Glutathione Transport in Immortalized HLE Cells and Expression of Transport in HLE Cell Poly(A)⁺ RNA-Injected Xenopus laevis Oocytes

Ram Kannan,¹ Yuzhou Bao,¹ Aravind Mittur,¹ Usha P. Andley,² and Neil Kaplowitz¹

PURPOSE. To determine reduced glutathione (GSH) transport in cultured human lens epithelial cells (HLE-B3) and plasma membrane vesicles and to study the expression of GSH transport in Xenopus laevis oocytes injected with poly(A)⁺ RNA from HLE-B3 cells.

METHODS. Confluent HLE-B3 cells pretreated with 10 mM dl-buthionine sulfoximine and 0.5 mM acivicin were used in GSH uptake studies. The uptake of ⁸⁶⁸GSH was performed for 30 minutes in either NaCl medium (Na⁺-containing) or choline chloride medium (Na⁺-free) at 37°C and 4°C. The molecular form of ³⁵S uptake was determined by high-performance liquid chromatography. GSH uptake kinetics were studied in acivicin and buthionine sulfoximine-treated HLE-B3 cells in NaCl medium in the concentration range 0.01 μM to 50 mM. The transport of GSH and the effect of Na⁺ on uptake also were determined in mixed plasma membrane vesicles from HLE-B3 cells. In oocyte expression studies, HLE-B3 poly(A)⁺ RNA was injected into X. laevis oocytes and GSH uptake experiments were performed 3 days after injection. The uptake of ⁸⁶⁸GSH and GSH efflux rates were determined in HLE-B3 poly(A)⁺ RNA-injected oocytes.

RESULTS. No significant difference was found in the uptake of 1 mM GSH ± acivicin (17.7 ± 4.3 versus 15.7 ± 1.4 picomoles/min⁻¹ per 10⁶ cells). However, GSH uptake was significantly lower in Na⁺-free medium compared with Na⁺-containing medium (10.3 ± 0.7 versus 16.8 ± 0.9 picomoles/min⁻¹ per 10⁶ cells; P < 0.01). GSH uptake in NaCl medium was carrier mediated. GSH uptake showed partial sodium dependency from 5 μM to 5 mM GSH in mixed plasma membrane vesicles from HLE-B3 cells. Oocytes injected with HLE-B3 poly(A)⁺ RNA expressed uptake and efflux of GSH. Uptake showed partial Na⁺ dependency at various GSH concentrations. The efflux rates were approximately 30-fold higher than those in water-injected oocytes (0.48 ± 0.03 versus 0.016 ± 0.005 nanomoles per hour⁻¹ per oocyte, respectively). The molecular form of uptake in cultured cells and in oocyte studies was predominantly as intact GSH.

CONCLUSIONS. HLE-B3 cells and plasma membrane vesicles transported GSH by a carrier-mediated process. HLE-B3 poly(A)⁺ RNA injected X. laevis oocytes expressed GSH transport. GSH uptake was partially Na⁺ dependent in all systems. HLE-B3 cells offer a useful model for characterizing GSH transport and for studying its regulatory role in the etiology of cataracts. (Invest Ophthalmol Vis Sci. 1998;39:1379 -1386)

The role of reduced glutathione (GSH) in protecting the lens from oxidant injury and cataractogenesis is well established.¹ GSH is present in millimolar quantities in the lens, but its levels in the lens decline progressively in the following order: epithelium, outer cortex, inner cortex, and nucleus.² Our interest in the last several years has been to study metabolism of GSH in normal and cataractous lenses. The in situ eye perfusion technique in the guinea pig and expression studies in oocytes injected with bovine and rat lens poly(A)⁺ RNA were used to study GSH transport in the lens.³⁻⁷ Our studies so far have shown that GSH is transported intact into the guinea pig lens by a saturable process. Oocytes injected with poly(A)⁺ RNA from a bovine lens express carrier-mediated GSH transport. The expression studies further reveal that two GSH transport systems are present in the lens. Although GSH transport by the cortical membranes is predominantly by a Na⁺-independent process, the lens epithelium exhibits Na⁺-dependent transport.⁴⁻⁷ In recent in vivo studies, the infusion of supraphysiological concentrations of GSH in guinea pig eye perfusion experiments produces an increase in lenticular GSH, with the epithelial GSH level showing a greater increase than the level in the cortex.⁵ This finding, coupled with the observation that de novo GSH synthesis from constituent amino acids is low,⁶ suggests that GSH transport in the lens may play an important role in the maintenance of cellular GSH. Thus, characterizing the proteins that mediate

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GSH transport in the lens is potentially of considerable physiological and pathophysiological importance.

Recently, the isolation and characterization of primary and immortalized cell cultures of human lens epithelial (HLE) cells was reported. The availability of human lens epithelial cell line has made it possible to undertake studies on effect of oxidant stress such as UVB exposure on HLE cell physiology. The aim of the present study was to investigate whether the HLE cell line of extended life span (HLE-B3) is a suitable model for the characterization and functional evaluation of the recently identified Na⁺-dependent GSH transport system. Therefore, studies on GSH transport were conducted in cultured HLE cells and membrane vesicles and in Xenopus laevis oocytes injected with poly(A)⁺ RNA from HLE-B3 cells.

**METHODS**

**Cultured Cells**

HLE cells in culture were grown by isolating epithelium fragments from infant human lenses from patients who underwent treatment for retinopathy of prematurity and by allowing epithelial cells to grow from explants. To immortalize cells, the cultures were infected with simian virus 40. The protocol for obtaining confluent cultures, serial passages, and characterization of HLE cells has been described previously. Briefly, primary HLE cells were grown in minimum essential medium with glutamine containing 20% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells from passages 8 to 12 were used in all cellular uptake and RNA isolation studies. HepG2 and Cos-1 cells were obtained from American Type Culture Collection (Manassas, VA). HepG2 cells are derived from human hepatoblastomas and retain many of the differentiated features of mature hepatocytes. Cos-1 cells, derived from monkey kidney, exhibit fibroblast-like characteristics. Growth media and culture conditions of HepG2 and Cos-1 cells are as described previously. The total glutathione content of HLE-B3 cells was measured by Tietze assay. Gamma glutamyltranspeptidase (GGT) activity was determined according to the method of Leier et al. with modifications as described below. Approximately 3 × 10⁶ cells were harvested by centrifugation (1200g, 8 minutes, 4°C). The pellet was suspended in 40 ml hypotonic lysis buffer (1 mM sodium bicarbonate, pH 7.0, supplemented with protease inhibitor cocktail P8340 from Sigma Chemical, St. Louis, MO) and was stirred gently at 4°C for 1 hour. The suspension then was centrifuged at 100,000g for 30 minutes at 4°C. The pellet was suspended in 20 ml lysis buffer and was homogenized in a Potter-Elvejhem (S818 pestle, 4°C, 900 rpm, approximately 4 strokes/min). The homogenate was diluted twofold in a buffer containing 520 mM sucrose, 0.4 mM CaCl₂, 10 mM HEPES-Tris, pH 7.4, and subsequently was centrifuged (1200g, 8 minutes, 4°C). The supernatant was diluted with suspension buffer (260 mM sucrose, 0.2 mM CaCl₂, 5 mM HEPES-Tris, pH 7.4) and was centrifuged at 100,000g for 45 minutes at 4°C. The pellet was suspended in 12 to 15 ml suspension buffer and was layered over 8 ml 38% (wt/wt) sucrose. The tubes were spun at 280,000g for 2 hours at 4°C. Mixed plasma membranes at the interface of the 38% layer were pooled, diluted in 40 ml suspension buffer, and centrifuged at 100,000g for 45 minutes at 4°C. The final membrane pellet was suspended in 500 μl suspension buffer, was vesiculated by 20 passages through a 25-gauge needle, and was stored at −80°C. The purity of mixed plasma membrane vesicles was assessed using alkaline phosphatase as the plasma membrane enzyme marker; alkaline phosphatase in isolated vesicles showed a 19-fold enrichment compared with the HLE-B3 cell homogenate.

**Reduced Glutathione Transport by Human Lens Epithelial B3 Cells**

Confluent HLE-B3 cells (0.5-1 × 10⁶ cells/well in 12-well plates) were pretreated for 30 minutes with 0.5 mM acivicin and 10 mM α-buthionine sulfoximine (BSO) before uptake studies were performed. In initial experiments, the optimal incubation time (for deriving linear initial rates) was determined by incubating cells pretreated with BSO and acivicin in an NaCl buffer for 5, 10, 15, 30, and 60 minutes at 37°C with 35S-GSH (>98% pure by high-performance liquid chromatography [HPLC]; NEN, Boston, MA) containing 0.1, 1, or 2 mM GSH. The NaCl buffer contained 100 mM NaCl, 1.2 mM MgCl₂, 0.81 mM MgSO₄, and 25 mM HEPES-Tris, pH 7.4. Cellular radioactivity was determined after three washes and treatment with trypsin-EDTA (0.05% and 0.02%, respectively). Trapping was estimated by measuring radioactivity at 4°C. Because the time course of uptake was linear up to 30 minutes, all subsequent experiments were carried out for 30 minutes.

To determine whether uptake exhibited sodium dependency at varying GSH concentrations, uptake was performed for 30 minutes at 0.05, 0.1, and 1 mM GSH in 10 mM dithiothreitol containing approximately 1 μCi 35S-GSH per well in NaCl buffer (Na⁺-containing buffer) or choline chloride buffer (Na⁺-free buffer). The molecular form of uptake was verified by HPLC of the cell homogenate in studies in which cellular GGT either was uninhibited or was inhibited by acivicin. The kinetics of GSH uptake also were studied in NaCl buffer containing dithiothreitol using GSH concentrations from 0.01 to 50 mM. Precaution was taken to adjust the pH of stock solutions of GSH (5 mM and above, which are acidic) to 7.4. The kinetics of GSH uptake in HLE-B3 cells was analyzed, as described previously. A computer program (SAAM II, version 1.0.3; SAAM Institute, University of Washington, Seattle) was used to perform weighted, nonlinear, least-squared regression fits to the kinetic data. SEMs were used to weigh the mean values of data to be fitted. Briefly, the best fit to net uptake required two kinetic components: a high-affinity Michaelis-Menten unit, and a low-affinity Hill unit. Because initial fitting analyses indicated a Hill coefficient (nH) that was greater than 2 but less than 3, the final analysis was performed at nH = 3.

In separate experiments, the effect of various compounds and GSH analogs on GSH uptake was measured. GSH uptake was measured in NaCl medium at 37°C for 30 minutes at 1 mM GSH in the presence or absence of 2 mM dibromosulfophthalein (DBSP), 0.1 mM 4,4′-diisothiocyanato stilbene-2,2′-disulfonic acid, 2 mM S-ocetyl GSH, and 2 mM S-ethyl GSH.

The rate of efflux of GSH was determined in 60 × 15-mm culture dishes (5 × 10⁶ cells per dish) in incubations in Krebs-Henseleit buffer supplemented with 12.5 mM HEPES (pH 7.4). Cells were pretreated with 0.5 mM acivicin for 30 minutes, and efflux was determined from the analysis of GSH in aliquots of supernatant at 5, 15, 30, 60, and 90 minutes. Supernatant...
fractions were analyzed by HPLC to determine the molecular form of efflux.6

**GSH Transport in Human Lens Epithelial B3 Cell Membrane Vesicles**

Transport of 35S-GSH was measured by a rapid filtration technique (Millipore, Bedford, MA).17 Briefly, frozen membranes were thawed rapidly by immersion in a water bath for approximately 2 minutes at 25°C and were vesiculated by 15 passages through a 25-gauge needle. The suspension was diluted (2-3 mg protein/ml) in an appropriate resuspension buffer and was mixed by five more passes through a 25-gauge needle. All membrane fractions were pretreated with 3 mM acivicin for 30 minutes at 25°C. The vesicles were pretreated with valinomycin (10-12 μg/mg protein) and were preloaded with 100 mM KCl before uptake determination. GSH uptake was measured under a valinomycin-induced gradient of K+in > K+out (100-20 mM). To study GSH uptake in the presence of Na+, in addition to the outwardly directed K+ gradient, a gradient of Na+in > Na+out (100-0 mM) was applied. Typically, 20 μl membrane suspension (30-50 μg protein) was added to a glass test tube and was incubated for 3 minutes at 37°C. The uptake of 35S-GSH was initiated by adding 80 μl incubation buffer, which contained 35S-GSH, unlabeled GSH, and the appropriate ionic gradient. At the desired time, uptake was terminated by the addition of 1 ml ice-cold stop buffer. The stop buffer was similar in composition to the incubation buffer, but without GSH. 35S-GSH, transported and bound to membranes, was trapped on a 0.45-μm cellulose acetate filter. The filters were washed twice with 4 ml ice-cold stop buffer. Radioactivity retained on the filters was measured in a liquid scintillation counter. Care was taken to ensure uniform low quenching and high efficiency. "Nonspecific" binding was the amount of radioactivity bound to membranes at 4°C and zero time, determined by addition of a chilled aliquot of membranes to 1 ml ice-cold stop solution with the radiolabel.

**Oocyte Studies**

Poly(A)+ RNA was isolated from HLE-B3, HepG2, and Cos-1 cells using a kit (Fastrack Kit; Invitrogen, San Diego, CA). The A280 was used to calculate RNA concentration. The isolation of defolliculated oocytes from stages 5 and 6, the microinjection of mRNA, and the maintenance of injected oocytes in modified Barth’s medium were accomplished as described previously.5,18 We assessed the functional quality of oocytes by observing the expression of sodium taurocholate transport protein and organic anion transport protein after injecting oocytes with the cRNA of respective clones (made available through the Molecular Biology Core of the USC Center for Liver Diseases). In addition, water-injected controls were included in all experiments to check whether the oocytes were "leaky" or not.

Pilot studies were performed to determine that maximal expression of GSH transport required 3 days after poly(A)+ RNA injection. The incubation time for uptake (1 hour) also was established from experiments on the time course of cellular GSH uptake for 15, 30, 60, 90, and 120 minutes. The uptake of radioactivity was linear up to 60 minutes (data not shown).

Oocytes were injected with equal amounts of poly(A)+ RNA (45 ng/oocyte) from HLE-B3, Cos-1, or HepG2 cells. Water-injected oocytes served as the control. Oocytes were washed three times in either NaCl buffer (NaCl) or choline chloride buffer (Na+-free). The NaCl buffer contained 100 mM NaCl, 1.2 mM MgCl2, 0.81 mM MgSO4, and 25 mM HEPES-Tris, pH 7.4, and choline chloride replaced NaCl iso-osmotically in the Na+-free buffer. Oocytes were resuspended in 600 μl (3-4 oocytes) of either buffer in the presence of 1 μCi 35S-GSH (containing 0.05 mM or 2 mM GSH) in 10 mM dithiothreitol. Before the addition of the tracer, oocytes were pretreated with 0.5 mM acivicin for 30 minutes to inhibit GGT activity. GGT activity in stage 6 oocytes was below the detection limit of the assay method.5,18

**RESULTS**

### Reduced Glutathione Levels and Gamma Glutamyltranspeptidase Activity in Human Lens Epithelial B3 Cells

The total glutathione content of HLE-B3 cells (mean ± SEM, n = 3) as measured by the Tietze assay was 12.4 ± 0.8 nanomoles/10^6 cells or 81 ± 7.8 nanomoles/mg protein. The mean GGT activity in HLE-B3 cells was 9.9 nanomoles/min^-1 per 10^6 cells (75 nanomoles/min^-1 per mg protein). GGT activity decreased to levels below the detection limit of the assay (<2 nanomoles/min^-1 per mg protein) after treatment of cells with 0.5 mM acivicin for 30 minutes at 37°C.

### Reduced Glutathione Uptake by Human Lens Epithelial B3 Cells: Linearity of Uptake and Effect of Gamma Glutamyltranspeptidase Inhibition

Linearity of uptake was at first established at two GSH concentrations by measuring uptake at 5, 15, 30, and 60 minutes. Uptake was linear up to 30 minutes (data not shown), and all further studies were performed for 30 minutes. Table 1 shows GSH uptake in HLE-B3 cells in 30-minute incubation studies at 37°C. To block GSH synthesis, the cells were pretreated with 10 mM BSO, and the effect of inhibiting GGT-mediated hydrolysis with 0.5 mM acivicin was determined at 0.05 mM and 1 mM GSH. The uptake of GSH at 4°C, which was caused by binding/trapping, was negligible, and the net uptake is shown.

<table>
<thead>
<tr>
<th>Condition</th>
<th>0.05 mM GSH (pmol/min^-1 per 10^6 cells)</th>
<th>1 mM GSH (pmol/min^-1 per 10^6 cells)</th>
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<tbody>
<tr>
<td>No acivicin</td>
<td>2.1 ± 0.33</td>
<td>17.7 ± 4.3</td>
</tr>
<tr>
<td>+ Acivicin</td>
<td>2.4 ± 0.30</td>
<td>15.7 ± 1.0</td>
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GGT, gamma glutamyltranspeptidase; GSH, reduced glutathione; HLE-B3, human lens epithelial B3 cells; and ns, not significant compared with no acivicin group.

Data are mean ± SEM from three preparations per group. Uptake was performed for 30 minutes in NaCl buffer with 0.05 mM GSH or 1 mM GSH containing 35S-GSH in the incubation buffer. HLE-B3 cells were pretreated with 10 mM N-acetyl-L-cysteine for 30 minutes, whereas GGT was either uninhibited (no acivicin) or inhibited with 1 mM acivicin (+acivicin) during the pretreatment period.
FIGURE 1. The effect of the replacement of sodium with choline on net reduced glutathione (GSH) uptake by human lens epithelial B3 cells. GSH uptake was performed at 1 mM GSH either in NaCl buffer or in choline chloride buffer at 37°C or 4°C with dl-buthionine sulfoximine- and acivicin-pretreated cells. The data represent the net uptake (uptake at 37°C minus the uptake at 4°C) and are the mean ± SEM from 4 or 5 preparations that were each performed in duplicate. The uptake in choline chloride buffer showed significantly decreased (P < 0.02) GSH uptake compared with the NaCl buffer.

GGT inhibition did not cause a significant difference in GSH uptake at either GSH concentration. The molecular form of uptake determined by HPLC was predominantly (>94%) as intact GSH under GGT-inhibited and uninhibited conditions (data not shown).

Sodium Dependency of Reduced Glutathione Uptake

Uptake of GSH by HLE-B3 cells was inhibited partially by the replacement of sodium with choline in the incubation medium at 1 mM GSH (Fig. 1). The mean percentage of inhibition of uptake at 1 mM was 37%. The percentage of inhibition of uptake on Na+ replacement at 0.05 mM and 2 mM GSH was 37% and 32%, respectively (not shown). The uninhibited portion of GSH uptake in Figure 1 probably represents the Na+-independent transport, which we have documented previously in the whole lens or cortex.7 To confirm the specificity of the ion requirement for uptake by HLE-B3 cells, GSH uptake also was performed in Cos-1 cells that were pretreated with BSO and acivicin in parallel experiments. GSH uptake at 1 mM GSH in the presence and absence of sodium in the incubation media was not statistically different from each other in Cos-1 cells (40 ± 1.5 versus 37 ± 1.8 pico-moles/min−1 per 106 cells; P = ns, mean ± SEM, n = 3) which is consistent with our previous findings.12

Concentration Dependence of Reduced Glutathione Uptake by Human Lens Epithelial B3 Cells

GSH uptake was concentration dependent (Fig. 2). These studies were conducted in NaCl and choline chloride buffers at 37°C after the pretreatment of cells with BSO and acivicin. GSH uptake (pico-moles/min−1 per 106 cells) increased with increasing concentrations in the presence or absence of Na+ in the incubation medium. Detailed studies of the kinetics of GSH uptake were not performed simultaneously in both buffers. However, the effect of a range of GSH concentrations (0.01–50 mM) on GSH uptake was studied in NaCl buffer (Fig. 3). Computer fits to the uptake data showed the presence of two components with $K_m$ 1.83 ± 0.51 mM and 11.7 ± 0.93 mM, respectively. These are preliminary estimates from limited studies; a more detailed analysis of kinetic parameters will be undertaken when the Na+-dependent and Na+-independent transporters are cloned and are available for such studies.

The effect of some 5-alkyl compounds and organic anions on GSH uptake was determined. GSH uptake was measured at 1 mM GSH concentration in the presence of either 2 mM DBSP, 2 mM S-octyl GSH, 2 mM S-ethyl GSH, 2 mM GSH-ethyl ester, or 0.1 mM 4,4'-diisothiocyanato stilbene-2,2'-disulfonic acid. Only DBSP inhibited GSH uptake significantly (>46%) in NaCl buffer, whereas all other compounds were without effect.

GSH Transport by Human Lens Epithelial B3 Plasma Membrane Vesicles

Figure 4 shows the GSH uptake at 30 seconds in a mixed plasma membrane vesicle preparation isolated from HLE-B3 cells. As in the case of HLE-B3 cells, net GSH transport in mixed plasma membrane vesicles increased with increasing GSH concentration. GSH uptake in the transport buffer in the absence of NaCl was substantially lower (33%-59%) compared with transport in the presence of NaCl.

Reduced Glutathione Uptake by Oocytes Injected with Human Lens Epithelial B3 Cell Poly(A)+ RNA: Sodium Dependence

The sodium dependence of GSH transport in HLE was confirmed by expression studies in X. laevis oocytes. The uptake of GSH by oocytes was carried out as described in our earlier work.
FIGURE 3. The kinetics of reduced glutathione (GSH) uptake by human lens epithelial B3 cells. 35S-GSH uptake was performed for 30 minutes in l-buthionine sulfoximine-and acivicin-treated cells in NaCl buffer. The data (mean ± SE, n = 3) represent the net uptake at various GSH concentrations ranging from 10 μM to 50 mM. The bold curve indicates the best fit to net uptake, obtained by the sum of the Michaelis-Menten (high-affinity, $K_\text{m} = 1.83 ± 0.51$ mM, mean ± SE) and sigmoid Hill (low-affinity, $n_H = 3$, $K_\text{m} = 11.7 ± 0.93$, mean ± SE) equations (see the Methods section for further details).

Publications.6'7 All uptake studies were carried out for 1 hour, during which GSH uptake was established to be linear. Figure 5 shows the expression of GSH uptake in oocytes injected with poly(A)+ RNA isolated from HLE-B3 and HepG2. Equal amounts of poly(A)+ RNA from these two cell lines were injected into the oocytes, and on day 3 after poly(A)+ RNA injection the oocytes were pretreated with acivicin and uptake studies were performed at 18°C in either NaCl or choline chloride media. There was a significant decrease in GSH uptake by oocytes injected with HLE-B3 poly(A)+ RNA when Na+ was replaced by choline. Uptake by oocytes injected with HepG2 poly(A)+ RNA was sodium independent, which is consistent with previous results in cultured HepG2 cells.12 Water-injected oocytes, which were used as controls in uptake studies, had negligible uptake (not shown). The data presented in Figure 5 are for uptake with 1 mM GSH in the incubation medium. A similar pattern of sodium dependency for HLE-B3 poly(A)+ RNA-injected oocytes was obtained with 0.05 and 2 mM GSH in the incubation medium.

Verification of the Molecular Form of Reduced Glutathione Uptake by Oocytes

The molecular form of the radiolabel taken up by oocytes injected with HLE-B3 poly(A)+ RNA after 1 hour of incubation with 35S-GSH and 1 mM GSH in NaCl medium was analyzed by HPLC. The uptake was predominantly in the form of intact GSH (Fig. 6). The figure shows the cellular uptake profile of poly(A)+ RNA-injected oocytes pretreated with acivicin. Uptake was also predominantly in the form of GSH in oocytes in which GGT was not inhibited by acivicin.

FIGURE 4. Reduced glutathione (GSH) uptake by mixed plasma membrane vesicles from human lens epithelial B3 cells. 35S-GSH uptake was determined at 30 seconds in the presence or absence of an inwardly directed Na+ gradient, as described in the Methods section. Inset: The uptake of 5 μM and 100 μM GSH in the presence and absence of Na+ gradient. The membranes were pretreated with 3 mM acivicin for 30 minutes at 25°C before uptake measurements. Each bar represents the mean of duplicate determinations. GSH uptake in the absence of Na+ (batched bars) was substantially lower at all four GSH concentrations studied than in the presence of Na+ (solid bars).

FIGURE 5. Reduced glutathione (GSH) uptake by oocytes injected with human lens epithelial B3 cell (HLE-B3) poly(A)+ RNA. Poly(A)+ RNA from HLE-B3 or HepG2 cells (36.8 nl) was injected into oocytes, and uptake was performed on day 3 in 1 mM GSH for 1 hour after acivicin pretreatment, as described in the Methods section. Oocytes injected with water served as controls, and uptake in controls was less than 10% of poly(A)+ RNA-injected oocytes (not shown). The data are the means ± SEM from three individual oocyte preparations. The asterisk indicates a significant difference at $P < 0.02$ with choline chloride buffer (batched bars) compared with NaCl buffer (solid bars).
Efflux of Reduced Glutathione by Oocytes Injected with Human Lens Epithelial B3 Cell Poly(A)⁺ RNA

Evidence for the efflux of GSH by oocytes injected with HLE-B3 poly(A)⁺ RNA also was obtained (Table 2). The efflux rate, expressed as nanomoles/h⁻¹ per oocyte, was 30-fold higher in oocytes injected with HLE-B3 poly(A)⁺ RNA than in oocytes injected with water. As in previous studies with rat lens epithelial mRNA,⁷ we have confirmed that the efflux is in the form of GSH, and not oxidized glutathione, as determined by HPLC (not shown).

DISCUSSION

In the present study, we have identified for the first time saturable and inhibitable GSH transport in an HLE cell line, that is, HLE-B3, using cultured cells and membrane vesicles that indicate carrier mediation. Furthermore, we have found that this transport in cells and vesicles is partially sodium depen-

(not shown). To examine the capacity of oocytes expressing HLE-B3 poly(A)⁺ RNA to synthesize GSH, we incubated the oocytes with 50 μM ³⁵S-cysteine (in 10 mM dithiothreitol) and examined the molecular form of radioactivity after 1 hour. The bulk (approximately 85%) of the radioactivity was found in two peaks that corresponded to cysteine and sulfate (Fig. 7). Sulfate was identified as the breakdown product of cysteine, using ³⁵S-sulfuric acid as the standard in an HPLC assay. It is known that when the pathway to GSH synthesis is blocked (with BSO), some cysteine is converted to sulfate.¹⁹ A small peak (approximately 4%) of radioactivity was in the form of GSH. These results confirm that the bulk appearance of ³⁵S-GSH in oocytes incubated with ³⁵S-cysteine cannot be accounted for by the breakdown of GSH, the uptake of cysteine, and the resynthesis of GSH, because virtually no ³⁵S-cysteine was found in oocytes incubated with GSH and negligible ³⁵S-GSH was found in oocytes incubated with labeled cysteine.
TABLE 2. GSH Efflux by Oocytes Injected with HLE-B3 Poly(A)+ RNA

<table>
<thead>
<tr>
<th>Group</th>
<th>Efflux Rate (nmol/h⁻¹ per oocyte)</th>
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<tr>
<td>Control (water-injected)</td>
<td>0.016 ± 0.005*</td>
</tr>
<tr>
<td>HLE-B3 poly(A)+ RNA-injected</td>
<td>0.48 ± 0.03</td>
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GSH, reduced glutathione; and HLE-B3, human lens epithelial B3 cell.
* Mean ± SEM from four determinations. Efflux was studied on day 3 after injection with poly(A)+ RNA or water. Oocytes were pretreated with acivicin, and efflux was measured 1 hour after the administration of 35S-GSH + 10 mM GSH in NaCl buffer.

dent, which suggests the existence of two systems: a sodium-coupled secondary active transport and a facilitated sodium-independent transport. We also have obtained evidence for the expression of both GSH transport systems in oocytes injected with HLE-B3 poly(A)+ RNA. The present findings thus extend earlier observations from our laboratory that suggested the presence of a Na⁺-dependent GSH transporter in the guinea pig and rat lens epithelium to also include the human lens.²,⁵

HLE-B3 has been shown to retain an epithelial morphology and to be capable of expressing endogenous crystallin genes.³ We found that these cells contained significant amounts of reduced GSH. Because these cells also express appreciable GGT activity, it was important to exclude degradation and resynthesis for the observed GSH uptake. As in our previous studies,⁷ we confirmed the uptake of intact GSH by determining the molecular form of the radiolabel transported in the presence of a Na⁺-independent transport system. We also have obtained evidence for the expression of both GSH transport systems in oocytes injected with HLE-B3 poly(A)+ RNA. The present findings thus extend earlier observations from our laboratory that suggested the presence of a Na⁺-dependent GSH transporter in the guinea pig and rat lens epithelium to also include the human lens.²,⁵

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Acknowledgments

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References


