Mass spectrometric and theoretical studies on dissociation of the S—S bond in the allicin: Homolytic cleavage vs heterolytic cleavage

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ABSTRACT

On the basis of the tandem mass spectrometry (ESI-MS/MS) technique and DFT calculations, an experimental and theoretical investigation has been conducted into the gas-phase dissociation of the S_1—$S_{1'}$ bond in the allicin as well as that of the S—C (S_1—C_2, $S_{1'}$—$C_{2'}$) bond. Meanwhile, the influence of protonation, alkali metal ion and electron transfer on the dissociation of the S_1—$S_{1'}$ bond has been taken into account. ESI-MS/MS experiments and DFT calculations show that in the neutral allicin, [allicin + Li]$^+$ and [allicin + Na]$^+$, the S_1—$S_{1'}$ bond favors homolytic cleavage, while in the allicin radical cation and protonated allicin, the S_1—$S_{1'}$ bond prefers heterolytic cleavage. In addition, alkali metal ions can strengthen the S_1—$S_{1'}$ bond in the allicin, while protonation or the loss of an electron will weaken the S_1—$S_{1'}$ bond.

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1. Introduction

It is well known that allicin (2-propenyl-2-propenethiosulfinate) is the major biologically active component in garlic. Allicin has exhibited anti-microbial, anti-thrombotic, anti-hypertensive and anti-hyperglycemic properties. Recently, it has been proved that allicin may reduce some cancer risk [1–8]. Evidence suggests that the biological activities of allicin are attributed to its antioxidant activity or rapid reaction with thiol-containing proteins (thiol disulfide exchange) [9]. As for the former, Okada et al. has stated that the antioxidant activity of allicin occurs through a mechanism involving the abstraction of the allylic H atom adjacent to the divalent sulfur atom [10–12]. As for the latter, allicin also exerts its biological effects by rapidly reacting with intracellular free thiols, such as reduced glutathione (GSH), cysteine and sulfhydryl groups of proteins, as are shown in Fig. 1. The reaction of the allylthio group with those cellular components constitutes the major beneficial effects of allicin [13–16]. Furthermore, this character has also been applied in the quantitative analysis of allicin [17] and the synthesis of novel antihypertensive drug [18].

Up to now, many theoretical and experimental works have centred upon the study of the antioxidant activity of allicin. In fact, the essence of the research lies in identifying the reaction activity of allylic H atom in the allicin with other radicals, and this reaction activity is also determined by the dissociation energy of the corresponding C—H bond. Pratt et al. [19] and Annia et al. [12] have performed some significant works on these aspects. Recent work even

indicates that the peroxyl-radical-trapping activity of garlic is not due to the allicin, but primarily 2-propenesulfenic acid formed by the decomposition of allicin [19].

On the other hand, the study on the reaction mechanisms of allicin with free thiols is relatively rare. As this kind of reaction relates to the dissociation of the S—S bond in the allicin (Fig. 1), therefore, the study on the S—S bond in the allicin benefits the deep insight into the reaction mechanism of the allicin with free thiols. In this paper, we decide to explore the gas-phase dissociation of the S—S bond in the allicin from both computational and experimental perspectives. Gas-phase study is beneficial to the grasp of the reaction essence, for cluster effects, counterions, and solvent molecules can be ignored in the gas phase. At the same time, the influence of protonation, alkali metal ion and electron transfer on the dissociation of the S—S bond have been taken into account. It is known to all that alkali metal ion, proton and electron transfer process widely exist in organism [20–22]. So study on the influence of these factors on the dissociation of the S—S bond will help to make sure whether or not protonation, alkali metal ion and electron transfer affect the biological effects of the allicin. In addition, the dissociation of the S—C bond in the allicin has also been given a thorough study.

2. Experimental

2.1. Material

Allicin was obtained from the Sigma–Aldrich Company. Analytic grade lithium chloride and sodium chloride were purchased from Shanghai Experiment Reagent Co. Ltd.
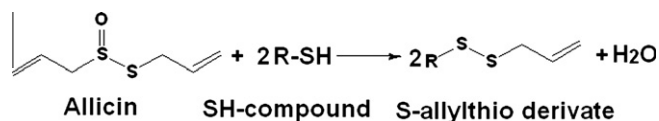
E-mail address: zhangxiang@mail.zjgsu.edu.cn

Fig. 1. Reaction between the allicin and free thiols.

2.2. Mass spectrometry

The mass spectral data were acquired on a LCQ ion trap mass spectrometer from ThermoFinnigan (San Jose, CA, USA) equipped with an electrospray ionization (ESI) interface operated in the positive ion mode. ESI of a CH_3CN/H_2O (3:1) solution containing the allicin (about 500 μM) and a small amount of MCl (M = Li and Na) was carried out at a flow rate of 15 μL/min into the ESI source. The operation parameters are listed as follows: spray tip potential 4000 V; capillary voltage: ~30 V; capillary temperature: ~240 °C; Sheath gas flow rate: 9.28 arbitrary units; Tube lens: 60 V. In the ESI-MS/MS experiments, the parent ions have been isolated mono-isotopically in the ion trap and collisionally activated by different collision energies so as to find the optimal collision energies for a distinct fragmentation. Helium (99.99%) was used as the trapping and collision gas. It is important to note that the operation is very fast considering the stability of the allicin.

2.3. Computational methods

All structures were computed on the basis of the hybrid density functional theory ((U)B3LYP) [23–25] and the 6-31+G (d, p) basis set which were implemented in Gaussian 09 [26] program package. All the gas phase minima and transition structures (here also referred to as transition states) were characterized by frequency analysis. Frequency calculations identify minimum structures with all real frequencies, while transition states with only one imaginary frequency. Zero point energy (ZPE) corrections were applied at the same level of theory and basis set superposition errors

(BSSE) were subtracted from the computed dissociation energies in the full counterpoise correction [27]. To confirm the transition states connecting the designated intermediates, intrinsic reaction coordinate (IRC) calculation was carried out. Furthermore, to obtain more reliable energetic data, higher-level single-point energy calculations were performed at (U)B3LYP/6-311++G(3df, 2p) level by using the (U) B3LYP/6-31+G(d, p) optimized geometries. Spin contamination for all of the stationary points was minimal (i.e., $0.75 < S^2 < 0.78$) [28].

3. Results and discussion

The dissociation of C_2—H and $C_{2'}$—H bonds in the allicin has been studied in many works [12,19]. This paper will focus on the gas-phase dissociation of the S_1—$S_{1'}$ bond as well as that of the S_1—C_2 and $S_{1'}$—$C_{2'}$ bonds, as are shown in Fig. 2. As for the S_1—$S_{1'}$ bond (Fig. 2a), homolytic cleavage and heterolytic cleavage have been both taken into account. On the other hand, as for the S_1—C_2 and $S_{1'}$—$C_{2'}$ bonds (Fig. 2b), only homolytic cleavage has been considered. In addition, we have also examined the influence of protonation, alkali metal ion and electron transfer on the dissociation of these chemical bonds.

First of all, the dissociation of the similar chemical bonds of the neutral allicin is examined, as is shown in Fig. 3a. The dissociation enthalpy of the S_1—C_2 bond is 24.7 kcal/mol, 14.8 kcal/mol less than that of the $S_{1'}$—$C_{2'}$ bond, which means the dissociation of the S_1—C_2 bond is prior to that of the $S_{1'}$—$C_{2'}$ bond. On the other hand, the homolytic cleavage enthalpy of the S_1—$S_{1'}$ bond is 33.1 kcal/mol, while the heterolytic cleavage enthalpy of the S_1—$S_{1'}$ bond is as high as 160.8 kcal/mol. It is obvious that the S_1—$S_{1'}$ bond of the neutral allicin favors homolytic cleavage.

In Fig. 3b, we have subsequently examined the dissociation of the S_1—$S_{1'}$, S_1—C_2 and $S_{1'}$—$C_{2'}$ bonds in the allicin radical cation. Similarly, the dissociation of the S_1—C_2 bond leading to the formation of ion **A** (m/z = 121) is more favorable than the dissociation of the $S_{1'}$—$C_{2'}$ bond, for the former has a relatively low dissociation

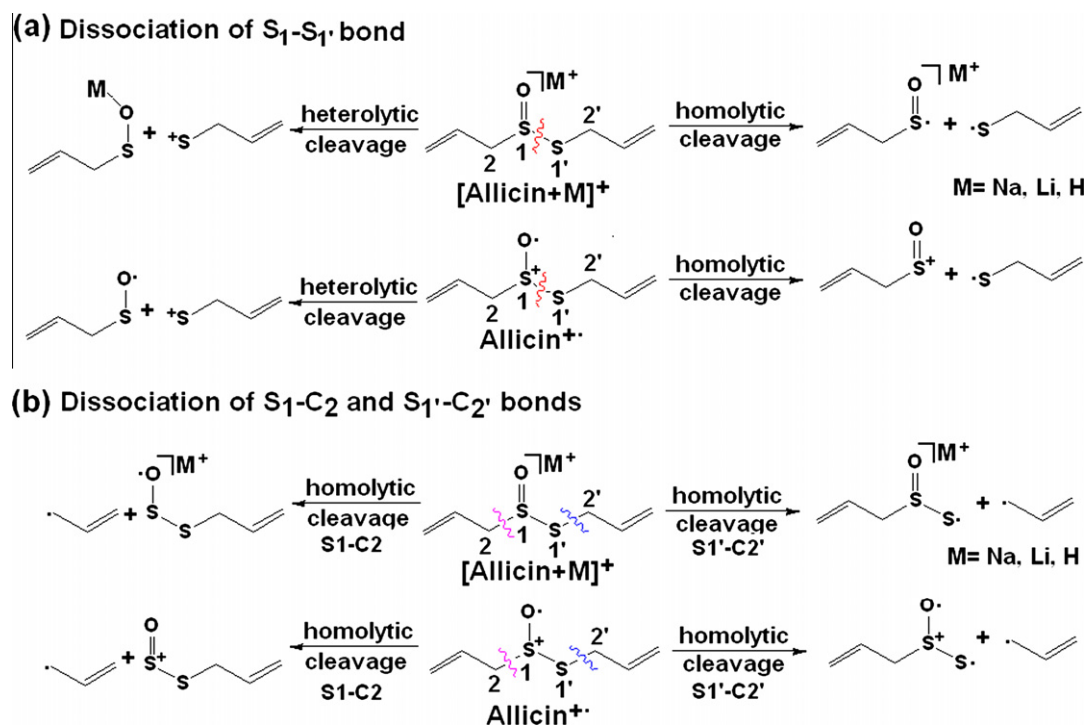Fig. 2. Dissociation of the S_1—$S_{1'}$, S_1—C_2 and $S_{1'}$—$C_{2'}$ bonds in allicin.