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A new HPLC method for the simultaneous determination of oxidized and reduced plasma aminothiols using coulometric electrochemical detection

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A new method has been developed that is capable of providing a complete profile of the most common monothiols and disulfides present in plasma or tissue extracts. The method utilizes reversed phase ion-pairing high performance liquid chromatography coupled with coulometric electrochemical detection to simultaneously quantify free oxidized and reduced aminothiols or total aminothiols after chemical reduction. The method is extremely sensitive, with limits of detection in the 5 fmol/mL range for monothiols and 50 fmol/mL for dithiols. The interassay and intraassay coefficients of variation for total and free aminothiols ranged between 1.2 and 5.8%. The mean recoveries for total and plasma aminothiols ranged between 97.1 and 102.8%. The aminothiols are quantified directly, without derivatization, and include methionine, homocysteine, homocystine, cystathionine, cysteine, cystine, cysteinylglycine, and oxidized and reduced glutathione. Because a complete aminothiol profile of metabolites in both the remethylation (anabolic) and transulfuration (catabolic) pathways of homocysteine metabolism can be determined simultaneously, this new method should be useful in determining the metabolic etiology of homocysteinemia and in designing appropriate nutritional intervention strategies. Basic research applications of this method should lead to an increased understanding of the metabolic pathology of aminothiol imbalance. (J. Nutr. Biochem. 10:490–497, 1999) *Published by Elsevier Science Inc.*

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Introduction

Moderate elevation in plasma homocysteine levels has become a useful clinical biomarker for deficiencies in folic acid, vitamin B_{12}, and vitamin B_6, as well as for several inborn errors of folate, cobalamin, or methionine metabolism.[1,2] In addition, mild to moderate elevations in homocysteine have recently become established independent risk factors for cardiovascular disease and certain birth defects.[3–5] Because homocysteine occurs at a pivotal metabolic juncture between pathways of methionine remethylation and transulfuration, elevated plasma homocysteine generally reflects the relative activity of these two pathways.[6,7] Appropriate clinical management depends on an understanding of the biochemical determinants of homocysteinemia and would be facilitated by a method to simultaneously quantify, within the same plasma sample, the aminothiols in both the transmethylation and transulfuration pathways. For example, homozygous mutations in methylenetetrahydrofolate reductase (MTHFR) gene and heterozygous mutations in cystathionine beta synthase (CBS) gene can both lead to mild elevations in homocysteine[8] (*Figure 1*). Differential diagnosis of these two inborn errors of metabolism may be made on the basis of the relative plasma methionine levels, which tend to be elevated with CBS deficiency and reduced with MTHFR deficiency. Thus, the

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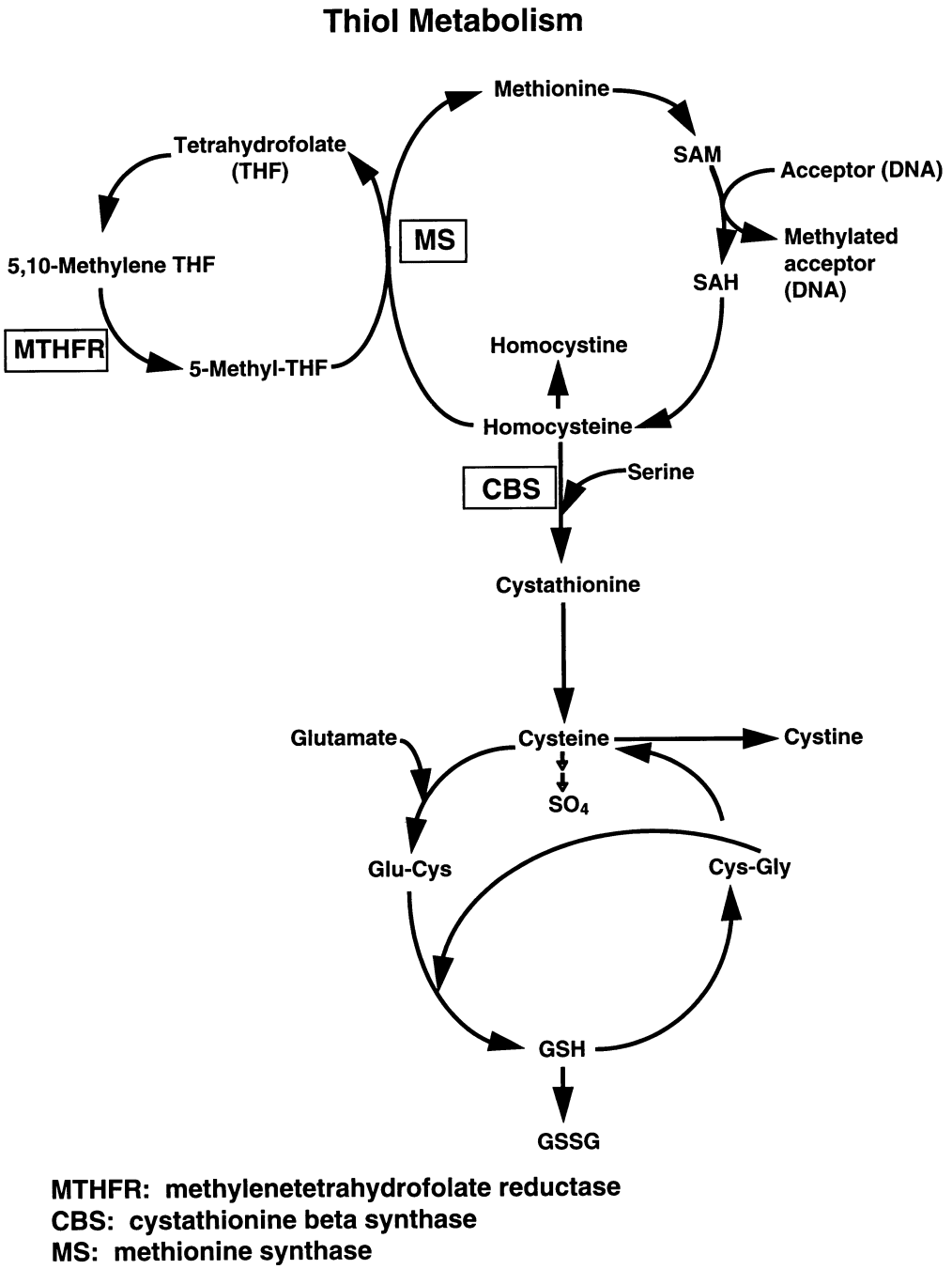

Figure 1 Flow diagram of aminothiol metabolism including the anabolic homocysteine remethylation pathway and the catabolic homocysteine transulfuration pathway. SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; MTHFR, methlyenetetrahydrofolate reductase; CBS, cystathionine beta synthase; Glu-Cys, glutamylcysteine; Cys-Glu, cysteinylglycine; MS, methionine synthase; GSH, reduced glutathione; GSSG, oxidized glutathione.

simultaneous detection of total homocysteine, methionine, and cystathionine in plasma would allow rapid differential diagnosis of these two inborn errors of one-carbon metabolism with or without a methionine loading test. Furthermore, the ability to simultaneously monitor aminothiol levels in the anabolic remethylation and the catabolic transulfuration pathways would provide a means to monitor the metabolic impact of nutritional intervention strategies. Finally, perturbation of the redox status of plasma and tissue aminothiols is an important indicator of chronic oxidative stress and a prooxidant state that may accompany homocysteinemia associated with cardiovascular disease, antioxidant deficiencies, and/or renal failure.[9,10] A low ratio of reduced to total levels of homocysteine, cysteine, cysteinylglycine, and/or glutathione would be an indication that antioxidant intervention may be warranted.[11,12]

In this article, we present a new method of high performance liquid chromatography (HPLC) that uses coulomet-

ric electrochemical (EC) detection to provide a complete profile of plasma aminothiols including methionine, homocysteine, homocystine, cysteine, cystine, cystathionine, cysteinylglycine, and oxidized and reduced glutathione within the same plasma sample. The application of the various ratios between these metabolically-interrelated compounds should facilitate differential diagnosis and nutritional management of the many inborn errors of methionine metabolism and the various disease states associated with abnormal one-carbon metabolism.

Material and methods

Reagents

L-Cysteine, L-cystine, L-cystathionine, D,L-homocysteine, L-homocystine, L-methionine, glutathione (oxidized and reduced forms), cysteinylglycine, and sodium borohydride were obtained from Sigma Chemical Co. (St. Louis, MO USA). Sodium phosphate monobasic, monohydride was purchased from EM Science (Gibbstown, NJ USA). HPLC-grade meta-phosphoric acid, acetonitrile, and 1-octanesulfonic acid (OSA) were obtained from Fluka (Milwaukee, WI USA). Deionized HPLC-grade water was prepared by passage through a Syrbon/Barusted NANOpure II (Boston, MA USA) filtration system and subsequent passage through C_{18} Sep-Pak cartridges (Millipore Corp., Milfold, MA USA).

Sample preparation

Blood was collected from healthy fasted adult females (mean age, 29 years) into EDTA-Vacutainer tubes and immediately centrifuged at 1,500 rpm for 15 minutes at 4°C. Aliquots of the plasma layer were transferred into cryostat tubes and stored at −20°C until analysis. For determination of total aminothiols, the disulfide bonds were reduced and protein-bound thiols were released by the addition of 50 μL freshly prepared 1.43 M sodium borohydride solution containing 1.5 μM EDTA, 66 mM NaOH, and 10 μL *n*-amyl alcohol to 200 μL plasma. After gentle mixing, the solution was incubated in 40°C water bath for 30 minutes with gentle shaking. To precipitate plasma proteins, 250 μL ice-cold 10% meta-phosphoric acid was added and the sample was incubated for 10 minutes on ice. After centrifugation at 14,000 rpm for 15 minutes at 4°C, the supernatant was filtered through 0.2 μ filter (PGC Scientific, Frederick, MD USA) and a 20 μL sample was injected into the HPLC system described below. To determine the free (non-protein-bound) aminothiol levels, an equal volume of freshly prepared 10% meta-phosphoric acid was added directly to 200 μL plasma followed by a 30-minute incubation on ice. After centrifugation for 15 minutes at 14,000 rpm at 4°C, the supernatant was filtered before injecting 20 μL of the sample into the HPLC.

HPLC Chromatography

The plasma aminothiols were separated by HPLC coupled with a Shimadzu solvent delivery system (model 580). A reverse phase C_{18} NBS column (5 μ; 4.6 × 150 mm; MCM, Inc., Tokyo, Japan) was obtained from ESA, Inc. (Chelmsford, MA USA). Isocratic elution with a mobile phase, which consisted of 50 mM sodium phosphate monobasic, monohydrate, 1.0 mM ion-pairing reagent OSA, 2% acetonitrile (v/v), adjusted to pH 2.7 with 85% phosphoric acid, was performed at ambient temperature at a flow rate of 1.0 mL/min and a pressure of 120 to 140 kgf/cm^2 (1,800–2,100 psi). Plasma extracts were directly injected onto the column using a Beckman autosampler model 507E (Beckman Inst. Inc., Fullerton, CA USA). To assure standardization between sample runs, calibration standards were interspersed at intervals and duplicate reference plasma standards were included.

Coulometric EC detection

Aminothiols are detected following HPLC separation with a model 5200A Coulochem II EC detector (ESA, Inc.) equipped with a dual analytical cell (model 5010) and a guard cell (model 5020). The guard cell is connected in line before the injector and is used to remove oxidizable impurities in the mobile phase that may interfere with baseline stability. The dual analytical cell contains two porous graphite electrodes in series. The first electrode (E1) is set at a lower voltage than the second electrode (E2), and is used as an oxidative screen to eliminate interfering compounds that oxidize at lower potentials than the compounds of interest. For selectivity, E2 is set at or above the established oxidation potential of the compounds of interest. For optimum detection of aminothiols, the electrode potentials for the guard cell, E1, and E2 were set at +950 mV, +350 mV, and +880 mV, respectively. The current generated at E2 results from the oxidation of the active species between +350 mV and +850 mV, a range that encompasses the peak oxidative range for aminothiols. These potentials provide peak area response with minimum background and are the basis for quantification. Peak area analysis for each thiol was provided by GOLD Nouveau software (Beckman Inst. Inc.) based on calibration curves generated for each compound. To assure baseline stability before a sample run, the system was conditioned by application of −400 mV at each electrode for 30 minutes with the mobile phase at 1.5 mL/min, followed by a 30-minute water rinse at 1 mL/min with electrodes turned off and a 30-minute rinse with 4% acetonitrile (electrodes off). Before injection of the first sample, the potential at the electrodes was increased stepwise to the final working potential, and HPLC-EC system was equilibrated for approximately 1 hour with the mobile phase at 1 mL/min. A sample autoinjector is highly recommended for this procedure to allow continuous sampling overnight. When not running samples overnight, the mobile phase was set at a rate of 0.2 mL/min with lower voltages of +50 mV, +100 mV, and +200 mV at E1, E2, and the guard cell, respectively.

Calibration curves and limits of detection

Linear calibration curves consisting of 4 to 5 points for each compound were generated in the following biologic ranges for each compound: 0.5 to 200 nmol/mL methionine, 0.5 to 100 nmol/mL homocysteine, 0.1 to 50 nmol/mL cystathionine, 5 to 350 nmol/mL cysteine, 5 to 200 nmol/mL

cystine, 5 to 200 nmol/mL cysteinylglycine, 0.5 to 100 nmol/mL reduced glutathione, and 0.5 to 500 nmol/mL oxidized glutathione. Before sample analysis, standard curves were examined each day using frozen aliquots of stock calibration solution. The limit of detection for standard calibration standards was defined as the concentration that produced a signal-to-noise ratio greater than 5.

Recovery, precision, and statistical analysis

For analytical recovery, known concentrations of cysteine, cystine, homocysteine, homocystine, methionine, cystathionine, cysteinylglycine, and reduced and oxidized glutathione were added to reference plasma samples. The total thiol concentration in the spiked samples was determined in five independent samples, and the mean quantitative recoveries were calculated. To determine the intraassay precision, 10 replicates of the same sample were analyzed in a single run. The interassay precision was determined by analyzing aliquots from a single control plasma sample on 10 different days over 1 month. The coefficient of variation (CV) was calculated as the standard deviation expressed as a percentage of mean values. Statistical differences between means were calculated using the Student's *t*-test and Sigmastat software (Jandel Scientific, San Rafael, CA USA).

Results

Optimization of thiol reduction and protein precipitation

Because the majority of plasma aminothiols are covalently linked in disulfide form to other thiols or to cysteine sulfhydryls in plasma proteins, the bound aminothiols must be released by chemical reduction of disulfide bonds. The selection and concentration of the reducing agent can affect subsequent chromatographic characteristics depending on the type of detector utilized. We compared two commonly used reducing agents, tri-*n*-butylphosphine and sodium borohydride, for compatibility with coulometric EC detection. The tri-*n*-butylphosphine has an advantage over sodium borohydride in that it does not produce gas during the reduction reaction. The tri-*n*-butylphosphine resulted in a large early peak that caused significant baseline drift and interfered with the resolution of the early-appearing thiols. In contrast, reduction with a mixture of 1.43 M sodium borohydride with 1.5 μM EDTA and 66 mM NaOH resulted in 100% reduction of bound thiols with no interfering peaks and excellent baseline stability. The anti-foaming agent *n*-amyl alcohol was added to the samples before the sodium borohydride and was sufficient to control excessive foaming and sample volume loss. Higher concentrations of sodium borohydride resulted in drift and baseline instability. For the determination of free thiols, plasma protein-bound thiols must first be removed by precipitation. With EC detection, the specific organic acid used to precipitate protein can affect chromatogram resolution, electrode sensitivity, and column stability. We compared meta-phosphoric acid, trichloroacetic acid (TCA), and perchloric acid (PCA) for optimum characteristics. Both TCA and PCA generated significant baseline elevation, large early peaks, and reduced sensitivity. Protein precipitation with meta-phosphoric acid produced optimal results and did not interfere with peak resolution or baseline stability.

Chromatography

The ion-pairing reagent OSA at a concentration of 1 mM in the mobile phase was critical for the separation of oxidized and reduced aminothiols and significantly prolonged the retention times in the presence of 2% acetonitrile. Resolution of the individual thiols was compromised at lower concentrations of OSA or at higher concentrations of acetonitrile. Peak resolution was also significantly affected by pH, with optimum characteristics achieved at pH 2.7. Typical chromatograms of a standard solution of oxidized and reduced aminothiols before and after reduction are presented in *Figures 2A* and *2B*, respectively. *Figures 2C* and *2D* are typical chromatograms of free (protein-precipitated) and total (reduced and protein-precipitated) aminothiols, respectively, obtained from a normal plasma sample. The order of elution was cysteine (4.3 min), cystine (5.6 min), cystathionine (7.3 min), reduced glutathione (9.5 min), homocysteine (12.1 min), cysteinylglycine (13.8 min), methionine (20.1 min), homocystine (29.9 min), and oxidized glutathione (40.8 min). Total reduced aminothiols eluted within 20 minutes (*Figures 2A* and *2C*), whereas the complete profile of free oxidized thiols including homocystine and oxidized glutathione required 41 minutes. The complete reduction of oxidized thiols to nondetectable peaks is apparent in *Figures 2B* and *2D*. It should be noted that the peak for cystathionine, an aminothiol, disappeared after sodium borohydride treatment in both plasma and standard preparations. We have no explanation for this phenomenon; however, because free cystathionine is measured before sodium borohydride treatment, the apparent distruction during the reduction step will not affect its measurement.

Sensitivity, accuracy, precision, and recovery

The limit of detection, defined as a signal to noise ratio of greater than 5, was approximately 5 fmol/mL for the monothiols and approximately 50 fmol/mL for the dithiols, which is lower than most methods using fluorescent ultraviolet detection. To achieve this level of sensitivity and baseline stability, given the high electrical potential at the electrodes, it is essential that the water be of highest purity (not less than 18 megohm-cm), that the mobile phase be continually degassed, and that a surge protector and dedicated electrical outlet be utilized. Because most of the background current is due to trace levels of electrochemically reactive components in the mobile phase, the coulometric guard cell effectively removes these impurities before the sample injector and thereby dramatically reduces background noise. The selectivity for aminothiols is greatly enhanced at E2 by the oxidation and elimination of nonthiol components of plasma at E1.

The interassay and intraassay precision for total and free plasma aminothiols are presented in *Table 1*. The within run intraassay CV for the total plasma aminothiols ranged between 1.5 and 4.5%; for free thiols, the CV range was between 4.1 and 5.8%. The corresponding interassay CV

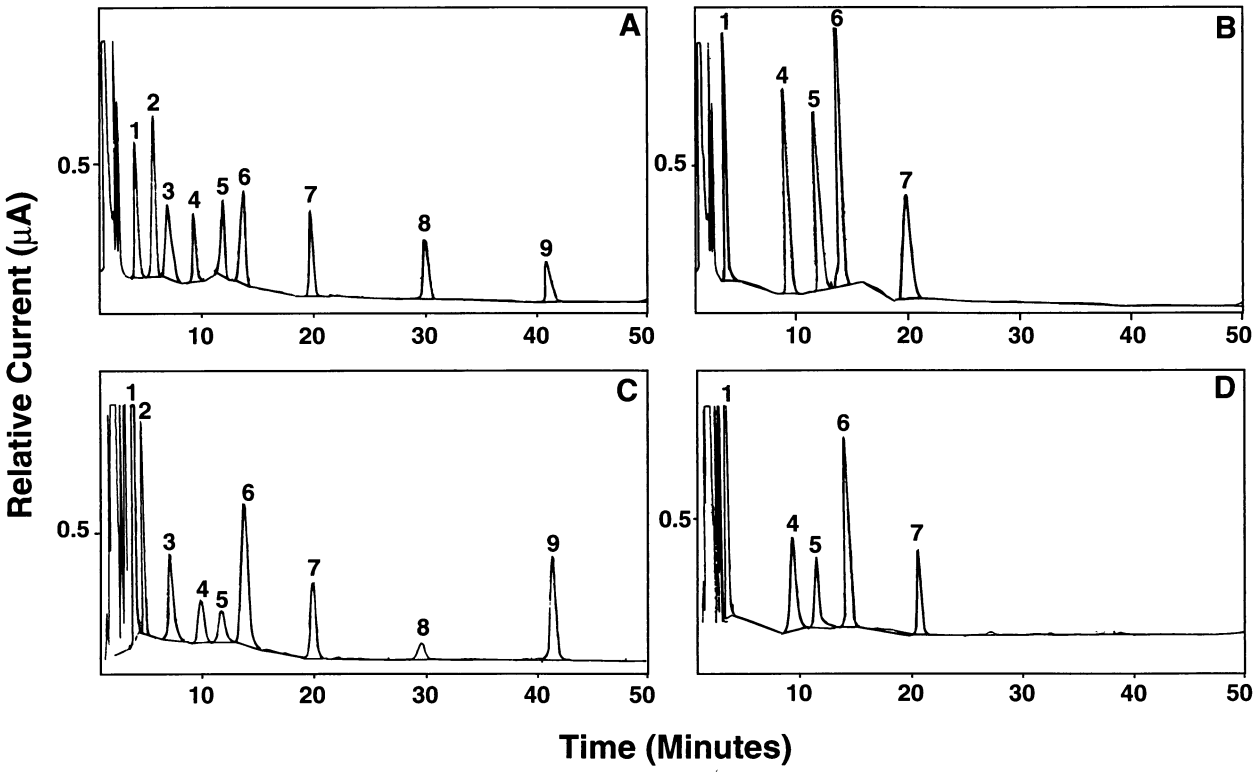

Figure 2 Representative high performance liquid chromatography-electrochemical chromatograms of aminothiols in order of elution: 1, cysteine (4.3 min); 2, cystine (5.6 min); 3, cystathionine (7.3 min); 4, reduced glutathione (9.5 min); 5, homocysteine (12.1 min); 6, cysteinylglycine (13.8 min); 7, methionine (20.1 min); 8, homocystine (29.9 min); and 9, oxidized glutathione (40.8 min). *(A)* Calibration standard solution of free oxidized and reduced aminothiols. *(B)* Calibration standard solution of total reduced aminothiols (after sodium borohydride reduction). *(C)* Free plasma aminothiols (after protein precipitation). *(D)* Total plasma aminothiols (after reduction and protein precipitation).

ranges were 1.2 to 3.8% for total plasma aminothiols and 3.5 to 5.8% for free plasma thiols.

The results of recovery studies are presented in *Table 2*. Recovery experiments were carried out by adding known amounts of each thiol to whole blood samples before protein precipitation. The mean range of recoveries for total and free plasma aminothiols was 97.1 to 102.8%. The excellent recovery indicates that the processing and analytical methods were accurate for the quantitative determination of plasma thiols. Regression analysis of the linearity of detection for each thiol yielded a mean correlation coefficient of greater than 0.99. The linear relationships between peak area (y) and thiol concentration (x) for each thiol were: cysteine, $y = 3.02x + 0.068$; cystine, $y = 0.64x + 0.071$; homocysteine, $y = 2.59x + 0.184$; homocystine, $y = 0.322x + 0.185$; reduced glutathione, $y = 4.63x + 1.255$; oxidezed glutathione, $y = 0.44x + 0.154$; cystathionine, $y = 0.59x + 0.041$; methionine, $y = 2.54x + 0.815$; and cysteinylglycine, $y = 2.03x + 0.673$. The lowest concentrations used for the linearity studies were all above the limit of detection for each compound.

Table 1 Precision of the assay

	Intraassay (n = 10)			Interassay (n = 10)		
	Mean	SD	CV (%)	Mean	SD	CV (%)
Total plasma thiols (μmmol/L)						
Homocysteine	7.7	0.3	3.9	7.5	0.3	4.0
Methionine	35.4	1.4	4.0	33.8	1.2	3.6
Cysteine	228.5	9.5	4.2	215.8	8.3	3.9
Cys-Gly	52.8	2.4	4.6	51.4	2.2	4.3
GSH	6.3	0.3	4.8	6.1	0.2	3.3
Free plasma thiols (μmmol/L)						
Homocysteine	2.4	0.1	4.2	2.4	0.1	4.2
Homocystine	1.9	0.1	5.3	2.0	0.1	5.0
Methionine	20.4	1.2	5.9	20.1	0.9	4.5
Cysteine	40.3	1.7	4.2	38.4	1.5	3.9
Chstine	33.4	1.8	5.4	32.5	1.6	4.9
Cystathionine	2.3	0.1	4.3	2.4	0.1	4.2
Cys-Gly	13.5	0.5	3.7	12.4	0.4	3.2
GSH	2.0	0.1	5.0	1.8	0.1	5.6
GSSG	1.2	0.1	8.3	1.3	0.1	7.7

Cys-Gly–cysteinylglycine. GSH–reduced glutathione. GSSG–oxidized glutathione.

Plasma aminothiol concentrations

The mean values for plasma aminothiols obtained from 11 healthy female volunteers is presented in *Table 3*. The plasma levels determined for each aminothiol are in excellent agreement with values previously reported for healthy females using HPLC, gas chromatography-mass spectrometry (GC/MS), or amino acid analyzer methodology.[11,13,14]

Table 2 Recovery of the assay

	Sample	Added	Measured Mean	Measured SD	Mean % recovery
Total plasma thiols (μmmol/L)					
Homocysteine	7.5	20.0	26.8	0.8	97.5
Methionine	34.5	50.0	84.1	1.2	99.5
Cysteine	225.8	100.0	319.4	2.3	98.5
Cys-Gly	44.5	50.0	95.6	1.2	102.1
GSH	7.1	20.0	27.1	1.1	100.0
Free plasma thiols (μmmol/L)					
Homocysteine	2.3	10.0	12.4	0.3	100.8
Homocystine	2.0	10.0	12.1	0.3	100.8
Methionine	24.5	50.0	74.3	0.7	98.4
Cysteine	38.4	50.0	88.1	1.4	99.7
Cystine	32.5	50.0	81.8	1.2	99.2
Cystathionine	2.3	10.0	12.1	0.4	98.4
Cys-Gly	14.2	20.0	35.1	0.5	102.6
GSH	1.8	10.0	11.6	0.2	107.4
GSSG	1.2	10.0	11.1	0.2	99.1

*Means from five independent experiments.
Cys-Gly–cysteinylglycine, GSH–reduced glutathione, GSSG–oxidized glutathione.

Discussion

Advantages of coulometric EC detection

Although there are several excellent HPLC methods currently available for measuring various combinations of aminothiols in plasma,[12,15–20] none combines the complete aminothiol profile provided by the ion-pairing HPLC method with coulometric EC detection presented in this report. The major advantages of EC detection over the HPLC with ultraviolet detection are elimination of the time-consuming precolumn derivatization step, increased sensitivity, and increased range of oxidized and reduced thiols quantifiable in a single run. There are also several excellent GC-MS methods available that are capable of quantifying cystathionine, cysteine, homocysteine, and methionine in plasma samples.[21–23] However, the 20-fold quantitative difference in homocysteine and cysteine levels in plasma obviates analysis of both aminothiols within a single GC-MC run. To our knowledge, the simultaneous resolution of oxidized and reduced aminothiols has not been shown using GC-MS methods.

Table 3 Plasma aminothiol profile using HPLC-EC detection (μmmol/L)*

Homocysteine	7.8 ± 0.3†
Homocystine	2.1 ± 0.1
Methionine	41.1 ± 2.5
Cysteine	227.1 ± 4.8
Cystine	36.5 ± 1.3
Cystathionine	2.4 ± 0.2
Cys-Gly	47.1 ± 2.8
GSH	6.9 ± 0.5
GSSG	1.5 ± 0.1

*Mean from 11 healthy adult female donors.
†SEM.
HPLC-EC–high performance liquid chromatography-electrochemical. Cys-Gly–cysteinylglycine. GSH–reduced glutathione. GSSG–oxidized glutathione.

The ESA coulometric EC detector offers several advantages over the commonly used amperometric EC detector.[24,25] In the amperometric mode, between 1 and 5% of the analyte is oxidized on the surface of a mercury-gold electrode. With a coulometric detector, close to 100% of the analyte is oxidized in dual flow-through porous graphite electrodes. In the amperometric mode, detection is based on the oxidation of mercury on the surface of the electrode, whereas in the coulometric mode, measurement is based on oxidation of the sulfhydryl group itself. Advantages of the coulometric detector include minimal electrode oxidation, longer electrode half-life, and excellent baseline stability. For routine maintenance of electrodes, at the end of each week, a negative potential of −400 mV is applied to all electrodes for 30 minutes with the mobile phase at 1.5 mL/min, followed by a 30-minute water rinse at 1 mL/min with the electrodes turned off. To prevent bacterial contamination, the electrodes are then rinsed with 1:1 water/acetonitrile for 30 minutes with the column disconnected and the electrodes at zero potential. The column and electrodes are then reconnected and rinsed with 4% acetonitrile for 30 minutes with the electrodes turned off. It is highly recommended that the mobile phase buffer be continually pumped at a low rate (0.2 mL/min) between sample runs with the electrodes set at +200 mV at the guard cell, +50 mV at E1, and +100 mV at E2. The baseline should be stable for a sample run after increasing flow rate to 1 mL/min with stepwise increase to working electrode potentials.

Applications

Because components of both the anabolic (remethylation) and catabolic (transulfuration) pathways of homocysteine metabolism are quantified in the same sample, the method has useful clinical and basic research applications.[17,21,26] Simultaneous determination of aminothiols spanning both pathways can give important clues to the etiology and severity of abnormalities in homocysteine metabolism. In addition, it can provide a means to evaluate the mechanism and effectiveness of nutritional intervention strategies. The method is readily adaptable to quantify intracellular aminothiols in meta-phosphoric acid precipitated lymphocytes or in tissue extracts (data not shown). For basic research applications, tissue-specific aminothiol levels can be assessed to further the understanding of abnormal one-carbon metabolism and homocysteine toxicity under pathophysiologic conditions such as cardiovascular, neurologic, renal diseases, hepatic alcohol injury, and vitamin deficiencies.

A potential application of the method is presented in *Table 4*. The homocysteine/methionine ratio obtained from 26 normal individuals is compared with that obtained from 51 individuals with a 677 C→T mutation in one or both alleles of the MTHFR gene. The homocysteine/methionine ratio in the control samples was 0.25, whereas the same ratio in samples from individuals with the MTHFR mutation was 0.45 ($P < 0.01$), reflecting the higher plasma homocysteine and lower methionine levels. If, on the other hand, the elevation of plasma homocysteine was due to heterozy-

Table 4 Mean plasma homocysteine/methionine ratio in women with normal and mutant MTHFR genotypes

MTHFR 677C→T genotype	*n*	Homocysteine (μmmol/L)	Methionine (μmmol/L)	Homocysteine/ methionine ratio
Normal (C/C)	26	8.5 ± 0.4*	33.8 ± 1.9	0.25 ± 0.02
Mutant (C/T and T/T)	51	11.0 ± 0.3	26.3 ± 1.1	0.45 ± 0.03

*SEM.
MTHFR–methylenetetrahydrofolate reductase. C/C–homozygous MTHFR normal. C/T–heterozygous MTHFR mutation. T/T–homozygous MTHFR mutation.

gous mutation in the CBS gene, the methionine levels would tend to be higher than controls and a lower homocysteine/methionine ratio would confirm the differential diagnosis.

Reduced, free-oxidized, and protein-bound forms of homocysteine, cysteine, cysteinylglycine, and glutathione constitute the plasma redox status and are important components of the extracellular antioxidant defense system.[9,11] The high sensitivity and selectivity of the HPLC-EC method will allow accurate determination of reduced:total ratios of plasma aminothiols. Low ratios would be consistent with a prooxidant state and an indication that antioxidant intervention is warranted. However, for accurate determination of redox status, sample handling is of critical importance. Blood must be immediately chilled in ice water, centrifuged at 4°C, and stored at −20°C to prevent spontaneous oxidation of aminothiols.

This new method is not intended for routine analyses, but is best suited for refinement of diagnoses, for the design and interpretation of nutritional intervention, and for mechanistically-based research studies. Applications of this method should advance the understanding of abnormal one-carbon metabolism and aid in the design of successful therapeutic strategies.

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