SEPARATION BY GAS–LIQUID CHROMATOGRAPHY OF SILYLATED DERIVATIVES OF SOME SULFO- AND SELENOAMINO ACIDS AND THEIR OXIDATION PRODUCTS

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Selenium compounds are important to animal health and nutrition[1]. They occur in biological materials primarily as selenium analogs of sulfoamino acids[2], from which they are difficult to separate. The best reported analytical technique for their separation is paper chromatography[3]. Treatment of a mixture of sulfur and selenium containing compounds with hydrogen peroxide converts the selenium components to oxidized derivatives. These migrate at rates different from those of unchanged sulfur analogs. However, we required a rapid analytical technique which, ideally, could distinguish between sulfur, selenium, and sulfoselenium compounds. Gas–liquid chromatography seemed especially suited to these needs, provided volatile derivatives could be synthesized. This paper reports studies on the preparation and chromatography of silylated derivatives of sulfo- and selenoamino acids.

EXPERIMENTAL

Apparatus

Experiments were carried out on an F & M Gas Chromatograph, Model 609, with hydrogen flame ionization detector. Nitrogen was the carrier gas. Column temperatures ranged from 100 to 250°. Detector and injector temperatures were 60 to 90° above column temperature.

Columns

All columns were constructed of stainless steel. Several liquid and stationary phases were used during preliminary experiments; on most of these columns and under the conditions of the experiments, cystine and selenocystine either were not detected or exhibited relatively long retention times. Columns included: 1 ft. × 1/4 in., 5 % Apiezon L on 40/60 Firebrick; 8 ft. × 3 mm, 1.5 % FFAP on 70/80 Chromosorb G; 51.5 cm × 4.2 mm, 1.5 % SE-30 + 1.5 % QF-1 on 70/80 Chromosorb G; 4 ft. × 1/8 in., 3 % OV-1 on 100/120 Chromosorb G HP; 4 ft. × 1/8 in., 0.5 % SE-30 on 80/90 Chromosorb G.

The most suitable column was 4 ft. × 1/8 in., 2 % SE-30 on 90/100 Anakrom SD. All data reported here were obtained on this column.

Preparation of silyl derivatives

Bis(trimethylsilyl)acetamide (BSA) was obtained from Applied Science, Inc.

and from Pierce Chemical Co.; TRI·SIL/BSA in dimethylformamide was also obtained from Pierce. The latter is a special formulation of BSA, solvent and catalyst. In early experiments compounds were heated with excess BSA in either dry pyridine or dry acetonitrile. In attempts to silylate cystine or selenocystine in this manner, we often observed decomposition during derivatization or the presence of multiple peaks on chromatograms; the latter was probably owing to incomplete silylation.

Best results were observed with TRI·SIL/BSA. Accordingly, subsequent experiments and all data reported here were obtained with this reagent. Compounds could be derivatized by heating them in excess reagent at 90–100° for 1–15 min. From 5 to 100 mequiv. reagent per mequiv. of replaceable hydrogen were generally used. Samples were injected into the column without further treatment or purification. Several silyl derivatives, especially that of cysteine, may be prepared by dissolving the amino acid in the reagent at room temperature. Cystine, methionine and its oxidized derivatives and selenium analogs required from 5–15 min heating. Cysteine and related compounds may be heated 1–5 min. Silyl derivatives prepared for this work decompose over several hours; therefore, samples were immediately injected onto the column. Stability of some compounds seemed improved when a large excess of reagent was used.

RESULTS AND DISCUSSION

Table I shows the retention times on 2 % SE-30 at several temperatures of ten pure compounds and one tentatively identified peak. Early attempts to resolve the ten-component mixture either isothermally or by temperature programming were unsuccessful. This result was owing to: (1) the broad temperature range required for

TABLE I

RETENTION TIMES ON SULFO- AND SELENOAMINO ACIDS ON SE-30

Compound	*Molecular weight*	*Retention time (min)*[a]		
Group I		*170°*[b]	*180°*[b]	
Cystine	240	9.8	6.0	
Sulfoselenide?	—	12.3	7.5	
SeCystine	334	15.8	9.5	
Group II		*105°*[b]	*117°*	*128°*
Methionine	149	5.6	2.8	1.5
SeMethionine	196	7.6	3.8	2.0
Methionine sulfoxide	165	—	10.5	5.5
Methionine sulfone	181	—	14.3	7.6
Group III		*105°*[b]	*117°*	*128°*
Cysteine	121	6.6	3.5	1.8
Cysteinesulfinic acid (monohydrate)	171	—	5.8	3.5
Taurine	125	—	5.8	3.3
Cysteic acid	169	—	9.8	4.6

[a] Column temperature is given. Flow rate of carrier gas was 73 ml/min, unless otherwise indicated.

[b] Carrier gas flow rate was 52 ml/min.

complete elution of all compounds, (2) the absence of peaks for cystine and selenocystine when programming was begun at temperatures low enough to give good resolution of other compounds, and (3) the similarity of retention times for methionine- or cysteine-related derivatives. The most satisfactory results were obtained by making three isothermal runs on mixtures containing closely related compounds (Table I).

Fig. 1 shows chromatograms of cystine, selenocystine, their resolution and the effect of temperature on this resolution. Literature reports on the chromatography

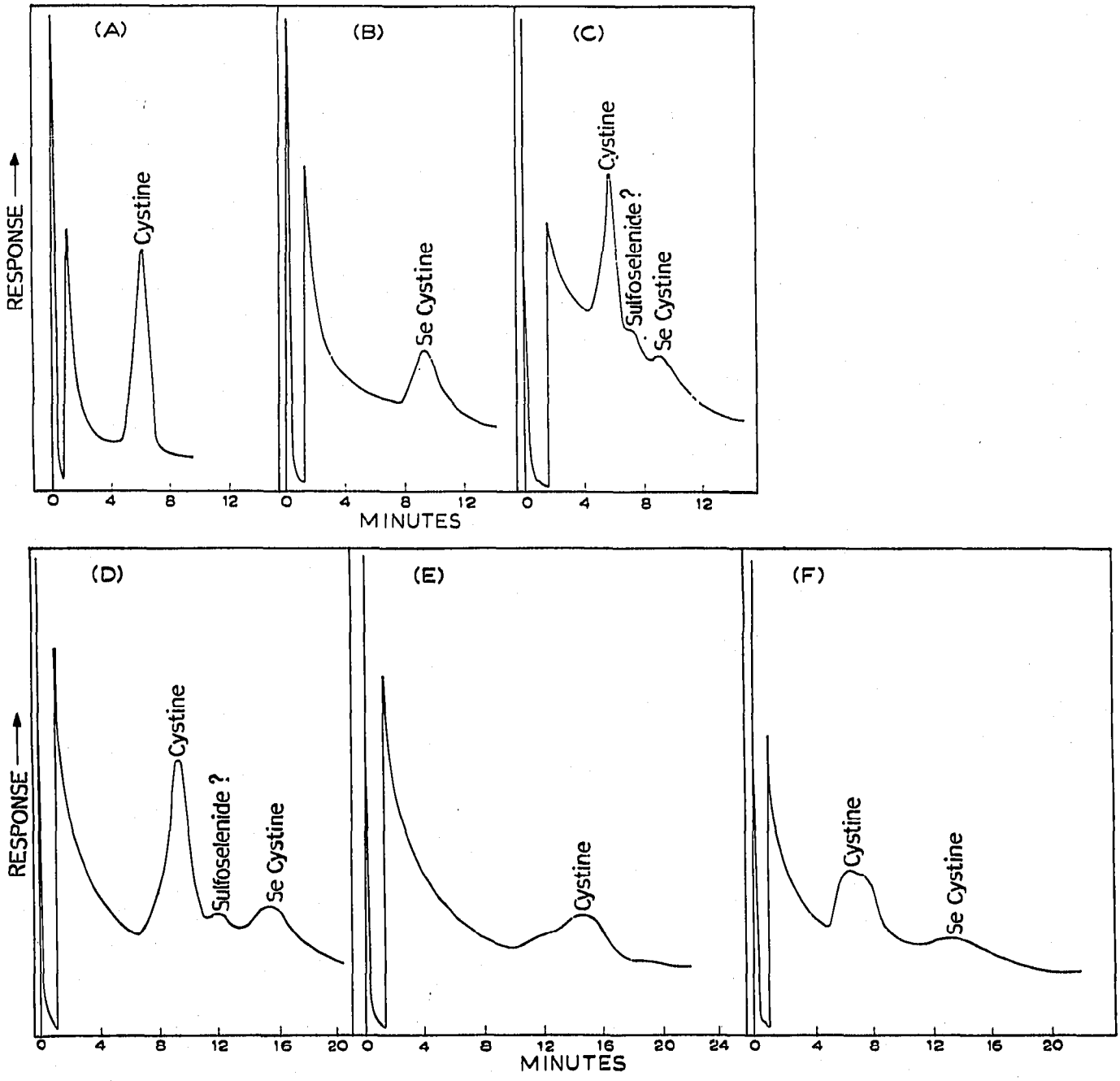

Fig. 1. Chromatograms on 2% SE-30 column showing effect of temperature on separation of cystine and selenocystine. Column temperature and carrier gas flow rate, respectively: (A), (B) and (C) 180°, 52 ml per min; (D) 170°, 51 ml per min; (E) 160°, 66 ml per min; (F) 165°, 98 ml per min. Attenuation for elution of silyl derivatives: (A) 100 × 8; (B) through (F) 100 × 4.

of other cystine derivatives indicate retention times of at least 30 min[4]. In contrast, the silylated derivative can be eluted in less than 10 min; selenocystine can be eluted in less than 20 min. Accordingly, unlike most analytical techniques, gas–liquid chromatography provides a simple method for resolution of sulfur and selenium isologs. Best resolution of cystine and selenocystine was obtained at 170°, Fig. 1D. At temperatures slightly higher than 170°, Fig. 1C, the resolution of these compounds from each other and from a third peak, tentatively identified as a sulfoselenide, is less effective. At temperatures slightly lower than 170°, broad and/or indistinct peaks were observed, which could not be sharpened appreciably by increasing the carrier gas flow rate. The sulfoselenide was observed on chromatograms both from separate preparation and subsequent mixing of the silylated derivatives as well as from simultaneous silylation of cystine and selenocystine. Therefore, the sulfoselenide does not arise owing to conditions associated with the simultaneous synthesis of silylated derivatives. Rather, a mixed sulfoselenide probably results from the interaction of disulfide and diselenide at temperatures necessary to effect their satisfactory chromatography and resolution. Attempts to obtain greater resolution by temperature programming were not successful.

Resolution of methionine, selenomethionine, methionine sulfoxide and methionine sulfone at a column temperature of 117° is shown in Fig. 2. Better separation of any two-component mixture of these compounds can be obtained by altering either the column temperature, carrier gas flow rate or both. However, results indicate that the most satisfactory separation of the four-component mixture was obtained under conditions specified in Fig. 2.

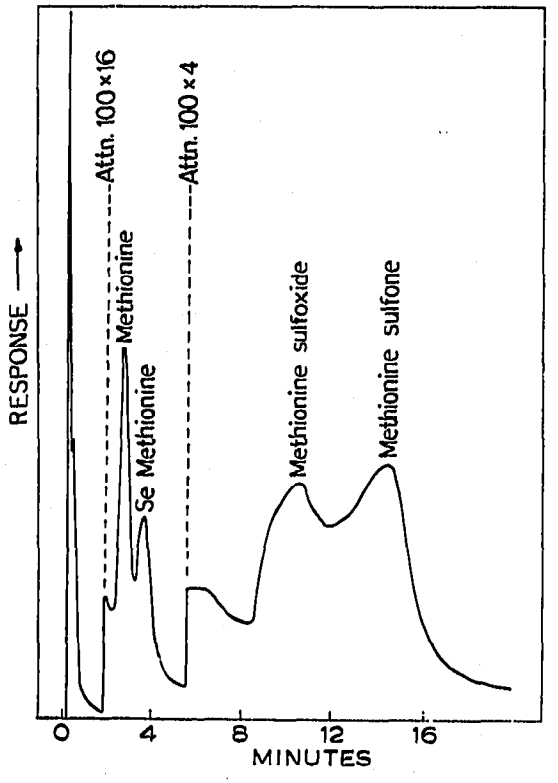

Fig. 2. Gas chromatogram of methionine and related compounds on 2% SE-30 column. Column temperature and carrier gas flow rate, respectively: 117°, 73 ml per min.

Table I shows that silylated derivatives of cysteinesulfinic acid and taurine have essentially the same retention times. Additionally, Figs. 3A and 3B show that a mixture of cysteine, cysteinesulfinic acid, taurine and cysteic acid could be resolved into only three peaks; these correspond to cysteine, cysteinesulfinic acid + taurine, and cysteic acid, respectively. We were unable to resolve cysteinesulfinic acid and taurine on the column used for these experiments.

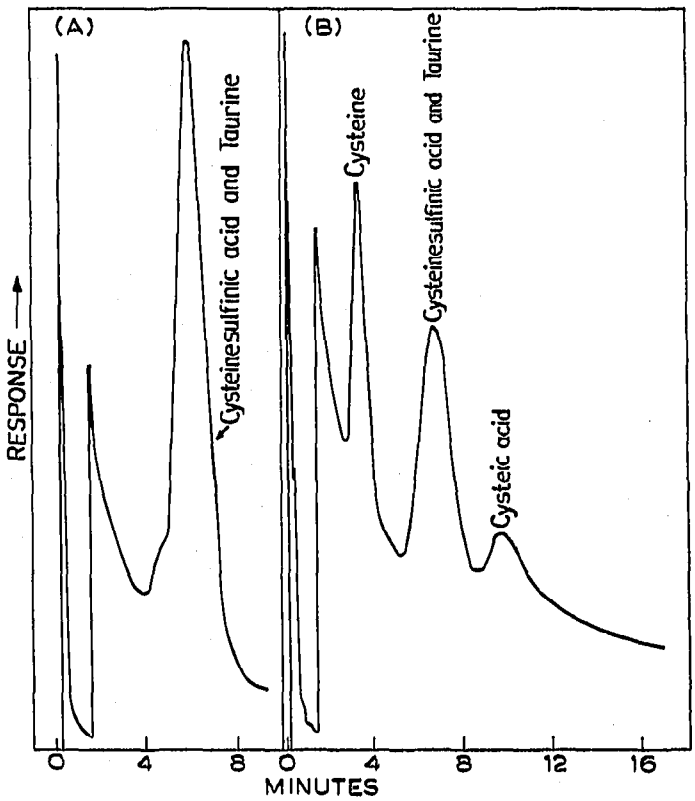

Fig. 3. Gas chromatogram of cysteine and related compounds on 2% SE-30 column. Column temperature and carrier gas flow rate, respectively: 117°, 73 ml per min. Attenuation for elution of silyl derivatives: 100 × 8.

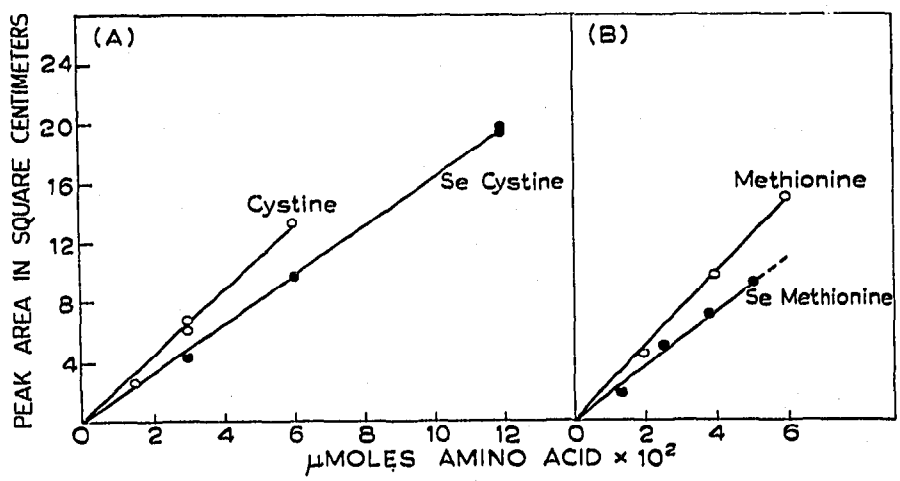

Fig. 4. Relative detector response to sulfur and selenium containing amino acids as demonstrated by calibration curves.

In efforts to quantitate results, we ran calibration curves for each of the compounds studied. These data revealed a linear relationship between peak area and amino acid concentration. However, we observed a significant difference in detector response to selenium compounds as compared to sulfur compounds, Fig. 4. Peak areas of 0.024, 0.015, 0.024 and 0.016 cm^2 per μmole cystine, selenocystine, methionine and selenomethionine, respectively were calculated.

In general, the results show that the specially formulated solution of BSA, solvent and catalyst known as TRI·SIL/BSA is an excellent reagent for converting sulfo- and selenoamino acids to highly volatile derivatives. These derivatives are to be recommended for their ease of preparation and subsequent chromatography. Resolutions obtained under the conditions of our experiments are good. It seems likely, however, that a still higher degree of resolution could be obtained on capillary columns. This technique may prove to be important in the analysis and study of sulfur and selenium compounds from biological sources.

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SUMMARY

Silylated derivatives of cystine and methionine were resolved from their selenium analogs. Peak areas are a linear function of amino acid concentration; however, detector response to sulfur compounds was about a third greater than to selenium compounds. Retention times and partial resolution of the following compounds were also obtained: methionine sulfoxide, methionine sulfone, cysteine, cysteinesulfinic acid, taurine and cysteic acid.

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