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The stability and degradation mechanism of sulforaphene in solvents

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ABSTRACT

Sulforaphene, a natural compound, has been investigated as a potential anticancer agent. However, the stability of sulforaphene, in various solvents, and its degradation pathway have not been appropriately reported. This instability impairs the preparation process, the biological evaluation experiments, and the applications of sulforaphene. In this study, the stability of sulforaphene stored at 26 °C was investigated in each of the following six solvents: two kinds of protic solvents (methanol and ethanol) and four kinds of aprotic solvents (acetonitrile, dichloromethane, ethyl acetate and acetone). Sulforaphene was found to be stable in aprotic solvents and unstable in the protic solvents. The degradation products of sulforaphene in protic solvents (methanol and ethanol) were purified by the preparative HPLC and identified by ESI/MS and NMR (^{1}H NMR). The degradation pathways of sulforaphene in methanol and ethanol were proposed. It was found that sulforaphene was degraded into two kinds of structural isomer in alcohols.
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1. Introduction

Studies have shown that consumption of cruciferous vegetables could reduce the risk of certain cancers, such as breast cancer (Ambrosone et al., 2004), prostate cancer (Joseph et al., 2004), lung cancer (Neuhouser et al., 2003; Voorips, Goldbohm, Verhoeven, et al., 2000), colorectal cancer (Voorips, Goldbohm, van Poppel, et al., 2000) and pancreatic cancer (Chan, Wang, & Holly, 2005) in humans. This is because these vegetables, including radish (*Raphanus sativus* L.), broccoli, cauliflower, kale, and Chinese cabbage, are rich in glucosinolates (Daxenbichler et al., 1991; Fahey, Zhang, & Talalay, 1997; Rosa, Heaney, Fenwick, & Portas, 1997). When the vegetables were ground, chewed or chopped, glucosinolates, which are stored in the plant vacuole, were released by the catalysis of myrosinase (ab-d-thioglucosidase), resulting in a variety of breakdown products (Kelly, Bones, & Rossiter, 1998; Bones, Thangstad, Haugen, & Espevik, 1991). The unstable thiohydroximate-O-sulfonate, one of the breakdown products, was then converted to the isothiocyanate in the Lossen rearrangement, or decomposed to the corresponding nitrile and elemental sulfur (Holst & Williamson, 2004; Franziska, Evelyn, Monika & Sascha, 2014). Isothiocyanates, the pungent flavor of several Brassica vegetables, were responsible for the strong anti-cancer activity and cardiovascular diseases (Beevi, Mangamoori, Subathra, & Edula, 2010).

Sulforaphene (4-methylsufinyl-3-butenyl isothiocyanate), derived from radish (Kuang, Song, Yuan, Yi, et al., 2013), has been considered as an anticancer agent (Papi, Orlandi, Bartolini, Barillari, & Iori, 2008). Research suggested that sulforaphene was able to induce apoptosis, in A549 cancer cell lines, by inhibiting tubulin polymerization (Wang et al., 2010). It was reported that sulforaphene could inhibit cell proliferation, in a dose-dependent manner, in K562 (O' Hare et al., 2011), HCT-116 (Pocasap, Weerapreeyakul, & Barusrux, 2013), LoVo, and HT-29 cancer cell lines (Papi et al., 2008). The cell proliferation of human and murine erythroleukemic cells, human T-lymphoid cells, human cervix carcinoma cells and H3-T1-1 cells (Nastruzzi et al., 2000) could be reduced by sulforaphene. Furthermore, sulforaphene has been proven to have 1.3–1.5 times stronger antimutagenicity activity than sulforaphane (SFN) (Shishu & Kaur, 2009).

A previous study has shown that sulforaphene was sensitive to heat (Tian et al., 2015) and could react with water forming a dimer of sulforaphene (Tian et al., 2016).

Few studies have reported about the stability of sulforaphene in various solvents and its degradation products. Different solvents were used either in the sulforaphene preparation process (Kuang, Song, Yuan, Yi, et al., 2013; Kuang, Song, Yuan, Lv, et al., 2013) or the biological evaluation experiments (Papi et al., 2008; Shishu & Kaur, 2009; Wang et al., 2010). The residual solvents were detected in the sulforaphene samples. It is believed that the study of the stability of sulforaphene in various solvents and its degradation products was of primary importance. Reports about the reactions of

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isothiocyanates could provide some reference to study the stability and degradation pathways of sulforaphene in solvents. It was reported that allyl isothiocyanate could interact with amino groups of lysine to form thiourea derivative and phenolic groups of tyrosine residues (Kishore, Murthy, & Rao, 1986). Other researchers have reported that allyl isothiocyanate could be attacked by the sulfhydryl group to form dithiocarbamate (Cejpek, Urban, Vel, & Hrabcovi, 1998). Furthermore, sulforaphane was found to form N, N′-di-(methylsulfinyl) butyl thiourea in an aqueous solution (Jin, Wang, Rosen, & Ho, 1999). The aforementioned degradation pathways of isothiocyanates lay a good foundation for this study.

The object of the present work was to study the stability of sulforaphene in methanol, ethanol, acetonitrile, dichloromethane, ethyl acetate and acetone during the storage at 26 °C. The resulting degradation products of sulforaphene in methanol and ethanol were separated by preparative HPLC and their structures were identified. Based on the structures of the isolated compounds, the degradation pathway of sulforaphene in protic solvents (methanol and ethanol) was proposed.

2. Materials and methods

2.1. Materials

Sulforaphene (purity >95%) was prepared and purified from radish seeds, by preparative high-performance liquid chromatography (HPLC). Moreover, its purity and chemical structure were identified by analytical HPLC, ESI-MS and NMR (Kuang, Song, Yuan, Yi, et al., 2013). Methanol and trifluoroacetic acid (TFA), used for analytical and preparative HPLC, were of HPLC grade and purchased from Fisher Scientific Co., LTD (Tustin, CA). Ultra-pure water was obtained by Q Millipore System (Millipore, Bedford, MA). All other reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

The analytical HPLC equipment was a Shimadzu LC-20AT system with two LC-20AT solvent delivery units, a SPD-M20A DAD detector and an analytical reversed phase C18 column (250 mm × 4.6 mm × 5 μm, Dikma). The preparative HPLC equipment used in the preparation experiment was a Waters Prep 4000 liquid chromatography system equipped with a 2487 dual-wavelength absorbance detector and a preparative reversed phase C18 column (19 × 300 mm, 7 μm, Symmetry Prep). Electrospray ionization mass spectrum (ESI/MS) were recorded by a Waters Xevo G2 Qt Liquid Chromatography–Mass system at 25 eV from 50 m/z to 500 m/z. NMR spectrum of the degradation product were obtained on a Bruker high-resolution AV600 NMR spectrometer.

2.2. The stability of sulforaphene in various solvents

The studied solvents containing sulforaphene at the concentration of 2 mg/ml were placed in 2 ml sealed glass sample bottles. The samples were placed at 26 °C in a drug stability test chamber and taken out daily to analyze for a week. The collected samples were filtered by 0.22 μm membrane filter. The filtrate was tested by reversed-phase HPLC system. The mobile phase system consisted of methanol as mobile phase A and 0.02% (v/v) TFA in ultra-pure water as mobile phase B. A linear change of methanol remained from 20% to 80% in first 20 min, and then raised to 100% methanol immediately and maintained for 2 min to purge the column. The flow rate was 1 ml/min and the detection wavelength was 254 nm. The temperature of the column oven was 30 °C. All samples were tested in triplicate.

2.3. Separation and purification of the degradation product

Sulforaphene (1 g) with the purity of 95% at the concentration of 75 mg/ml in the methanol and ethanol were placed at 26 °C in a drug stability test chamber for 7 days to produce the degradation product, respectively. To obtain the pure degradation product, the degradation product-rich mixture diluted with ultra-pure water, to 25 mg/ml, was subjected into the preparative HPLC system after being filtrated with 0.22 μm of membrane. The preparative HPLC parameters of the experiment were as follows: the mobile phase system consisted of 35% (v/v) or 25% methanol in ultrapure water, the flow rate was 10 ml/min, the detection wavelength was 254 nm and the injection volume was 2 ml. The peak fraction of the degradation product was collected manually according to the preparative HPLC chromatogram, and then dried at 45 °C under vacuum, with a rotator evaporator. Then the product was freeze-dried.

2.4. ESI/MS and NMR

The identification of the degradation product was carried out by ESI/MS and NMR (^{1}H NMR). The purified degradation product with 100 ppm was detected by ESI/MS with an ion source temperature of 200 °C and a probe temperature of 25 °C. Deuterium oxide and deuterated DMSO, with 550 μl containing 20 mg of the degradation product in methanol and ethanol, was subjected to a Bruker high resolution AV600 NMR spectrometer at 600 MHz using tetramethylsilane (TMS) as internal standard.

3. Results and discussion

3.1. The storage stability of sulforaphene in various solvents

Since solvents dissolving sulforaphene were always involved in the biological evaluation experiments (Talalay et al., 2007) and preparation process (Kuang, Song, Yuan, Yi, et al., 2013; Kuang, Song, Yuan, Lv, et al., 2013), the study of the stability of sulforaphene in various solvents is needed. The knowledge of the stability in various solvents would allow the appropriate solvent for storage to be chosen. According to the solvents used in the preparation process and biological evaluation experiments, the stability of sulforaphene stored at 26 °C was investigated in two kinds of protic solvents (methanol and ethanol) and four kinds of aprotic solvents (acetonitrile, dichloromethane, ethyl acetate and acetone), respectively.

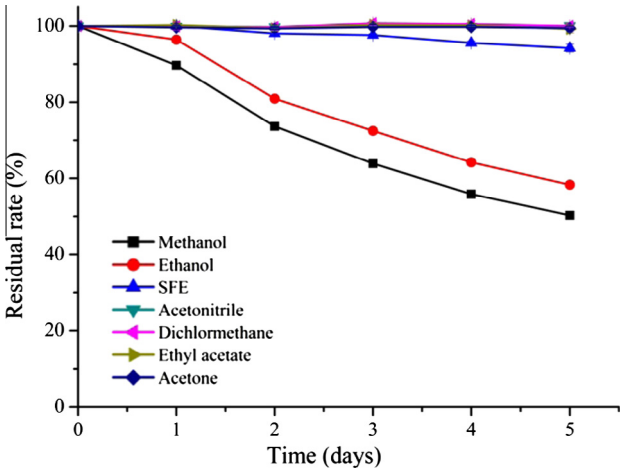

Fig. 1. The stability of SFE in various solvents at 26 °C.

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