The Evidence for the Production Mechanism of cis-S-1-Propenylcysteine in Aged Garlic Extract Based on a Model Reaction Approach Using Its Isomers and Deuterated Solvents

Yukihiro Kodera, Toshiaki Matsutomo, Kenji Itoh
Drug Discovery Laboratory, Wakunaga Pharmaceutical Co., Ltd., Akita, Japan

Abstract

cis-S-1-Propenylcysteine has been identified in aged garlic extract, however, its production mechanism is still unclear. The content of cis-S-1-propenylcysteine in aged garlic extract at 22 months of aging was more than twice the molarity of cis-S-1-propenylcysteine in raw garlic, a plausible precursor of cis-S-1-propenylcysteine. We found that cis-S-1-propenylcysteine was generated through the isomerization of trans-S-1-propenylcysteine in the model reactions. These results suggested that the isomerization was induced by the electron-releasing conjugative effect of the sulfur atom in S-1-propenylcysteine.

Key words
Allium sativum · Amaryllidaceae · aged garlic extract · isomerization · cis/trans-S-1-propenylcysteine

Abbreviations

AGE: aged garlic extract
cis-S1PC: cis-S-1-propenylcysteine
13C-NMR: carbon 13 nuclear magnetic resonance
D-NMR: deuterium nuclear magnetic resonance
GS1PC: γ-glutamyl-cis-S-1-propenylcysteine
1H-NMR: proton nuclear magnetic resonance
HPLC: high performance liquid chromatography
LC: liquid chromatography

Results and Discussion

S-Alk(en)ylcysteine sulfoxides and γ-glutamyl-S-alk(en)ylcysteines were found in garlic as its characteristic sulfur storage compounds [1]. Over two decades, many scientists have come to pay more attention to S-alk(en)ylcysteines, such as S-methylcysteine, S-allylcysteine, and trans-S1PC, which are produced from γ-glutamyl-S-alk(en)ylcysteines [1,2]. Recently, we have reported that cis-S1PC is included in AGE [3]. The content of cis-GS1PC, a plausible precursor of cis-S1PC, in raw garlic was less than 50% of cis-S1PC content in AGE on a molar basis [3,4]. These differences suggested that cis-S1PC was produced not only from the precursor, γ-glutamyl peptide, but also from the other compounds. Recently, we demonstrated that cis-S1PC was produced from trans-S1PC through the isomerization reaction [3]. However, the mechanism of isomerization reaction is unclear. To reveal the production mechanism of cis-S1PC, we used the model reaction in which S1PC was incubated in the mixture of deuterated solvents to detect the hydrogen-deuterium exchange within the propenyl group.

Fig. 1 The chemical structures of cis-GS1PC and trans-GS1PC, and mass chromatogram of cis/trans-GS1PC in LC-MS analysis. A. The chemical structures of cis-GS1PC (1) and trans-GS1PC (2). B. Mass chromatogram of authentic 1 and 2. C. Mass chromatogram of 1 and 2 in AGE.
period, and reached about 5% of content in raw garlic after 22 months of aging (Table 1) [3]. The content of cis-S1PC in AGE gradually increased and reached to more than twice the molarity of cis-S1PC in raw garlic during the 22-month aging period, 1.80 ± 0.01 µmol/g (dry-AGE) [3].

We hypothesized that cis-S1PC is produced via isomerization of its trans form. As a result of an experiment using the model reaction, we found that cis-S1PC was produced from trans-S1PC by the isomerization reaction, although its mechanism was unclear [3]. Divalent sulfides have the electron-releasing conjugative effect when an unsaturated group or carbonium ion directly binds to sulfur [5, 6]. This causes the cleavage of the π bond between C1 and C2 in the unsaturated group. The isomerization reactions in the deuterated solvents were performed to confirm the cleavage of the C1–C2 bond in the propenyl group. The analyses of reaction mixtures were performed by LC-MS under the selected ion monitoring trace of monodeuterated S1PC (S1PC-d, C₈H₁₀D₉N₅S; [M + H]+ = m/z 163.0646 ± 0.0016). The selected ions of S1PC-d, both the cis form and trans form, were detected in the reaction mixtures, and the ratios of cis/trans forms were cis/trans = 24.2/75.8 (Fig. 2A) and cis/trans = 25.1/74.9 (Fig. 2B). However, multideuterated compounds, such as dideuterated S1PC, were not observed (data not shown). The mass signal intensity of S1PC-d was less than 10% of that of non-deuterated S1PC.

1H-NMR spectra of isomerization reaction mixtures were measured at day 0, 5, 15, and 30. The proton signals of C1, C2, and C3 in the propenyl group were gradually changed, and the signals of both cis- and trans-S1PC were detectable in the reaction mixtures after 5 days during the 30-day incubation period, while only one of the signals was observed in each mixture before incubation (Fig. 3). These results indicated that the isomerization between the cis form and trans form is reversible. We also performed the 1H-NMR and D-NMR analyses of the deuterated S1PC to confirm the deuterated position of S1PC. However, the change of proton integration by the deuteration was not clearly observed in the 1H-NMR spectra. The deuteron signals of deuterated S1PC were also not detected in the D-NMR spectra (data not shown).

Although the deuterated position in S1PC under the model reaction using deuterated solvents was not confirmed by NMR analysis, the detection of a mass signal of monodeuterated cis/trans-S1PC suggested that more than half a portion of cis-S1PC in AGE is produced from the trans form by the electron-releasing conjugative effect of a sulfur atom in the S-propenyl group (Fig. 4) [5, 6]. These results can help us understand the complex sulfur chemistry in the processing of garlic such as the aging process.

### Materials and Methods

**Chemicals and reagents**

Chemicals for synthesis were obtained from Tokyo Chemical Industry. Solvents for LC, LC-MS, and NMR analyses were purchased from Wako Pure Chemicals Industry. γ-Glutamyl-cis/trans-S-1-propenylcysteines were synthesized and purified according to previous reports [7–10].

**Analyses of γ-glutamyl-cis/trans-S-1-propenylcysteines in raw garlic and aged garlic extract**

AGE was prepared according to a previous report [11]. The contents of cis/trans-GS1PC were analyzed according to a previous report [3]. Identification of cis/trans-GS1PC was performed by LC-MS.

**Isomerization reaction of S-1-propenylcysteine**

cis-S1PC and trans-S1PC were dissolved in deuterium oxide separately, and acetic acid-d₄ was added to each solution to adjust the pH to be between 3 and 5. The solutions were dispersed into glass sample vials and NMR tubes, and stored at 60°C. The solutions in the glass vials were lyophilized after incubation for 30 days. The isomers of S1PC in the solution were isolated by preparative HPLC according to the previous report [3], and analyzed by LC-MS and NMR. 1H-NMR analyses of the solutions in the NMR tubes were performed on days 0, 5, 15, and 30.

**Liquid chromatography-mass spectrometry analysis**

The LC-MS analysis was carried out on a system consisting of an Ultimate 3000 (Dionex-Thermo Fisher Science) coupled to a Q-Exactive (Thermo Fisher Scientific). The column utilized for separation was a Cadenza CD-C18 column (2.0 mm × 150 mm, 3 µm, Intakt Corporation) with a flow rate of 0.2 mL/min. The chromatographic separation of cis/trans-GS1PC was achieved using a mobile phase consisting of water containing 0.1% (v/v) formic acid (solvent A) and acetonitrile (solvent B) with isocratic elution (A : B = 97 : 3, v/v). The qualitative analysis of S1PC-d was performed using a mobile phase consisting of water containing 0.3% (v/v) heptafluorobutyric acid (solvent A) and 80% (v/v) methanol containing 0.3% (v/v) heptafluorobutyric acid (solvent B) with isocratic elution (A : B = 73 : 27, v/v). The MS analysis was carried out using the following conditions: ionization mode: ESI+ (positive mode), mass range: m/z 50–750, resolution: 70000, maximum IT: 200 ms, isolation width: 4.0 m/z. The quantitative analysis of cis/trans-GS1PC was carried out by the LC method according to a previous report [3].

**Nuclear magnetic resonance analysis**

1H-, D-, and 13C-NMR spectra of the compounds were taken in D₂O, D₂O containing acetic acid-d₄, and H₂O on a VNMRS-500 spectrometer (VARIAN, Inc.) at 500 MHz and 125 MHz, respectively.
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Conflict of Interest

The authors report no conflict of interest.

Fig. 2 Chromatogram of LC-MS analysis for model reaction samples using deuteride solvent. A Mass chromatogram of monodeuterated cis/trans-S1PC on the isomerization sample using the trans form. B Mass chromatogram of monodeuterated cis/trans-S1PC on the isomerization sample using the cis form.

Fig. 3 Change of $^1$H-NMR signals of S-propenyl groups in cis/trans-S1PC. A–D $^1$H-NMR for C1, C2, and C3 in the S-propenyl groups of cis/trans-S1PC in the reaction mixture using cis-S1PC (A 0 days, B 5 days, C 15 days, D 30 days). E–H $^1$H-NMR for C1, C2, and C3 in the S-propenyl groups of cis/trans-S1PC in the reaction mixture using trans-S1PC (E 0 days, F 5 days, G 15 days, H 30 days).

Fig. 4 Production mechanism of cis-S1PC through isomerization from the trans form.
References


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Correspondence

Dr. Yukihiro Kodera
Drug Discovery Laboratory
Wakunaga Pharmaceutical Co., Ltd.
1624 Shimokotachi
Koda-cho, Akitakata-shi
Hiroshima 739–1195
Japan
Phone: + 81 8 26 45 23 31
Fax: + 81 8 26 45 43 51
kodera_y@wakunaga.co.jp

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