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Note

Analysis of γ-carboxyglutamic acid by gas–liquid chromatography*

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γ-Carboxyglutamic acid (Gla), formed by a vitamin K-dependent post-translational modification, confers the ability to bind Ca^{2+} on certain blood coagulation proteins (factors VII, IX, X and prothrombin), osteocalcinin and a number of other proteins from serum, lung, kidney, and spleen and other tissues. The determination of Gla is necessary for the investigation of these Gla-containing proteins and is also useful for the study of a variety of clinical situations induced by vitamin K deficiency.

Since Gla is not stable under the conditions usually used for acid hydrolysis of proteins, it is assayed after alkaline hydrolysis. A number of different methods are available for this purpose. For example, ion-exchange chromatography[1–4], colorimetry[5], and high-performance liquid chromatography[6,7], have been used.

The strategy of most methods is to determine the molar ratio of Gla to glutamic acid. In some methods, Gla is converted to glutamic acid by decarboxylation and the total glutamic acid is determined. An important principle of any assay is that the quantity being measured represents only the compound of interest. Price[4] stressed this point, particularly with reference to assays for Gla using ninhydrin, and also emphasised the need to have "independent evidence for the presence of Gla in the alkaline hydrolysate".

We herein report the development of a gas–liquid chromatographic (GLC) assay for Gla.

EXPERIMENTAL

The N-heptafluorobutyryl (HFB) isobutyl ester of Gla was prepared by a previously described procedure[8,9]. Chromatography was performed on a 15 m × 0.25 mm I.D. SPB-1 capillary column installed in a Varian Vista 6000 gas chromatograph and operated in the splitless mode. The oven temperature was programmed from 75°C to 265°C at 6°C/min.

Mass spectra were obtained using a Finnigan Model 3000 mass spectrometer operated in the chemical ionization (CI) mode using methane as the reagent gas.

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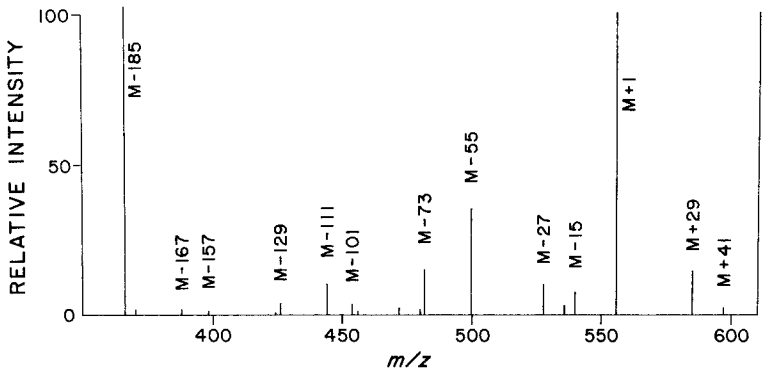

Fig. 1. CI mass spectrum of γ-carboxyglutamic acid N-heptafluorobutyryl isobutyl ester.

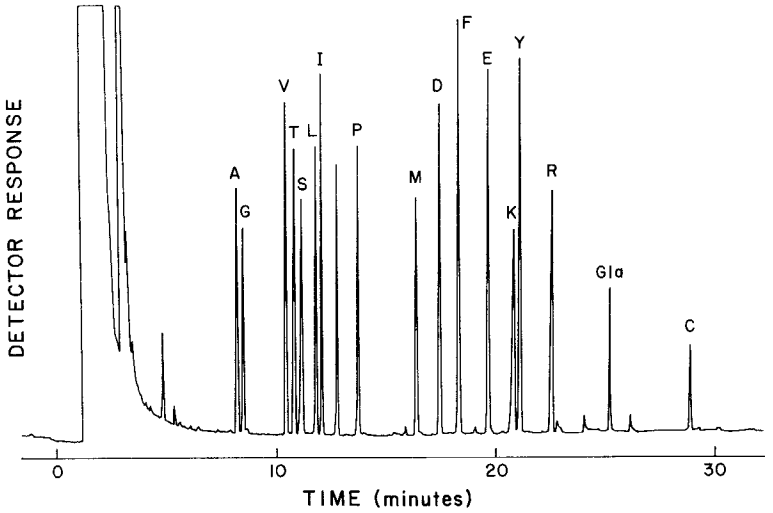

Fig. 2. Chromatogram illustrating resolution of γ-carboxyglutamic acid N-heptafluorobutyryl isobutyl from the corresponding derivatives of the standard proteic amino acids. The single letters represent the conventional code for these amino acids.

RESULTS AND DISCUSSION

The derivatization procedure resulted in the expected product as demonstrated by the CI mass spectrum illustrated in Fig. 1. The molecular mass was identified by the protonated molecular ion at m/z 556 and by the adduct ions having m/z 585 (M + C_2H_5) and m/z 597 (M + C_3H_5). The series of ions characteristic of a dicarboxylic acid ester[10] were augmented by an ion at M − 185 reflecting a tricarboxylic acid ester.

The N-HFB isobutyl ester of Gla was well resolved from the corresponding derivatives of the standard proteic amino acids (Fig. 2) and had a molar response of 0.62 relative to norleucine.

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