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Note

Gas–liquid chromatographic analysis of glutathione*

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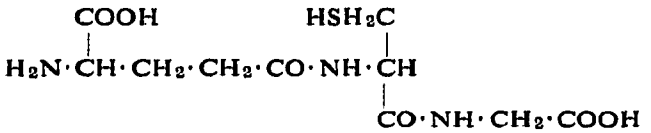
Glutathione (GSH) is a tripeptide γ-L-glutamyl-L-cysteinyl glycine (I), which exists in yeast, plants, and animal tissue. It has been recognized as a characteristic substance of living cells, where it occurs primarily intracellularly and in the reduced form. The concentration of GSH in a given tissue, however, is under the influence of growth, nutritional state, and hormonal balance of the organism[1,2]. GSH of whole blood has been related to growth rate in several species of rabbits, poultry and rats[3–5]. Some important metabolic functions of GSH are (1) controlling the oxidation–reduction potential of the cells, (2) transferring amino acids from soluble RNA to microsomal protein, (3) acting as a cofactor for several enzymatic reactions, and (4) serving as a substrate for other γ-glutamyl transfer reactions[6]. Its role in metabolism may probably be ascribed, at least in part, to its sulfhydryl group.



Mol. wt. = 301.33

(I)

The chemical methods of analysis, *e.g.*, iodometric titration, nitroprusside colorimetry, and the amperometric titration, etc., are popular but lack of specificity and sensitivity[7,8]. Bioassay and enzymatic measurements of GSH generally are specific but again are subject to interferences by closely related compounds[9,10]. Paper chromatographic procedures have shown the separation of GSH from other amino acids[11]. Liquid chromatographic evidence has identified GSH at 400-min elution time on a resin column[12].

The purpose of this report is to show that glutathione can be quantitatively analyzed by gas–liquid chromatography (GLC) as peptide in a unique separation on the same columns used for plasma amino acid analysis.

EXPERIMENTAL

Pure glutathione (Nutritional Biochemicals, Cleveland, Ohio, U.S.A.) was dissolved in 0.1 *N* HCl to make up a final concentration of 2.5 m*M*. An aliquot

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of 10 μl glutathione standard or 10 μl swine plasma and 10 μl of internal standard (L-α-aminocaprylic acid, 2.5 m*M*) were introduced directly into a cone-shaped micro-vial (0.5-ml volume). The sample vials were placed on derivatizing apparatus[13] and ready for direct esterification and acylation[14] (forming the glutathione N-TFA *n*-butyl ester). A microTek Model MT-220 gas chromatograph equipped with a Coulson electrolytic conductivity (N mode) detector (Austin, Texas, U.S.A.) and a Vidar 6300 digital integrator (Autolab, Mountain View, Calif., U.S.A.) were used in this study. A $6 \times {}^{1}/_{4}$ in. I.D. U-glass column packed with 0.325% (w/w) EGA on 80–100 mesh Chromosorb W HT AW (pre-heated at 140° for 12 h), and another with 1.5% (w/w) OV-17 on 80–100 mesh Chromosorb G HP AW, were used for the separation of peptides as well as amino acids. Various parameters used on GLC operation in this study are outlined in Table I.

TABLE I

GLC (NITROGEN DETECTION) CONDITIONS FOR GLUTATHIONE AND AMINO ACIDS SEPARATION

Column I	0.325% (w/w) EGA* on 80–100 mesh Chromosorb W HT AW (pre-heated at 140° for 12 h)
Column II	1.5% (w/w) OV-17** on 80–100 mesh Chromosorb G HP AW
Helium flow, ml/min	
Carrier	60
Pyrolyzer	10
Hydrogen flow, ml/min	
Pyrolyzer	50
Pyrolyzer temperature, °C	820
Initial temperature, °C	90 or 140
Temperature programming, °C/min	5
Final temperature, °C	230
Inlet temperature, °C	220
Chart speed, in./min	0.5

* EGA = Ethylene glycol adipate
** OV-17 = Phenyl methyl silicone

RESULTS AND DISCUSSION

The GLC data on the analysis of GSH and oxidized glutathione (GSSG) studied on the EGA and OV-17 columns are summarized in Table II. The elution temperatures for GSH are 171° or 178° at an initial temperature of 90° on EGA or OV-17. The average relative retention is 1.06 or 1.19 for each column. These data showed that the better separation was with OV-17. The relative molar response of GSH to the internal standard (I.S.) is not in proportion with the nitrogen number (3:1) but is constant and proportional to the concentration.

Fig. 1 illustrates the relative retention and separation of GSH from I.S. on OV-17 and EGA columns. It appears to be sensitive with EGA but they are well separated (Chromatogram B, Fig. 1).

To give a practical example of plasma analysis, a gas chromatogram of plasma

TABLE II

GAS CHROMATOGRAPHIC DATA ON GLUTATHIONE ANALYSIS

Sample load in each micro-vial was 10 to 20 μl of equal molar concentration of 2.5 m*M*.

Components	*Column*	*Initial temperature (°C)*	*Relative retention**	*Relative molar response***	
				1:1	*2:1*
GSH	EGA	90	1.057 (171°)	0.488	
			1.057	0.402	
			1.058	0.481	
			1.057		0.952
			1.059		0.970
		140	1.148 (175°)	0.480	
			1.148	0.485	
	OV-17	90	1.186 (178°)	0.483	
			1.187	0.484	
			1.187		0.952
			1.187		0.962
		140	1.407 (182°)	0.510	
			1.410	0.492	
GSSG	EGA	140	1.140 (175°)		1.729
	OV-17	140	1.398 (180°)		1.450

* Internal standard (I.S.) = 1.000.

** Relative molar response = $\left(\frac{\text{GSH}}{\text{I.S.}} \text{ or } \frac{\text{GSSG}}{\text{I.S.}}\right)$ peak area.

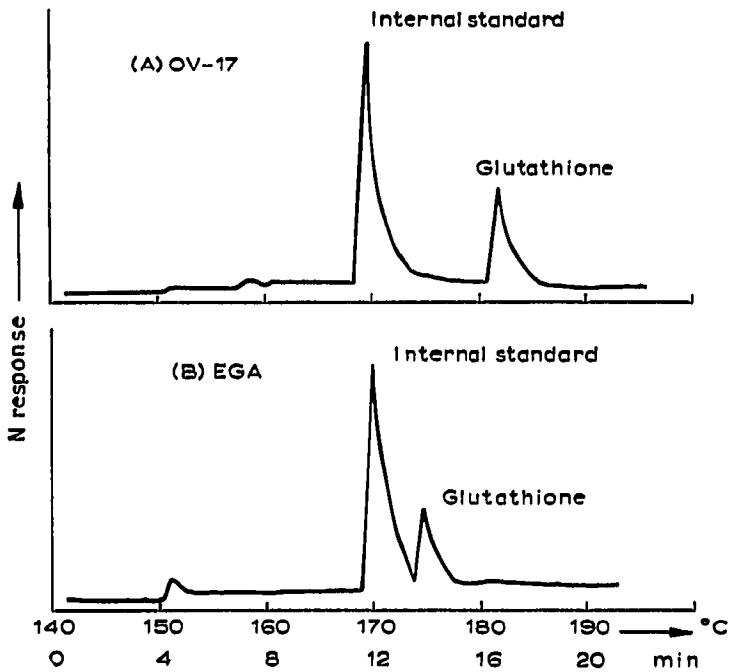

Fig. 1. Gas–liquid chromatograms of glutathione N-TFA *n*-butyl ester on an OV-17 (A) and an EGA column (B). The injected mixture contained *ca.* 0.4 μg of internal standard, L-α-amino-caprylic acid.

amino acid and GSH from swine blood at 90 days is presented in Fig. 2. Results of analysis of GSH are shown in Table III. Plasma-free GSH level ranges from 1.5 to 18 mg per 100 ml were noticed.

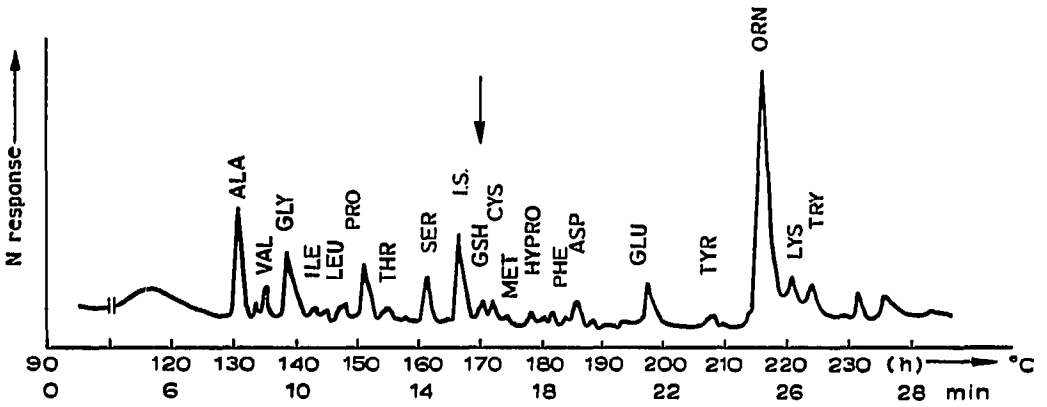

Fig. 2. Gas-liquid chromatogram for N-TFA *n*-butyl esters of plasma-free amino acids and glutathione in swine. Column, 0.325% (w/w) EGA. Temperature programming, 5°/min.

TABLE III

PLASMA GLUTATHIONE (GSH) IN SWINE

Swine (90 days)	*Peak area ratio (GSH:I.S.)*	*GSH level* (mg per 100 ml)*
66	0.05	3.72
67	0.02	1.49
146	0.15	11.15
189	0.24	17.83
194	0.16	11.89
207	0.18	13.37
256	0.17	12.63

* mg/100 ml plasma = peak area ratio $\times \frac{7.43}{10} \times 100$.

As a sulfhydryl compound, GSH is subject to non-enzymatic oxidation to the disulfide, GSSG. It is interesting to note that GSSG gave the same elution temperature as GSH under the same experimental conditions on both EGA and OV-17. However, the relative molar response of GSSG showed twice as high as that of GSH, suggesting the possibility that both GSH and GSSG be modified into an identical derivative product. Mass spectrometric analysis failed to identify such modified compound.

It is generally believed that blood glutathione is in the erythrocytes and the majority of it to be in the reduced form, GSH[15]. The determination of plasma glutathione along with plasma amino acids should provide extra information useful to nutritionists, geneticists, and many other investigators of the life sciences.

ACKNOWLEDGEMENT

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