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Analysis of glutathione in rat airway surface liquid by capillary zone electrophoresis with conductivity detection

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Abstract

Glutathione (GSH) is an important component of antioxidant defenses in airway surface liquid (ASL), a thin layer (10–30 μm) of liquid covering the epithelial cells lining the airways of the lung. Decreased levels of ASL GSH have been reported in cystic fibrosis (CF), potentially contributing to the severe oxidative stress seen in this disease. To help investigate the role of GSH in ASL, we developed a technique suitable for analysis of GSH and its oxidized form (GSSG) in microliter samples using capillary sampling followed by capillary zone electrophoresis (CZE) analysis with conductivity detection. CZE was carried out in 100 m*M* CHES and 40 m*M* lithium hydroxide with 5 m*M* spermine at pH 9.1 under an applied electric field of -416 V cm^{-1}. To prevent any autooxidation of GSH during sample manipulations, the samples were treated with *N*-ethylmaleimide (50 m*M*) to alkylate free thiol (–SH). Under these conditions, GSH and GSSG were cleanly separated without interference from common anions (e.g. Cl^-, PO_4^{3-}, HCO_3^-, etc.) and the limit of detection for ASL analysis was 11 μ*M* for GSH and 8 μ*M* for GSSG ($S/N=3$). GSH and GSSG were also measured in rat plasma. Baseline values of 897 ± 210 μ*M* (GSH) and 215 ± 61 μ*M* (GSSG) were obtained for rat ASL ($n=8$), whereas 12.4 ± 2.7 μ*M* (GSH) and 14.8 ± 6.7 μ*M* (GSSG) were obtained for rat plasma ($n=5$).

Keywords: Glutathione

1. Introduction

Glutathione (γ-L-glutamyl–L-cysteinyl–glycine, GSH) is the major free thiol present in virtually all animal cells. Its thiol group is a potent reducing agent (-0.2 V) and hence, an important component of antioxidant defense in the cells. GSH occurs predominantly intracellularly at concentrations that range from about 0.5 to about 10 m*M*. In contrast, the extracellular level of GSH, such as in plasma, is in the micromolar (μ*M*) range [1]. Usually, most of the intracellular GSH is in the reduced form (>95%). However, the levels of oxidized (GSSG) and reduced GSH can change significantly upon oxidative stress, and their evaluation provides useful information about the redox status of cells and tissues [2–4].

In lung airway surface liquid (ASL), GSH is thought to play a major role in antioxidant defense. Apart from GSH, the ASL has also a range of antioxidant defenses such as urate, ascorbate and α-tocopherol that help to maintain a balanced redox

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status. An imbalance between the amounts of reactive oxygen species (ROS) and antioxidant defenses is characteristic of lung diseases. Decreased levels of GSH has been proposed as contributing to the pathogenesis of lung disease in cystic fibrosis (CF) [5–8], the most common fatal genetic disease in North America. In the lung, CF leads to a chronic inflammatory disorder characterized by the accumulation of alveolar macrophages and neutrophils in the lower respiratory tract with destruction and remodeling of airways. GSH deficiency in ASL has also been associated with other pulmonary diseases such as acute respiratory distress syndrome (ARDS) [9], chronic obstructive pulmonary disease (COPD) [10], and infant respiratory distress syndrome (IRDS) [11]. Furthermore, GSH deficiency has also been reported in the lungs of HIV-positive patients [12,13]. There is also a direct relationship with the aging process and reduction in GSH levels in intracellular fluid suggesting a role for oxidant stress in aging [14,15]. Hence, the measurement of GSH in intracellular and/or extracellular fluids is important to the understanding of various biological functions in cells and tissues. Several methods have been described for the quantitation of reduced (GSH) and oxidized (GSSG) forms of glutathione from different biological sources [16–25]. Most of the spectroscopic methods require relatively large sample volumes (μl), an impediment to analyzing ASL (available only in nanoliters quantity) or other biological fluids present in only small volumes.

Bronchoalveolar lavage (BAL) techniques that involve instillation into the lung airways of saline solutions that are then recovered by aspiration was used for ASL sampling. BAL is then analyzed for glutathione, but this reflects GSH/GSSG production in both airways and the lung tissues [19,22]. It would be useful to have an index of GSH/GSSG level in the airway epithelium to better assess the oxidant stress in the airways. However, relatively little is known about the composition of ASL due to its limited quantity (ASL is present as a coating of 10–30 μm thickness overlying the airway epithelium) and difficulties in collecting samples from such an inaccessible area without disturbing the underlying epithelium. We have previously described a technique for harvesting ASL that involves the introduction of polyethylene capillary into the airways. ASL is then collected by capillary action. Although, this results in the harvesting of only 100–300 nl of ASL, we have successfully used capillary electrophoresis (CE) to analyze salt, protein and NO metabolites such as nitrite and nitrate [26–30]. In the present study, we investigate the possible use of capillary sampling or tracheal lavage coupled with CE with conductivity detection as a method to analyze GSH and GSSG in submicroliter quantity of ASL with minimum dilution.

2. Experimental

2.1. Chemicals

Glutathione (reduced, GSH and oxidized, GSSG), CHES [2-(*N*-cyclohexylamino)ethanesulfonic acid], lithium hydroxide, spermine [*N,N′*-bis(3-aminopropyl)-1,4-butanediamine], *N*-ethylmaleimide (NEM) and 63-UV BUN reagent were obtained from Sigma (St. Louis, MO, USA). Perchloric acid (60%) and sodium hydroxide from BDH (Toronto, Canada). Buffer solution, consists of 100 m*M* CHES [2-(*N*-cyclohexylamino) ethanesulfonic acid], 40 m*M* lithium hydroxide and 5 m*M* spermine at pH 9.1, was prepared from distilled and deionised water (Milli-Q50 unit, Millipore, Montreal, Canada) and was used for the analysis. All chemicals used were of analytical grade.

2.2. Animals

Sprague–Dawley rats (6–8 weeks male, ~250 g) were purchased from a commercial source (Charles River, St. Constant, Canada) and housed in a conventional animal care facility at the Meakins-Christie Laboratories. Protocols were approved by the local (McGill University, Montreal, Canada) animal ethics committee.

2.3. Instrumentation

Glutathione was analyzed using a Crystal CE 1000 system with a Concap capillary (60 cm×365 μm O.D.×50 μm I.D.) and Contip conductivity detector from ATI Unicam (Boston, MA, USA). At the

beginning of the analysis, capillaries were washed with 0.5 *M* sodium hydroxide for 30 min, deionized water for 10 min, and background electrolyte (BGE) for 30 min. Between runs the capillary was washed with 0.5 *M* sodium hydroxide (2 min), followed by deionised water (2 min) and then, with BGE (5 min). Samples were introduced into the capillary by applying a pressure at 25 mbar for 0.2 min (~6 nl) at the inlet end. A special glass vial insert (ATI Unicam) was used at the inlet to allow sampling from ~15 μl liquid volume. A high voltage (-416 V cm^{-1}) was applied across the capillary and a conductivity detector was used to analyze the migrating charged species. Data were collected with an integrator (Model SP4600, Spectra-Physics, San Jose, CA, USA) and Spectra-Physics WINNER software was used for data storage and manipulation. For all analyses, the peak areas were normalized with migration time to correct any variations in the peak area due to changes in the migration time.

2.4. Limits of detection, calibration range and reproducibility

Standard solutions of GSH and GSSG were prepared at a concentration of 10 m*M* in 100 μ*M* perchloric acid solution containing 50 m*M* NEM. To avoid any matrix effect of ASL, the standard solutions of GSH and GSSG were diluted in ASL matrix to prepare calibration curve and to study intra- and inter-day reproducibility of the analysis. Although ASL matrix is not well characterized, its composition is known to contain inorganic ions (Na^+, Cl^-, K^+, HCO_3^-, etc.) [26,27,29,31], mucins (a heterogeneous class of large glycoproteins) [31], glycosamioglycons, lysozyme, lactoferrin, serum proteins (albumin, transferrin and globulins) [28,31] and lipids (alveolar surfactants) [31]. The limits of detection (LODs) for GSH and GSSG with pressure injection (25 mbar for 0.2 min, ~6 nl) and conductivity detection were also examined in the ASL matrix. Since the peak areas are used for quantitation, the linear relationship of peak areas with concentrations of GSH and GSSG around the LOD were also analyzed. The recoveries of added GSH and GSSG standards to ASL matrix were evaluated by analyzing their aqueous standards.

2.5. Sampling rat airway surface liquid

ASL samples were collected from Sprague–Dawley rats by the method described in earlier publications [26–30]. Briefly, the rats were sedated with xylazine (0.08 ml/100 g body mass, i.p.) and then anaesthetized with pentobarbital (0.053 ml/100 g body mass, i.p.). An intubation tube (6 cm×1.67 mm I.D.×242 mm O.D.; Becton-Dickinson, Sparks, MD, USA) was inserted into the trachea and the animal placed supine. Then, a polyethylene sampling capillary (10 cm×280 mm I.D.×610 mm O.D.; Becton-Dickinson) was passed through the intubation tubing and left in contact with epithelium near the main carina for a period of 3–5 min before being pulled out. The ASL samples obtained in this way were typically around 100–300 nl in volume per collection. Three to five collections (~1 μl) were pooled together and diluted to 15 μl with 100 μ*M* perchloric acid solution containing 50 m*M* NEM. The samples were centrifuged at 14 000 *g* for 10 min to remove any insoluble particles and than analyzed by CE.

Alternatively, ASL samples were also collected as tracheal lavage. Rats were anesthetized as described above and then following a midline neck incision, the trachea was isolated and tied near the carina. After tracheotomy, 50 μl of saline containing 50 m*M* NEM and 100 μ*M* perchloric acid were instilled into the trachea and recovered by aspiration. The ASL tracheal lavage was centrifuged at 14 000 *g* for 10 min to remove any insoluble particles and than analyzed by CE. GSH values in both sampling methods were corrected for dilution by measuring urea in these samples and compared with plasma urea.

2.6. Sampling rat plasma

Blood was collected from the same rats used for ASL sampling by cardiac puncture and collected into 10-ml sterile, heparinised containers (Vacutainer tube, Becton-Dickinson). The blood samples were spun at 2000 *g* for 6 min at 4 °C. Then, the plasma was removed and treated with NEM in perchloric acid to a final concentration of 50 m*M* (100 μ*M* acid). The plasma samples were centrifuged again at 14 000 *g* for 10 min to remove any insoluble particles and analyzed immediately.

2.7. Urea measurements

Since it is assumed that the urea concentration in vascular and ASL compartments are equivalent due to free diffusion of urea [32,33], the dilution factors for ASL sampling were calculated from the urea content in diluted ASL sample in comparison to plasma. Urea concentrations in the samples were determined with a commercially available reagent (63-UV BUN reagent, Sigma). Briefly, 10 μl of the samples were mixed with 1000 μl of 63-UV reagent and then the absorbance of these solutions were measured at 340 nm after 30 s at room temperature (A1) and after 60 s at 37 °C (A2). The decreases in absorbance (A1−A2) were used to calculate the urea concentration from a standard curve prepared from known standard solutions of urea (0.5–16 m*M*).

3. Results and discussion

3.1. Selection of conductivity detection, electrolyte, electroosmotic flow modifier and pH

CE with conductivity detection has been mostly used to analyze inorganic ions and this is the first time, to our knowledge, that it has been used for analyzing a peptide such as glutathione. The physical properties of glutathione present important advantages for using conductivity detection. Since glutathione is a tripeptide with a molecular mass (M_r) of 307 (for GSH, monomer) or 612 (GSSG, dimer), it should exhibit reasonable conductivity in its deprotonated form [pK_a 2.35 (α-COO^-), pK_a 8.0 (–S^-) and pK_a 9.4 (α-$NH3^+$)], which can be detected by using a conductivity cell. In contrast, proteins (M_r>5000) have very low conductivities and hence, the interferences from various protein macromolcules can be minimized while analyzing glutathione in biological samples such as ASL and plasma. This is an advantage compared to UV detection where we encountered protein interference on analyzing ASL samples for GSH. Selection of CHES with lithium hydroxide buffer (pH 9.1) provides a low conducting electrolyte and thereby, minimises any background noise in the electropherograms. This in turn helps to improve the LOD of glutathione with a reasonable signal-to-noise (S/N) ratio. Alkaline pH (9.1) generates negative charges on the glutathione and in turn, creates charge over mass differences between the reduced (GSH) and oxidized (GSSG) form of glutathione. This leads to difference in their mobilities and hence, separation of these two glutathiones. The resolution in the separation can further be improved by modulating the electroosmotic flow (EOF) with use of spermine [34].

Under the conditions used (BGE: 100 m*M* CHES and 40 m*M* LiOH with 5 m*M* spermine, pH 9.1. $E=-416$ V cm^{-1}), GSH eluted first at 11.0 min followed by GSSG at ~12.0 min Fig. 1A. The extra peak at ~3.5 min appears to represent an impurity from glutathione samples. However, lack of complete baseline resolution between GSH and GSSG indicates that there may be a small percentage of the

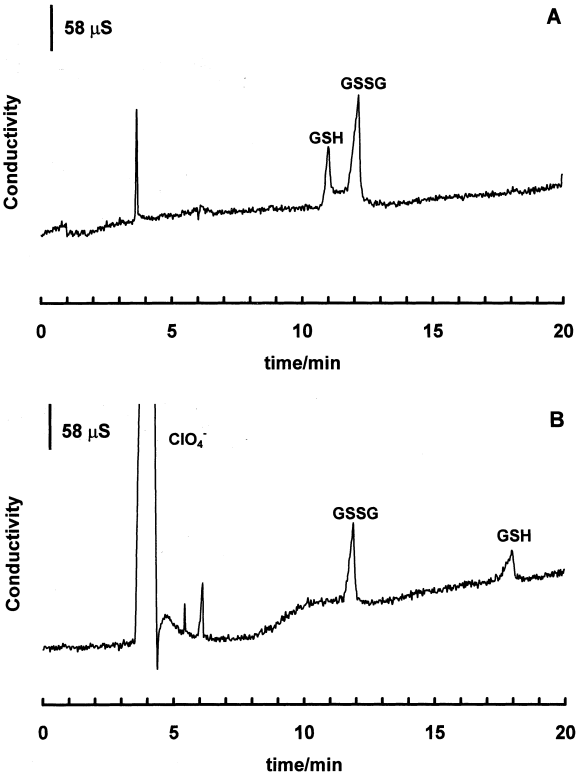

Fig. 1. Electropherograms for the separation of 500 μ*M* GSH and 500 μ*M* GSSG. (A) Aqueous standard solution of glutathiones, and (B) standard solution glutathiones in 100 μ*M* perchloric acid with 50 m*M* *N*-ethylmaleimide. BGE: 100 m*M* CHES and 40 m*M* lithium hydroxide with 5 m*M* spermine, pH 9.1. Applied voltage: −416 V cm^{-1} (I=~31 μA).

molecules that exists in equilibrium with GSH and GSSG in solution due to autooxidation of the free thiol group in GSH (Fig. 1A). To prevent this autooxidation, we pretreated GSH with 50 m*M* NEM to alkylate the free thiol (–SH) group [21,35]. This has the additional advantage of reducing the negative charge on GSH and its mobility compared to GSSG and hence, improving the baseline resolution between them significantly. After NEM treatment, GSH took longer to elute (~18.0 min) due to alkylation of the thiol (–SH) group, whereas GSSG elution was unaffected (Fig. 1B). The higher resolution (~6 min) obtained between GSH and GSSG by this method is advantageous in quantitating higher concentrations (~ m*M*) of GSH–GSSG without any overlap of their peaks, which is highly desirable in analyzing intracellular fluid samples where glutathione levels are expected to be on the order of several m*M* [1]. GSH and NEM are known to be more stable under acidic conditions [21,35] and hence alkylation was done using perchloric acid (100 μ*M*). We found perchloric acid to be superior to sulfosalicylic acid, which is generally used for glutathione extraction and storages, as the perchlorate ion has faster mobility than GSH and GSSG and coelutes with chloride without interfering with the elution of GSH and GSSG. Under the experimental conditions (BGE: 100 m*M* CHES and 40 m*M* LiOH with 5 m*M* spermine, pH 9.1. $E = -416$ V cm^{-1}) used for the CE analysis, there was no interference from excess NEM from the samples and hence, there was no need to remove the unreacted reagent.

3.2. Limits of detection, calibration ranges and reproducibility

In our analysis, the LODs ($S/N = 3$) for GSH and GSSG in the ASL matrix were found to be 11.0 and 8.0 μ*M* (~6 nl sample injection), respectively, with the corresponding mass detection limits of 66 and 48 fmol. This conductivity detection method is a few orders of magnitude more sensitive than the spectroscopic method, but 1–2 orders of magnitude less sensitive than the other electrochemical detection using a Au–Hg amalgam or quinone modified electrodes [23–25] for similar analysis. A calibration curve was constructed using ASL samples spiked with authentic standards of GSH and GSSG in 100 μ*M* perchloric acid containing 50 m*M* NEM, over the range 11–841 μ*M*. With GSH and GSSG concentrations expressed in μ*M*, the equations of typical standard curves are as given in Table 1. Based on the analysis of aqueous standards of GSH and GSSG, the average recoveries of spiked GSH and GSSG standards in ASL matrix were 95.3±6.2% and 93.6±1.8% (means±SD), respectively. Intra- and inter-day reproducibility measurements for ASL samples spiked with GSH and GSSG standards (500 μ*M*) were carried out. The intra-day relative standard deviations (RSDs) ($n = 7$) for peak area were 8.7 and 9.5%, and inter-day RSDs (10 days, $n = 10$) were 10.8 and 11.2% for GSH and GSSG, respectively. The intra-day RSDs for migration time were 1.3 and 1.5%, and for inter-day runs were 5.9 and 5.8% for GSH and GSSG, respectively.

3.3. Rat ASL and plasma GSH and GSSG analyses

Both reduced (GSH) and oxidized (GSSG) glutathione were measured in ASL as well as in plasma. GSSG and GSH in rat ASL as well as in plasma migrated approximately 12.0 and 19.5 min under applied electric field of -416 V cm^{-1} (Figs. 2A and 3), which were confirmed by spiking rat ASL with authentic standard GSH and GSSG (Fig. 2B). The concentrations of GSH and GSSG were calculated

Table 1
Composition of GSH and GSSG determined in ASL and plasma from Sprague–Dawley rats

Analyte	Standard curve[a]	ASL[b] (μ*M*)	Plasma[c] (μ*M*)
GSH	$y_{area} = (387 \pm 35)x_{[GSH]} - (2118 \pm 5610)$	897±210	12.4±2.7
GSSG	$y_{area} = (1118 \pm 46)x_{[GSSH]} + (2166 \pm 21874)$	215±61	14.8±6.7

[a] GSH and GSSG concentrations are expressed in μ*M*, $r^2 = 0.99$–0.997 ($n = 5$).
[b] $n = 8$.
[c] $n = 5$.

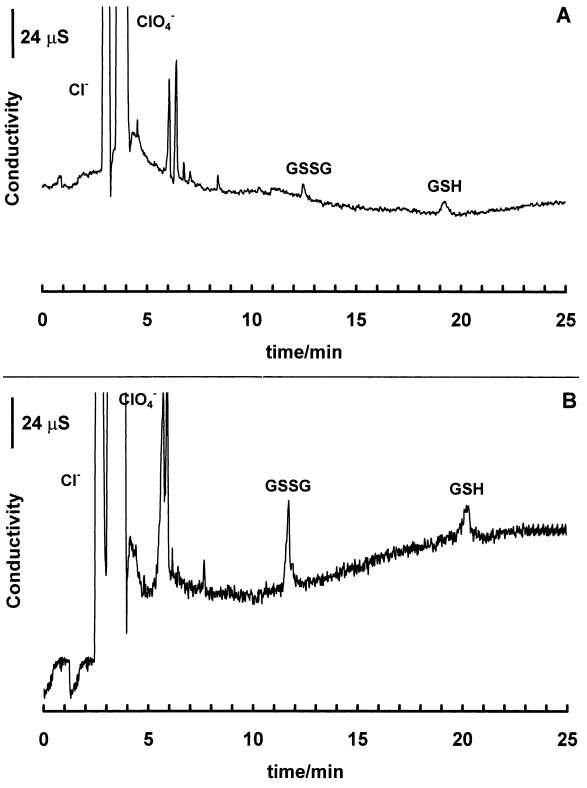

Fig. 2. Electropherograms for the analysis of GSH and GSSG in (A) diluted rat ASL containing 100 μ*M* perchloric acid and 50 m*M* *N*-ethylmaleimide and (B) the same sample after being spiked with 108 μ*M* of standard GSH and GSSG in 100 μ*M* perchloric acid with 50 m*M* *N*-ethylmaleimide. BGE: 100 m*M* CHES and 40 m*M* lithium hydroxide with 5 m*M* spermine, pH 9.1. Applied voltage: −416 V cm^{-1} (*I* = ~31 μA).

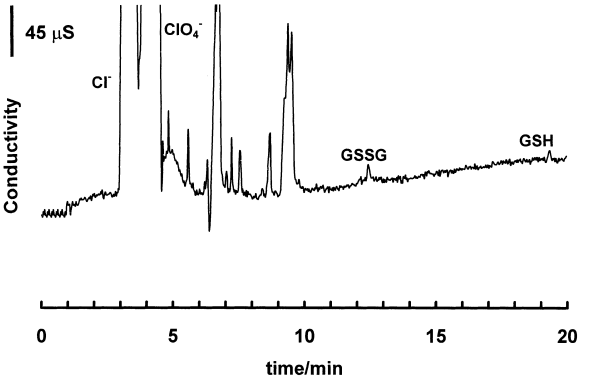

Fig. 3. Electropherograms for the analysis of GSH and GSSG in rat plasma containing 100 μ*M* perchloric acid and 50 m*M* *N*-ethylmaleimide. BGE: 100 m*M* CHES and 40 m*M* lithium hydroxide with 5 m*M* spermine, pH 9.1. Applied voltage: −416 V cm^{-1} (*I* = ~31 μA).

from the standard curve. By analyzing the urea concentration of ASL sample and comparing with plasma values, the dilution factors were calculated for ASL and used to estimate the exact concentration of glutathione in ASL. The concentration of GSH was found to be 897±210 μ*M* in rat ASL (*n* = 8) and 12.4±2.7 μ*M* in plasma (*n* = 5), whereas the GSSG concentration was found to be 215±61 μ*M* in rat ASL (*n* = 8) and 14.8±6.7 μ*M* in plasma (*n* = 5) (Table 1). The total glutathione determined in ASL is ~40 fold higher than that observed in plasma, a value generally consistent with other measurements [36–41]. A likely explanation for the higher amount of GSH in ASL compared to plasma is that several cell types known to export GSH including lymphocytes, macrophages and fibroblasts are present in the lung [19]. Furthermore, the concentration of γ-glutamyl transpeptidase, an enzyme that cleaves the γ-glutamyl bond of GSH during the uptake process [1], is reported to be expressed at a much lower level in the lung compared to the kidney, the major site of glutathione removal from plasma, likely resulting in a lower rate of glutathione metabolism in the airways [19]. However, there has been no previous measurement of glutathione directly from ASL; other approaches such as BAL have generally been used to estimate their concentrations. Using the BAL technique, Cantin et al. [19] determined the total glutathione values for human lung airways as 429 μ*M* and Velsor et al. [36] estimated values of 512 μ*M* of GSH and 106 μ*M* of GSSG for WT mice. Even though there were some minor differences, the values are very much in agreement. The difference may be due to the source of sampling and adaptation of different techniques to analyze the samples. Our method of CE analysis involves direct sampling of ASL, whereas other methods (using BAL) involve sampling of airways and lung tissues.

Although both GSH and GSSG occur in tissues, GSH is by far the predominant form. In the intracellular fluid, greater than 95% of total glutathione exists in the form of GSH. However, the levels of oxidized (GSSG) and reduced GSH can change significantly upon oxidative stress [2–4]. Extracellular fluids such as ASL are particularly prone to

oxidative stress due to the fact that the airways are exposed to the highest O_2 tension of any body tissue. It is not surprising to observe ~20% of total glutathione measured in rat ASL is in the oxidized form (GSSG). Increased O_2 tension also leads to higher ROS, and this may cause an increase in formation of GSSG and H_2O_2 [42]. GSSG is also formed by reaction of GSH with free radicals in the airways to prevent tissue injury by free radicals [43]. Although total glutathione values ranging from 3 to 28 m*M* have been reported for plasma samples of different species [17,37–41,44], our measurement of rat plasma values (~27 μ*M* total glutathione) are consistent with the values of 20–28 μ*M* for rat plasma reported by Meister and co-workers [39,40] and others [38,42]. Since the redox potential of the GSSG/2GSH (−0.137 V) in plasma was considerably more oxidized than values for tissues and cultured cells (−0.185 to −0.258 V), a rapid oxidation of GSH occurs upon release into plasma [44] and this may explain the equal amount of GSH and GSSG observed in our study for the plasma.

A simple CE method coupled with capillary sampling or tracheal lavage has been developed and used to analyze simultaneously both the reduced (GSH) and oxidized (GSSG) form of glutathione in biological sample such as ASL, which is available only in submicroliter quantity.

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References

[1] A. Meister, M. Anderson, Annu. Rev. Biochem. 52 (1983) 711.
[2] W.G. Siems, R. Brenke, A. Beier, T. Grune, Q. J. Med. 95 (2002) 803.
[3] M. Erden-Inal, E. Sunal, G. Kanbak, Cell Biochem. Funct. 20 (2002) 61.
[4] J.D. Adams, L.K. Klaidman, M.L. Chang, J. Yang, Curr. Top. Med. Chem. 1 (2001) 473.
[5] J.H. Roum, R. Buhl, N.G. McElvancy, Z. Borok, R.G. Crystal, J. Appl. Physiol. 75 (1993) 2419.
[6] A.V.D. Vliet, J.P. Eiserich, G.P. Marelich, B. Halliwell, C.E. Cross, Adv. Pharmacol. 38 (1997) 491.
[7] P. Linsdell, J.W. Hanrahan, Am. J. Physiol. 275 (1998) C323.
[8] L. Gao, K.J. Kim, J.R. Yankaskas, H.J. Forman, Am. J. Physiol. 227 (1999) L113.
[9] M. Moss, D.M. Guidot, M. Wong-Lambertina, T. Ten Hoor, R.L. Perez, L.A. Brown, Am. J. Respir. Crit. Care Med. 161 (2000) 414.
[10] I. Rahman, W. MacNee, Am. J. Physiol. 277 (1999) L1067.
[11] D. Boda, I. Nemeth, S. Pinter, Biol. Neonate 74 (1998) 281.
[12] F.J.T. Staal, S.W. Ela, M. Roederer, M.T. Anderson, L.A. Herzenberg, Lancet 339 (1992) 909.
[13] E.R. Pacht, P. Diaz, T. Clanton, J. Hart, J.E. Gadek, Chest 112 (1997) 785.
[14] C.A. Lang, S. Naryshkin, D.L. Schneider, B.J. Mills, R.D. Lindeman, J. Lab. Clin. Med. 120 (1992) 720.
[15] M. Julius, C.A. Lang, L. Gleiberman, E. Harburg, W. Di Franceisco, A. Schork, J. Clin. Epidemiol. 47 (1994) 1021.
[16] M.E. Anderson, Meth. Enzymol. 113 (1985) 548.
[17] A.M. Svardal, M.A. Mansoor, P.M. Ueland, Anal. Biochem. 184 (1990) 338.
[18] T.P.M. Akerboom, H. Sies, Meth. Enzymol. 77 (1981) 373.
[19] A.M. Cantin, S.L. North, R.C. Hubbard, R.G. Crystal, J. Appl. Physiol. 63 (1987) 152.
[20] T. Kuninori, J. Nishiyama, Anal. Biochem. 197 (1991) 19.
[21] Q. Yang, C. Krautmacher, D. Schilling, M.R. Pittelkow, S. Naylor, Biomed. Chromatgr. 16 (2002) 224.
[22] L.J. Smith, M. Houston, J. Anderson, Am. Rev. Respir. Dis. 147 (1993) 1461.
[23] W. Jin, W. Li, Q. Xu, Electrophoresis 21 (2000) 774.
[24] Y. Hiraku, M. Murata, S. Kawanishi, Biochim. Biophys. Acta 1570 (2002) 47.
[25] T. Inoue, J.R. Kirchhoff, Anal. Chem. 74 (2002) 1349.
[26] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, Anal. Chem. 69 (1997) 2793.
[27] E.A. Cowley, K. Govindaraju, D.K. Lloyd, D.H. Eidelman, Am. J. Physiol. 273 (1997) L895.
[28] K. Govindaraju, E.A. Cowley, D.H. Eidelman, D.K. Lloyd, J. Chromatogr. B 705 (1998) 223.
[29] E.A. Cowley, K. Govindaraju, C. Guilbault, D. Radzioch, D.H. Eidelman, Am. J. Physiol. 278 (2000) L1213.
[30] K. Govindaraju, M. Toporsian, M.E. Ward, D.K. Lloyd, E.A. Cowley, D.H. Eidelman, J. Chromatogr. B 762 (2001) 147.
[31] J.H. Widdicombe, J.G. Widdicombe, Respir. Physiol. 99 (1995) 3.
[32] S.I. Rennard, G. Basset, D. Lecossier, K.M. O'Donnell, P. Pinkston, P.G. Martin, R.G. Crystal, J. Appl. Physiol. 60 (1986) 532.
[33] T.W. Marcy, W.W. Merrill, J.A. Rankin, H.Y. Reynolds, Am. Rev. Respir. Dis. 135 (1987) 1276.
[34] K. Govindaraju, A. Ahmed, D.K. Lloyd, J. Chromatogr. A 768 (1997) 3.
[35] F. Tietze, Anal. Biochem. 27 (1969) 502.
[36] L.W. Velsor, A.V. Heeckeren, B.J. Day, Am. J. Physiol. 281 (2001) L31.

[37] H. Ono, A. Sakamoto, N. Sakura, Clin. Chim. Acta 312 (2001) 227.
[38] N.W. Solomons, A. Rosenthal, J. Lab. Clin. Med. 105 (1985) 585.
[39] O.W. Griffith, A. Meister, Proc. Natl. Acad. Sci. USA 76 (1979) 268.
[40] M.E. Anderson, A. Meister, J. Biol. Chem. 255 (1980) 9530.
[41] B.A. Neuschwander-Tetri, F.J. Roll, Anal. Biochem. 179 (1989) 236.
[42] B. Chance, H. Sies, A. Boveris, Physiol. Rev. 59 (1979) 527.
[43] J. Navarro, E. Obrador, J.A. Pellicer, M. Asensi, J. Vina, J.M. Estrela, Free Rad. Biol. Med. 22 (1997) 1203.
[44] D.P. Jones, J.L. Carlson, V.C. Mody Jr., J. Cai, M.J. Lynn, P. Sternberg Jr., Free Rad. Biol. Med. 28 (2000) 625.