Biotransformation of natural polyacetylene in red ginseng by Chaetomium globosum


Keywords: Chaetomium globosum Detoxification Fermented red ginseng NO inhibition Polyacetylene

Abstract

Background: Fermentation has been shown to improve the biological properties of plants and herbs. Specifically, fermentation causes decomposition and/or biotransformation of active metabolites into high-value products. Polyacetylenes are a class of polyketides with a pleiotropic profile of bioactivity.

Methods: Column chromatography was used to isolate compounds, and extensive NMR experiments were used to determine their structures. The transformation of polyacetylene in red ginseng (RG) and the production of cazaldehyde B induced by the extract of RG were identified by TLC and HPLC analyses.

Results: A new metabolite was isolated from RG fermented by Chaetomium globosum, and this new metabolite can be obtained by the biotransformation of polyacetylene in RG. Panaxytriol was found to exhibit the highest antifungal activity against C. globosum compared with other major ingredients in RG. The fungus C. globosum cultured in RG extract can metabolize panaxytriol to Metabolite A to survive, with no antifungal activity against itself. Metabolites A and B showed obvious inhibition against NO production, with ratios of 42.75 ± 1.60 and 63.95 ± 1.45% at 50 μM, respectively. A higher inhibitory rate on NO production was observed for Metabolite B than for a positive drug.

Conclusion: Metabolite A is a rare example of natural polyacetylene biotransformation by microbial fermentation. This biotransformation only occurred in fermented RG. The extract of RG also stimulated the production of a new natural product, cazaldehyde B, from C. globosum. The lactone in Metabolite A can decrease the cytotoxicity, which was deemed to be the intrinsic activity of polyacetylene in ginseng.

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1. Introduction

Red ginseng (RG), a widely used traditional herbal medicine, is reported to have enhanced pharmacological activities and stability compared with fresh ginseng because of changes in its chemical constituents [1], and it is one of the most popular herbal medicines to be used as a dietary supplement in recent years. Ginseng contains various pharmacologically active compounds such as ginsenosides, polysaccharides, polyacetylenes, and phytosterols. Among these compounds, ginsenosides are considered major bioactive compounds [2]. The fermentation process has been shown to improve the biological properties of plants and herbs. More specifically, fermentation causes decomposition and/or biotransformation of active metabolites into high-value products [3]. Previous work has reported that ginseng modified by fungal and bacterial fermentation has improved bioavailability and increased the amount of bioactive components [4]. However, only a few studies focused on the metabolites from fermented red ginseng (FRG), and most of this research focused on the metabolic mechanism of ginsenosides [5]. Therefore, in the present work, we investigated the compounds from RG fermented by Chaetomium globosum. Chaetomium is considered to be a rich source of active compounds. These metabolites produced by Chaetomium belong to diverse chemical groups, including chaetoglobosins, xanthones, anthraquinones, chromones, epidosides, terpenoids, and steroids, and many of these compounds showed enzyme inhibitory,

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cytotoxic, antitumor, antimalarial, antibiotic, and other activities [6]. A new metabolite (Metabolite A) associated with RG was isolated from FRG, and this is the first report on the biotransformation of natural polyacetylene. Polyacetylenes are a class of polyketides that occur in plants, fungi, marine organisms, and animals. These compounds show a pleiotropic profile of bioactivity [7,8]. A series of biologically active polyacetylenes were isolated from the Panax ginseng root. Among them, panaxynotriol (PXT) was reported to have in vitro cytotoxic activity against a range of human tumor cells [9]. The PXT-based compounds are markedly able to alleviate the toxic and neuropathy-inducing side effects associated with chemotherapeutic and radiation-based anticancer treatments [10]. A new natural product, cazaldehyde B (Metabolite B) (Fig. 1), was also found in this work, and it was proved to be produced by C. globosum through the induction of RG extract.

2. Materials and methods

2.1. General experimental procedure

Silica gel (200–300 mesh; Qingdao Marine Chemical Group Co., Shandong, China), the TLC plate (Qingdao Marine Chemical Group Co., Shandong, China), Lichroprep RP-18 (Beijing Greenherbs Science and Technology Development Co., Beijing, China), and Sephadex LH-20 (GE Healthcare Co., Buckinghamshire, UK) were used for column chromatography and chromatography analysis. 1D and 2D NMR spectra were obtained on Bruker AVANCE 500 MHz NMR instruments (Bruker, Karlsruhe, Germany). MS spectra were recorded with Agilent G3250AA (Agilent, Santa Clara, CA, USA) and AutoSpec Premier P776 spectrometers (Waters, Milford, USA). Optical rotations were obtained on a Jasco P-1020 polarimeter (Tokyo, Japan). HPLC analyses were performed on a Waters e2695 instrument (Milford, USA) with Agilent ZORBAX SB-C18 (Santa Clara, USA). Circular dichroism spectra were obtained on an Applied Photophysics Chirascan spectrometer (Applied Photophysics Ltd., Surrey, United Kingdom).

2.2. Chemicals

PXT and two ginsenosides, ginsenoside Rg3 and ginsenoside Rh1, were isolated from RG, and their structures were determined by NMR.

2.3. Biological material and cultivation of the fungal strain

RG was obtained from the Jianzhijia Pharmacy of Kunming in Yunnan Province in China. The fungus was isolated from Dendrobium officinale in Honghe in Yunnan Province. The species was identified as C. globosum (GenBank accession no. KX926579) on the basis of morphological and genetic internally transcribed spacer (ITS) characteristics. A voucher specimen was deposited at the School of Chemical Science and Technology, Yunnan University. The fermentation of C. globosum was carried out in 0.5-L Erlenmeyer flasks containing 120 mL of potato dextrose broth (PDB; potato infusion of 200 g of fresh potato, 15 g of dextrose, and 1.0 L of distilled water; pH 7.0) at 150 rpm and 28°C for 2 days for a seed culture. Aliquots of 20–25 mL of seed culture were transferred to a 1.0-L Erlenmeyer flask containing 200 mL of RG extract (5.5 kg of RG, 30 L of distilled water; pH 7.0) and incubated at 150 rpm and 28°C for 7 days. The routine fermentation of C. globosum was carried out in 0.5-L Erlenmeyer flasks containing 120 mL of PDB (potato infusion of 200 g of fresh potato, 15 g of dextrose, and 1.0 L of distilled water; pH 7.0) with the same method as that used for FRG. The precursor PXT was added in the routine fermentation at a concentration of 2 mg/200 mL.

2.4. Extraction and isolation

The culture medium was filtered to remove mycelia. The filtrate was partitioned with ethyl acetate (EtOAc) to produce an EtOAc layer and then dried under vacuum to afford a dark brown residue (21.6 g). The extract was subjected to column chromatography on silica gel and was eluted with a gradient of CH2Cl2–MeOH from 1:0 to 0:1 (v/v) to obtain five fractions (A–E). Fraction B (1.8 g) was separated using Sephadex LH-20 (CH2Cl2–MeOH at 1:1) to obtain seven subfractions (B1–B7). Fraction B1 was purified by Lichroprep RP-18 column chromatography with MeOH–H2O (80%) to produce Metabolite B (10 mg). Fraction B2 was separated repeatedly using Sephadex LH-20 (CH2Cl2–MeOH at 1:1) to obtain five subfractions (B21–B25). Fraction B23 was purified by Lichroprep RP-18 column chromatography with MeOH–H2O (20%) to obtain Metabolite A (7 mg).

Metabolite A. [α]22 D -78.5 (c 0.2, MeOH). High Resolution Electrospray Ionization Mass Spectrometry (HRESI-MS) m/z: 257.0785 [M+Na]+, calcd for C13H14O4Na+: 257.0790. 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz) in Table 1.

Metabolite B. HRESI-MS m/z: 301.1409 [M+Na]+, calcd for C16H22O4Na+: 301.1416. 1H-NMR (CDCl3, 500 MHz) and 13C-NMR (CDCl3, 125 MHz) in Table 2.

2.5. TLC and HPLC analysis method

The extracts of RG, FRG, and routine fermentation products using the PDB medium were obtained according to the aforesaid method. Similar sampling volumes of RG, FRG, routine fermentation products, PXT, and Metabolites A and B were prepared for use in the TLC test. TLC was used for qualitative identification of PXT and Metabolites A and B in extracts of RG, FRG, and routine fermentation products, using CHCl3–MeOH (10:1) as a developing solvent. The visualization reagents were iodine vapor and sulfuric acid–anisaldehyde.

Fig. 1. Structures of botanical Metabolite A associated with red ginseng from fermented red ginseng by biotransformation of endophytic Chaetomium globosum, and fungal Metabolite B produced by C. globosum induced by RG extract.

RG, red ginseng.
Bioactive assay

with a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay for cell viability. L-monomethylarginine (L-NMMA) was used as the positive control with a ratio of 54.07 ± 1.65% at a concentration of 50 μM.

The cytotoxicity of Metabolites A and B against tumor cells, HL-60, A-549, SMMC-7721, SW480, and MCF-7, was assessed in vitro by means of a 3-(4,5-dimethylthiazol-2-yl)-2(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium assay. The positive control of Taxol was used with IC50 < 0.008 μM.

3. Results and discussion

3.1. Structural elucidation

Metabolite A was obtained as a yellow oil and was determined to be C13H14O4 from its distortionless enhancement by polarization transfer (DEPT) spectrum and HRESI-MS (m/z 257.0785 for [M+Na]+). Analysis of its 13C-NMR data indicated the presence of one CO at δ: 176.9; one olefinic methine at δ: 135.9; one olefinic methylene at δ: 117.3; three methines at δ: 80.8, 71.6, and 63.5; two alkynyls at δ: 77.0, 75.2, 70.7, and 67.0; and three methylenes at δ: 24.2, 24.7, and 24.4. Based on the 7 degrees of unsaturation of C13H14O4, an additional ring existed in this compound. The analysis of correlation spectroscopy data revealed the C1-C2-C3 fragment and the C9-C8-C10-C11-C12 structural connection. The heteronuclear multiple bond correlations from H-3 to C-1, C-2, C-4, and C-5; H-8 to C-6, C-7, and C-10; H-9 to C-7; H-11 to C-10; H-11 to C-12 and C-13; and H-12 to C-13 confirmed the structure of Metabolite A (Fig. 2). The connection of C-10 and C-13 was confirmed by a downfield shift of H-10 and C-10 at 4.63 (m) and 80.8. Because of the high degree of similarity between the structures of Metabolite A and PXT, a crocin polyacetylene frequently found in ginseng. Metabolite A should be biotransformed from polyacetylene in RG by microbial fermentation (Fig. 3).

The 1H-NMR values, with OR values [α]D = 12.3 for (3R, 9R, 10R)-PXT and [α]D = 25.3 for (3S, 9R, 10R)-PXT [13]. The configuration of Metabolite A at C-3 was determined by comparing the NMR and OR values with those of PXT, with the biogenesis of Metabolite A being biotransformed from PXT. The OR value at -78.5 and circular dichroism spectrum of Metabolite A also confirmed the absolute configuration by comparing with those of natural PXT ([α]D -18.4) (Fig. S18, S19) [9]. The chemical transformation failed to determine the configuration for the low amount of Metabolite A.

Metabolite B was determined to be cazaldehyde B, which has been previously produced by synthetic biology methods [14]. The

![Fig. 2. 1H-1H correlation spectroscopy (COSY) correlations and the selected heteronuclear multiple bond correlation (HMBC) correlations of new metabolite (Metabolite A) produced from fermented red ginseng by the microorganism biotransformation of polyacetylene.](image-url)
The plausible biotransformation of Metabolite A produced from fermented red ginseng by microorganism biotransformation of C. globosum. RG, red ginseng.

3.2. The detoxication mechanism of C. globosum against the inhibition of active compounds from RG and evaluation of inhibition of NO and cytotoxicity of Metabolites A and B

In FRG, because the active metabolites in RG may affect the growth of C. globosum, C. globosum may possess a detoxication mechanism against such inhibition. To study the interaction of the active ingredients from RG and C. globosum in the FRG system, the antifungal activities of the new metabolite (Metabolite A), the main ginseng ingredients (PXT, ginsenoside Rg3, and ginsenoside Rh1) thought to inhibit C. globosum growth were investigated. PXT showed the highest antifungal activity with an MIC of 128 μg/mL, compared with those of Metabolite A (MIC > 512 μg/mL) and two ginsenosides (MIC > 512 μg/mL). These results suggest that C. globosum can biotransfer PXT to Metabolite A with less toxicity than PXT for its own survival. Metabolites A and B showed obvious inhibitions against NO production with ratios of 42.75 ± 1.60 and 63.95 ± 1.45% (IC50 at 40.12 ± 0.85 μM) at concentrations of 50 μM, respectively. A higher inhibitory rate on NO production was found in Metabolite B than in a positive drug. However, Metabolites A and B exhibited no obvious cytotoxicity at 40 μM.

3.3. The analysis of Metabolites A and B and PXT in different culture conditions by TLC and HPLC tests

Based on the TLC test, the new metabolite (Metabolite A) was found to be negative in RG extract but positive in FRG. PXT was positive in both RG and FRG extracts (Fig. S11, S12, S14 and S15). Polyacetylenes, including PXT in FRG, were not isolated; thus, Metabolite A was obtained by microbial fermentation. Metabolite B was positive in FRG extract but negative in C. globosum cultured in the PDB medium (Fig. S13). Thus, the RG extract induced the production of cazaldehyde B (Metabolite B). The content of PXT in the RG extract was 0.872 mg/mL by HPLC analysis (Fig. S16).

3.4. The biotransformation of polyacetylene in FRG

PXT is a major product from ginseng that exhibits significant cytotoxicity. The new metabolite (Metabolite A) was isolated from FRG, and the structures of Metabolite A and PXT exhibited high similarity. We also confirmed through TLC and HPLC analyses that Metabolite A does not exist in the RG extract (Fig. S11, Fig. S14). To study the biotransformation of polyacetylene by C. globosum, we added the precursor PXT to C. globosum cultured in the PDB medium. However, HPLC analysis showed that Metabolite A and PXT did not exist in the extract of the PDB medium using PXT as a precursor (Fig. S14, S15). Furthermore, Metabolite A could not be isolated by column chromatography in this medium (1 L sample). This suggests that the biotransformation from PXT to Metabolite A by C. globosum occurs in the RG medium. The genus Chaetomium has been shown to transfer other natural products to its oxylate in previous works [16,17].

4. Conclusion

The new metabolite (Metabolite A) is a rare example of a natural polyacetylene biotransformed by microbial fermentation. This biotransformation only occurs in FRG. The RG extract also stimulated the production of a new natural product, cazaldehyde B, from C. globosum. The fungus C. globosum cultured in RG extract can metabolize PXT to Metabolite A to survive with no antifungal activity against itself. The lactone in Metabolite A can decrease the cytotoxicity, which was deemed to be the intrinsic activity of polyacetylene in ginseng. Metabolites A and B showed obvious inhibition against NO production with ratios of 42.75 ± 1.60 and 63.95 ± 1.45% at 50 μM, respectively.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jgr.2019.06.007.

References