Selenolanthionine analysis by ion-exchange chromatography

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A procedure for the chromatographic separation and evidence of selenolanthionine is described. This amino acid is separated on strongly acidic cation exchanger in sodium citrate elution buffer of pH 3.25 on the columns 37 or 62 cm long. Separation from lanthionine and from the other amino acids is good. The dependence of the elution time of selenolanthionine on pH of the buffer has been found. During an acid hydrolysis selenolanthionine is partially (6 h) or completely (24 h) destroyed with the formation of some ninhydrine-positive compounds.

Описан метод хроматографического разделения и определения селенолантионина. Эта аминокислота делится на стиролдивинилбензольном катионите в цитраннатриевом буфере pH 3,25 на колоннах с длиной 37 или 62 см. Разделение от лантионина и от других аминокислот хорошее. Установлена зависимость времени удерживания селенолантионина от pH буфера. В течение кислотного гидролиза селенолантионин частично (6 часов) или полностью (24 часов) подвергается деструкции с образованием некоторых нингидрин-позитивных соединений.

The recognition of the biological role of selenium as a micronutrient and a toxic agent [1—3] stimulated studies of the selenium compounds present in plant and animal tissues or prepared synthetically. Selenoamino acids and related selenoproteins belong to the most important selenium-containing compounds. Besides selenocysteine, selenomethionine, and selenoethionine we also know selenolanthionine (3,3′-seleno-bisalanine). The latter compound has not been found in nature but was prepared by chemical synthesis [4—6]. However, its selenium-free counterpart, lanthionine, occurs in biological material such as wool or microbial peptidoglycans [7, 8].

The increased interest in the above substances is accompanied by a development of procedures for their analysis. Separation of selenoamino acids from other amino acids and their sulfoanalogues usually requires modifications of routine chromatographic procedures. Special approaches must be often used when only one selected amino acid is to be determined.

The separation and determination of selenocysteine, selenomethionine, and selenoethionine has already been reported [9—11]. In this paper we present a method for the separation of selenolanthionine by ion-exchange chromatography. The stability of the amino acid during hydrolysis in 6 M-hydrochloric acid was also studied.

Experimental

Selenolanthionine was synthesized according to *Zdansky* [4, 5]. DL- and *meso*-Lanthionine was purchased from Calbiochem (USA). A standard mixture of amino acids (Lachema, Brno) — lysine, histidine, arginine, aspartic acid, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine — was prepared in a sodium citrate buffer (pH 2.25, 0.2 mol dm^{-3} Na^+). 1 cm^3 of the buffer contained 2.5 μmol of each amino acid. The ninhydrine reagent used was composed of methylcellosolve (1500 cm^3), 4 M-acetate bufer (pH 5.5, 500 cm^3), ninhydrine (15 g), and $SnCl_2 \cdot 2H_2O$ (0.8 g).

Amino acids were separated on an automatic analyzer AAA 881 (Mikrotechna, Prague) operating on three columns (4.5 × 0.8 cm, 37 × 0.8 cm, and 62 × 0.8 cm) packed with Ostion LG KS 0803 using a buffer flow rate of 70 cm^3 h^{-1} and a ninhydrine reagent flow rate of 35 cm^3 h^{-1}. The colorimeters performed at 440 and 570 nm. The rate of registration was one point in 2.5 s. During the analysis the temperature was kept at 52 °C. The columns were regenerated with 0.2 M-NaOH and equilibrated with the elution buffer.

Selenolanthionine stability during hydrolysis in 6 M-HCl *in vacuo* at 110 °C for 6 or 24 h was examined at a concentration 5 mg per 4 cm^3 of the acid. The cooled hydrolyzate was prepared for analysis according to *Spitz* [12] and its aliquot was applied on a column.

Results

As shown in Figs. 1 and 2, selenolanthionine is eluted from the 37 cm long column in the 46th min and from the 62 cm long column in the 69th min, always between proline and glycine. This procedure also separates well selenolanthionine from lanthionine (Fig. 3).

The elution time of selenolanthionine was found to be dependent on pH of the elution buffer. However, the separation of selenolanthionine from proline is impaired at pH values lower than pH 3.25 and from glycine at higher pH values.

The constant of selenolanthionine (the peak area corresponding to 1 μmol [13]) was found to be 38.9 (aspartic acid 53.2, glutamic acid 62.4, glycine 72.7). The ratio of the peak heights recorded at 440 and 570 nm [14] was 0.447.

During heating in 6 M-HCl *in vacuo* at 110 °C for 6 h selenolanthionine is only partially decomposed (10—15 %). The compound is completely destroyed after 24 h heating. In this

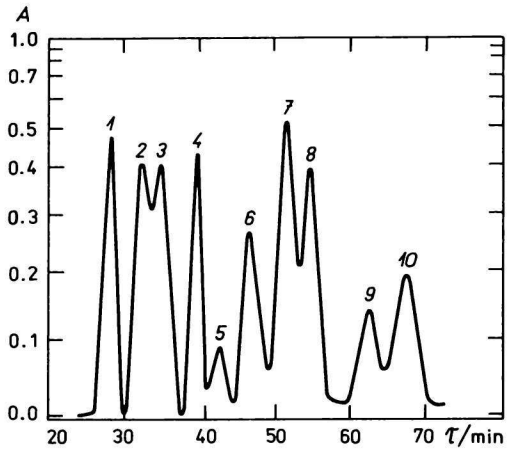

Fig. 1. Separation of a standard mixture of amino acids containing selenolanthionine on a catex column (37 × 0.8 cm) eluted with a pH 3.25 buffer.
1. Aspartic acid; *2.* threonine; *3.* serine; *4.* glutamic acid; *5.* proli *6.* selenolanthioni *7.* glycine; *8.* alanine; *9.* cystine; *10.* valine.

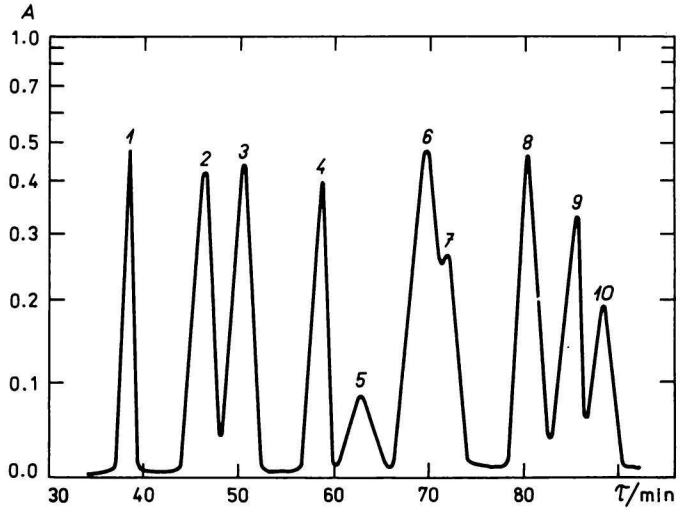

Fig. 2. Separation of a standard mixture of amino acids containing selenolanthionine in a catex column (62 × 0.8 cm) eluted with a pH 3.25 buffer.
1. Aspartic acid; *2.* threonine; *3.* serine; *4.* glutamic acid; *5.* proline; *6.* DL-selenolanthionine; *7. meso*-selenolanthionine; *8.* glycine; *9.* alanine; *10.* cystine.

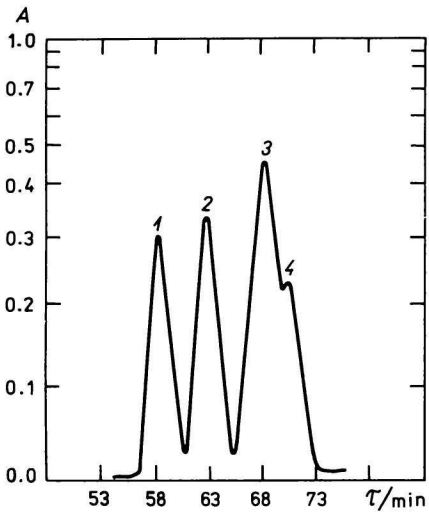

Fig. 3. Record of the separation of lanthionine and selenolanthionine on a 62 cm long catex column eluted with a pH 3.25 buffer. *1.* DL-Lanthionine; *2.* *meso*-lanthionine; *3.* DL-selenolanthionine; *4.* *meso*-selenolanthionine.

case the chromatography revealed a new NH_3 peak (the largest one) and other peaks in the position of glycine and valine.

Discussion

Suitable columns for the determination of selenolanthionine are those having the length of 37 and 62 cm. On column shorter than 30 cm selenolanthionine was not separated from other amino acids even under changed chromatography conditions (temperature and pH of the elution buffer). Selenolanthionine is well separated on the 37 cm column on which, however, some other amino acids are poorly resolved and cannot be determined quantitatively. This concerns particularly the twains threonine — serine and glycine — alanine. The column is therefore suitable for the determination of selenolanthionine itself.

The best results were achieved on the 62 cm long column from which selenolanthionine is eluted as two overlapping peaks. This is obviously due to its existence in two forms, DL and *meso*, which were described in the case of lanthionine. The peak corresponding to the *meso* form of selenolanthionine is the smaller one, which is in accord with the conclusions of *Zdansky* [4] that the synthetic route leads preferably to the DL form. The two forms are not distinguishable on the 37 cm column, but at high concentrations of the compound certain asymmetry of the peak may be observed.

The elution of selenolanthionine is strongly dependent on pH value of the elution buffer. The dependence is not so pronounced with proline and glycine.

Therefore, the precise value of the operating buffer is important particularly when using the shorter column on which the elution times of the amino acids are not long.

Chromatographic analysis confirmed that selenolanthionine undergoes decomposition during heating in 6 M-hydrochloric acid. A peak corresponding to ammonia and three unidentified peaks in the region of glycine and valine were observed on chromatograms. Similar results were reported by *Walter et al.* [10] analyzing a hydrolyzate of selenocysteine. Regarding the similarity between the structure of selenocysteine and selenolanthionine, their decomposition probably involves the same reaction mechanism. The extent of destruction of selenolanthionine during acid hydrolysis has not been so far followed in the presence of other amino acids or with peptides containing selenolanthionine. Behaviour of selenolanthionine in alkaline medium has not been examined either.

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