate (PFA), 2-hydroxy-5-nitro-benzaldehyde (HNBA), 4-morpholinepropanesulfonic acid (MOPS), *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (Hepes), and piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) were purchased from Sigma–Aldrich Chemical Co. [^{35}S]Na_2SO_4 was obtained from Perkin-Elmer Life and Analytical Sciences, and [^{14}C]glucose 6-phosphate from MP Biomedicals. S3483 was kindly supplied by Aventis Pharma. All other reagents were of analytical grade.

Transient expression of G6PT in COS-7 cells

A vector suitable to express human glucose 6-phosphate transporter (G6PT), containing an N-terminal flag peptide (DYKDDDDK) following the initial Met, was constructed by PCR from the human G6PT cDNA template. The oligonucleotide primers derived from nucleotides 170 to 190 (5′-GC TCT AGA GCC GCC ATG GAC TAC AAG GAC GAC GAT GAC AAG GCA GCC CAG GGC TAT GGC-3′, sense) and nucleotides 1439–1459 (5′-GGG GTA CCG TCA CTC AGC CTT CTT GGA CAC-3′, antisense) of the template were used. The sense primer was modified to include 24 nucleotides encoding the flag epitope and a restriction site for *Xba*I, while the antisense primer included the restriction site for *Kpn*I. The cDNA obtained was subjected to a double digestion with *Xba*I and *Kpn*I and cloned in the expression vector pShuttle (Clontech). The construct obtained was verified by DNA sequencing. COS-7 cells were grown in DMEM medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum, 100 IU/ml penicillin, 100 μg/ml streptomycin, and L-glutamine (2 mM) at 37 °C in a humidified atmosphere of 5% CO_2, and 95% air. The G6PT-pShuttle construct was transfected into COS-7 cells by the lipofectamine method (Lipofectamine 2000, Invitrogen). The presence of G6PT in COS-7 microsomes was proven with Western blot analysis using 12% polyacrylamide–SDS gel, nitrocellulose membrane (Amersham Biosciences), rabbit anti-G6PT antibody, and horseradish peroxidase-conjugated donkey anti-rabbit IgG (Amersham Biosciences). The immunocomplex was visualized using the enhanced chemiluminescence (Amersham Biosciences).

Preparation of rat liver microsomal vesicles

Microsomes were prepared from livers of overnight-fasted male Wistar rats (180–230 g), using fractional centrifugation [8]. The ER vesicles were washed and resuspended in MOPS–KCl buffer (100 mM KCl, 20 mM NaCl, 3 mM $MgCl_2$, and 20 mM MOPS, pH 7.0), and then immediately frozen in liquid nitrogen and kept in liquid nitrogen until use (within 2 months). The protein concentration in microsomal samples was determined using the method of Lowry et al. [9] with bovine serum albumin as a standard. The integrity of the microsomal membranes was assessed using the mannose-6-phosphatase assay [10], which showed latency greater than 95%.

Preparation of COS-7 cell microsomes

Cultured COS-7 cells (90% confluence) were harvested by plate scraping and washed with PBS. Cell pellets were resuspended (10%, w/v) in 300 mM sucrose, and 20 mM Hepes (pH 7.2) including a cocktail of protease inhibitors (Complete, Roche). Cell homogenates were obtained using a glass/Teflon homogenizer and centrifuged for 10 min at 10,000*g*. Microsomes were then recovered by ultracentrifugation of the supernatants for 60 min at 150,000*g*. Microsomal fractions were resuspended in MOPS–KCl buffer.

Rapid filtration transport measurements

The influx or efflux of sulfate was determined using a rapid filtration method at 20 °C. In case of influx measurements, the microsomal fractions were resus-