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Note

Chromatographic properties of peptides of cystine and glycine and some related derivatives

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The unsymmetrical disulfide, cystinylglycine (Cys_2Gly), was shown to occur in blood plasma[1]. Because of disulfide interchange and crystallization of cystine from concentrated solutions of the peptide it was not possible to prepare pure Cys_2Gly for comparison with the plasma compound. Therefore, indirect procedures were required to characterize the compound and to obtain a color yield with ninhydrin for it so that it could be quantitated. The mixture obtained by aerating a solution of equimolar amounts of cysteine and L-cysteinylglycine was subjected to ion-exchange chromatography with an amino acid analyzer and the eluate containing Cys_2Gly was collected, desalted and treated with four procedures: hydrolysis and measurement of the cystine and glycine formed; reduction with $NaBH_4$ followed by measurement of thiol formed or carboxymethylation and measurement of the S-carboxymethyl derivatives; and reduction with dithiothreitol, sulfation with tetrathionate and measurement of the S-sulfo derivatives. The rather unlikely possibility remained that the plasma compound might be N-glycylcystine which would yield the same results as Cys_2Gly with these procedures if the isomeric peptides and their carboxymethyl derivatives had similar chromatographic properties. It was therefore necessary to prepare glycylcystine to exclude this possibility.

EXPERIMENTAL

Ordinary chemicals and reagents were obtained from commercial sources. Cysteinylglycine (CysGly)[2] and N,N′-diglycylcystine[3] were prepared as described in the literature. Cystinyl-bis-(diglycine) was prepared by aeration of CysGly at pH 7. It proved essential to have seed crystals of the dihydrate[4] in order to obtain a pure product.

Monoglycylcystine was prepared by the method of Zervas *et al.*[5]. The properties of the product were in agreement with those reported for the sesquihydrate but examination by thin-layer and ion-exchange chromatography showed it to be contaminated with cystine (5.4%) and diglycylcystine (22.4%). S-Carboxymethyl-L-cysteinylglycine was prepared by treating CysGly with iodoacetic acid and was purified by absorption and elution from a column of Amberlite CG-120 (H^+) followed by absorption and elution from Bio-Rad AG 1-X8 (CH_3COO^-). N-Glycyl-S-carboxy-

methyl-L-cysteine was prepared from carbobenzoxyglycylcarboxymethylcysteine and purified in the same manner.

RESULTS AND DISCUSSION

The conditions used for ion-exchange and thin-layer chromatography and the data obtained are given in Table I. It is apparent that thin-layer chromatography is of little value in distinguishing between cystine and its peptides with glycine. High-voltage thin-layer electrophoresis likewise was ineffective in separating the compounds.

TABLE I

CHROMATOGRAPHIC PROPERTIES OF SOME DERIVATIVES OF CYSTINE AND GLYCINE

Compound	*Ion-exchange chromatography**			*Thin-layer chromatography***		
	Elution volume (ml)	*Color yield (cystine = 1.00)*	*Absorbance 570/440 nm*	*A*	*B*	*Color with ninhydrin (collidine)*
S-Carboxymethylcysteine	129	1.32	4.09	0.46	0.11	Blue
N-Gly-S-carboxymethylcysteine	302	1.06	8.35	0.40	0.11	Yellow
Glycine	306	0.95	8.11	0.36	0.18	Purple
S-CarboxymethylCysGly	338	1.02	5.38	0.44	0.13	Olive-gray
Cystine	389	1.00	3.01	0.19	0.15	Blue-purple
Monoglycylcystine	477	0.96***	3.64	0.22	0.21	Gray
Cystinylglycine	507	0.78§	2.20	0.24	0.18	Purple
N,N'-Diglycylcystine	588	1.75	8.51	0.24	0.25	Yellow
Cystinyl-bis-diglycine	639	—§§	3.93	0.23	0.23 (streaked)	Faint gray

* Chromatography was done with a Spinco Model 120 amino acid analyzer on a 150 × 0.9 cm column of Aminex Q-150S. Elution was effected initially with pH 3.15, 0.30 *N* lithium citrate and, after 14 h, pH 4.15, 0.30 *N* lithium citrate. Column temperature was increased from 30° to 60° after 9 h. Flow-rates of 30 ml/h of buffer and 15 ml/h of ninhydrin were used.

** Thin-layer chromatography was done on Polygram® Cel 300 (Brinkmann, Westbury, N.Y., U.S.A.). Solvent systems: A, 1-butanol–acetic acid–water (4:1:2); B, pyridine–acetone–3 *N* NH_4OH (50:30:25).

*** Value calculated after correction for contaminating cystine and diglycylcystine.

§ Value derived as described in text.

§§ Cystinyldiglycine is destroyed so rapidly during chromatography it is not possible to obtain a color yield.

Cystinyl-bis-(diglycine) forms a diketopiperazine (anhydro-bis-glycylcystine) readily[2] so that with the conditions used for ion-exchange chromatography it is rapidly destroyed. It was necessary to apply a 5-μmole sample to the 150-cm column of the amino acid analyzer in order for a small amount to persist and show the elution volume. With a 50-cm column and the conditions of Spackman *et al.*[6] cystine is eluted at 40 ml, Cys_2Gly at 53 ml and cystinyldiglycine at 89 ml. A trailing edge of the monodiketopiperazine follows the cystinyldiglycine. Cys_2Gly also probably forms a diketopiperazine to a lesser extent, since only 75–80% of the amount originally present was found when it was collected, desalted and rerun. This behavior is not

characteristic of other cystine peptides since the expected proportion of mixed disulfides was obtained by aeration of various mixtures of CysAla, CysVal and CysLeu[7].

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