Amino Acids (1992) 2: 285–288

Amino
Acids

Synthesis of S-farnesyl-L-cysteine methylester and purification by HPLC

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Accepted September 12, 1991

Summary. S-trans, trans-farnesyl-L-cysteine methylester, a post translational modified amino acid, was synthesized from farnesyl bromide and L-cysteine methylester hydrochloride salt in the presence of triethylamine. Its purification as well as separation from the other isomers by HPLC on RP Vydac C_4 and C_8 columns are reported here.

Keywords: Amino acids – S-Farnesyl – Cysteine – HPLC – Separation

Introduction

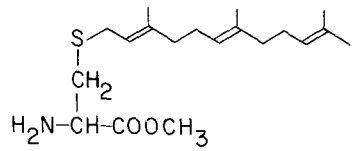
An increasing number of ras proteins, in mammalian system and in yeast, have been found to incur a number of post translational modifications including C-terminal proteolysis of three amino acids, carboxymethylation, polyisoprenylation and palmitoylation (James and Olson, 1990, Tamanoi et al., 1988; Gutierrez et al., 1989; Hancock et al., 1989; Betz et al., 1987).

It has been shown that the C-A-A-X sequence (where C= cysteine, A= aliphatic amino acid and X= any amino acid) directs polyisoprenylation rather than palmitoylation Hancock (1989) in combination with the finding that yeast α-factor precursors are isoprenylated at their C-termini Anderegg et al. (1988).

S-Farnesyl-L-cysteine was identified as a structural component of mating hormone α-factor Anderegg et al. (1988). In addition rhodotorucine A, which induces mating tube formation in *Rhodosporidium toruloides* Kamiya et al. (1978, 1979) as well as tremerogen from *Tremela brasiliensis* Ishibashi et al. (1984) were also found to contain S-farnesyl-cysteine as C-terminus.

The importance of having a simple and routine method for identification of S-farnesyl-cysteine in various peptides and proteins (such as amino acid analysis or determination of primary structure by Edman degradation) is really profound.

Here we report the synthesis as well as the purification by HPLC of S-trans, trans-farnesyl-L-cysteine methylester (main isomer):



Materials and methods

Farnesol, L-cysteine hydrochloride salt were obtained from Serva (Fein Biochimica, Heidelberg/New York). N-Bromosuccinimide, methyl sulfide and triethylamine were obtained from Sigma Chem, Co (W. Germany). All other solvents and reagents used in this paper were analytical grade.

L-Cysteine methylester hydrochloride salt was prepared by the classical procedure used for methyl or ethyl esters of amino acids.

Silica gel F_{254} thin layer plates (20 × 20 cm, d = 0.2 mm) were purchazed from Merck (W. Germany). Thin layer chromatography (TLC) solvent systems used were (ratio by volume): A; n-butanol-acetic acid-water (4:1:5, upper phase), B; chloroform-methanol-acetic acid (55:40:5) and C; chloroform-methanol-acetic acid (85:10:5). Spots were visualized a) by their absorption on UV b) by ninhydrin (0.2 g/100 ml acetone) and c) iodine vapors.

A Gilson HPLC with the columns described in the purification section, was used.

Synthesis

Farnesyl bromide

Prepared from N-bromosuccinimide and farnesol as reported by Corey (1972) with some modifications as follows: N-Bromosuccinimide 1.08 g (6 mmole) in 30 ml of methylene chloride reacted with methyl sulfide 0.5 ml (7 mmole) at 0° C under nitrogen atmosphere. To that mixture further cooled with ice/salt, farnesol 1.33 g (6 mmole) dissolved in the minimum volume of methylene chloride, was added in 10 min time. The reaction mixture was stirred for two hours at that low temperature and then for one hour more at room temperature (still under nitrogen). Pentane was then added to the solution which was further washed with water and brine. Organic layer was evaporated to dryness and the oil residue was chromatographed on a silica gel column using pentane as eluent. Fractions with farnesyl bromide, Rf_C 0.9 were combined and evaporated to give a yellow brown oil (yield 90%).

S-Farnesyl-L-cysteine methylester

L-Cysteine methyl ester hydrochloride salt 340 mg (2 mmole) and triethylamine 0.58 ml (4.2 mmole) were added in 40 ml n-butanol methanol-water (1:1:0.5). Farnesyl bromide 600 mg (2.1 mmole) was added to the solution which was stirred at room temperature for 24 hrs.

The mixture was diluted with water and extracted with n-butanol (3 × 50 ml). The emulsion formed in the interphase was removed with inorganic layer. Organic layers were combined, dried over $MgSO_4$ for 5 min, filtered off and evaporated to dryness to give a yellowish oil. Thin layer chromatography of the oil in the systems reported above showed the presence of one major spot of Rf_A 0.5 and Rf_B 0.6 respectively, and other minor ones of Rf_A 0–1. This oil called henceforth crude S-farnesyl-L-cysteine methylester was further purified and its isomers were separated by HPLC as it follows:

Purification by HPLC of S-trans, trans-farnesyl-L-cysteine methylester

An RP-Vydac C_4 column (250 × 4 mm) and a methanol (B)-water (A) gradient (45% B in 25 min and 100% in 60 min) was used. The flow rate was 0.75 ml/min, Aufs 0.2, A_{254}, and chart speed 2mm/min. Peaks with S-farnesyl-L-cysteine methylester were rechromatographed on a second more hydrophobic RP-column C_8, 5μm Exsil 300 A (250 × 4 mm). The gradient methanol (B)-water (A) was distributed as follows: 40% B in 20 min, 50% B in 35 min, 60% B in 45 min 70% B in 60 min and 100% in 70 min. All other conditions were as in the case of Vydac C_4 column.

Results and discussion

S-Farnesyl-L-cysteine methylester (I) was prepared from farnesyl bromide and cysteine methylester hydrochloride salt as reported by Kamiya et al (1978) with the modifications indicated above.

Farnesol (3,7,11-trimethyl-2,6,10-dodecatrien-1-ol) has four isomers, depending on the geometry of the double bonds at C-2 and C-6. Commercially available farnesol (Serva) comes as mixed isomers with the trans, trans one as the main constituent (around 90%). These isomers can be separated by GC as their TMS derivatives, Kamiya et al. (1979).

Pure trans, trans farnesol can be prepared by the method of Oguni and Uritani (1969). In our experiments we escaped this step, by using the commercially available farnesol. The farnesyl bromide synthesized according to Corey et al. (1972) with slight modifications was a mixture of all isomers too.

This mixture reacted with cysteine methylester hydrochloride salt in the presence of triethylamine. The yield of the reaction varied from 35 to 45%. Crude S-farnesyl-L-cysteine methylester, which upon thin layer chromatography showed the presence of various impurities was purified by HPLC on RP Vydac C_4 column.

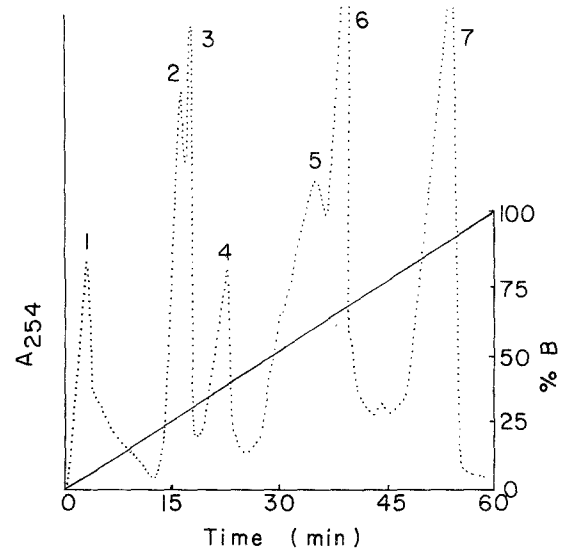


Fig. 1. HPLC analysis of crude S-farnesyl-L-crysteine methylester preparation on RP Vydac C_4 column

Fig. 1 shows a typical chromatogram obtained by this column. Peaks were identified by thin layer chromatography in the systems mentioned above. Peaks 5 and 6 were found to contain S-farnesyl-L-cysteine methylester. These fractions were further analyzed on a second more hydrophobic column C_8. S-trans, trans-Farnesyl-cysteine methylester was eluted with methanol gradient of 85%. The other three isomers (not characterized yet) were eluted with methanol gradient of 60–70%.

In conclusion, the synthesis of small peptides containing S-trans, trans-farnesyl-L-cysteine methylester as well as further characterization of the isolated isomers are in progress and will be reported elsewhere.

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Received August 5, 1991