Journal of Photochemistry and Photobiology A: Chemistry 219 (2011) 195–199

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology A: Chemistry

journal homepage: www.elsevier.com/locate/jphotochem

Characterization of transient species produced from laser flash photolysis of a new cardioprotective drug: S-propargyl-cysteine

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ARTICLE INFO

Article history:
Received 14 September 2010
Received in revised form 31 January 2011
Accepted 17 February 2011
Available online 23 February 2011

Keywords:
Laser flash photolysis
S-propargyl-cysteine
Cardioprotective drug
Sulfur-containing amino acid
Antioxidant

ABSTRACT

S-propargyl-cysteine (SPRC), a sulfur-containing amino acid, was proved to have cardioprotective effect mediated by the CSE/H_2S pathway. In this study, photo-ionization of SPRC was investigated by 266 nm laser flash photolysis. Irradiated by 266 nm laser pulse, SPRC in aqueous solution experienced photo-ionization via a bi-photon process to produce hydrated electron, anion radical ($SPRC^{\bullet -}$), and neutral radical (SPRC•(–H)). The reaction of SPRC with $SPRC^{\bullet -}$ was found and the pKa of $SPRC^{\bullet +}$ was determined. Oxidation of SPRC by $SO_4^{\bullet -}$ not only confirmed the assignment of SPRC•(–H), but also provided an evidence for the antioxidant activity of SPRC.

1. Introduction

Garlic is used traditionally as a complementary therapy in the treatment of several diseases such as diabetes, several forms of cancer, and neurodegenerative conditions [1,2]. A list of health-promoting and related biological effect has been ascribed to the organosulfur components of tissue preparations from garlic and onion [3]. S-allylcysteine (SAC), a sulfur-containing amino acid, is one of the major compounds in aged garlic extract [4]. SAC has been reported to have antioxidant activity [5,6], anti-cancer promoting activity [7–11], anti-hepatopathic activity [12,13], and neurotrophic activity [14,15]. Moreover, a recent study showed that the cardioprotective effect of SAC was associated with a hydrogen sulfide (H_2S)-mediated pathway in an acute myocardial infarction rat model (MI) [16]. SAC can undergo β-elimination and serve as the substrate of cystathionine-g-lyase (CSE) which is a pyridoxal-5-phosphate-dependent enzyme involved in endogenous hydrogen sulfide production [17]. Hydrogen sulfide might be the third endogenous signaling gasotransmitter, besides nitric oxide and carbon monoxide [18]. Hydrogen sulfide, at physiologically relevant concentrations, can alleviate myocardial ischemia-reperfusion injury [19–22].

S-propargyl-cysteine (SPRC) (Fig. 1), a structural analog of SAC, has the same cysteine-containing structure. Recent studies showed that SPRC had cardioprotective property in MI rats and preserved cell viability when cultured cells were exposed to hypoxia. Moreover, SPRC had better cardioprotective effect than SAC. The protective effect of SPRC was partly mediated by the CSE/H_2S pathway [23]. However, metabolism and pharmacology of SPRC have been ambiguously known by far. As we know, the pathogenesis of many cardio-vascular diseases is involved in the generation of active species such as ROS and nitrogen oxides. SPRC may react with these active species to produce many new transient species which may be associated with the metabolism of SPRC. Therefore, it is necessary to investigate these transient species resulting from SPRC in order to better understand the metabolism and pharmacology of SPRC.

In this work, laser flash photolysis (LFP) was employed to investigate photo-ionization of SPRC. Several new transient species produced from LFP have been identified. The antioxidant activity of SPRC was also proved by investigating reaction of $SO_4^{\bullet -}$ with SPRC. Meanwhile, a series of related kinetic parameters have been obtained.

2. Experimental

2.1. Chemical reagents

SPRC was kindly supplied by Prof. Yi Zhun Zhu (Department of Pharmacology, School of Pharmacy and Institute of Biomedical

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doi:10.1016/j.jphotochem.2011.02.009

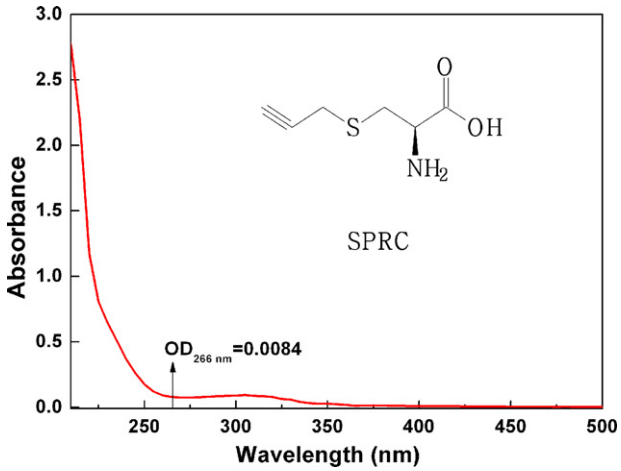

Fig. 1. The UV–vis spectrum of 5 mM SPRC aqueous solution at pH 6.7 and structural formula of SPRC.

Sciences, Fudan University, Shanghai, China) and used without further purification. Its purity (≥99%) was checked by HPLC. Tert-Butyl alcohol (t-BuOH) and acetonitrile were purchased from Sigma. $K_2S_2O_8$, NaOH, $HClO_4$ and phosphate (analytic grade reagent) were commercially available and used without further purification. The pH value of the solution was adjusted by adding NaOH and $HClO_4$ solution. The solutions were deaerated with high-purity N_2 (≥99.99%), N_2O, or O_2 (≥99.5%) for different purposes by bubbling for at least 20 min prior to experiments. Ground-state absorption properties were studied by using a UV–vis spectrometer (VARIAN CARY 50 Probe). All experiments were performed in a 1 cm quartz cuvette at room temperature.

2.2. Laser flash photolysis experiments

Laser flash photolysis experiments were carried out by using Nd:YAG laser of 266 nm light pulses with a duration of 5 ns and the maximum energy of 40 mJ per pulse used as the pump light source. A xenon lamp was employed as detecting light source. The laser and analyzing light beam passed perpendicularly through a quartz cell with an optical path length of 10 mm. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signal from the Agilent 54830B digital oscillograph was transferred to a personal computer for data treatment. The LFP setup has been previously described [24–26].

3. Results and discussion

3.1. Characterization of photo-ionization of SPRC in aqueous solution

SPRC shows a very low absorption at 266 nm (Fig. 1). However, after 266 nm LFP of N_2-saturated 5 mM SPRC aqueous solution, transient absorption spectra with two absorption bands centered at 300 and 330 nm and a strong and broad absorption band after 500 nm was obtained (Fig. 2). The transient species with the broad absorption band ($\lambda > 500$ nm) decayed fast. The kinetic decay curve observed at 700 nm can be efficiently quenched by N_2O with the addition of t-BuOH in the solution or O_2 (inset of Fig. 2). Therefore, it should be assigned to the hydrated electron (e_{aq}^-) [27,28]. The appearance of e_{aq}^- means that SPRC experienced photo-ionization to produce e_{aq}^- which can be captured by O_2 or N_2O (Eqs. (1)–(4)).

$$SPRC \rightarrow SPRC^{\bullet +} + e_{aq}^- \quad (1)$$

$$e_{aq}^- + O_2 \rightarrow O_2^{\bullet -} \quad (2)$$

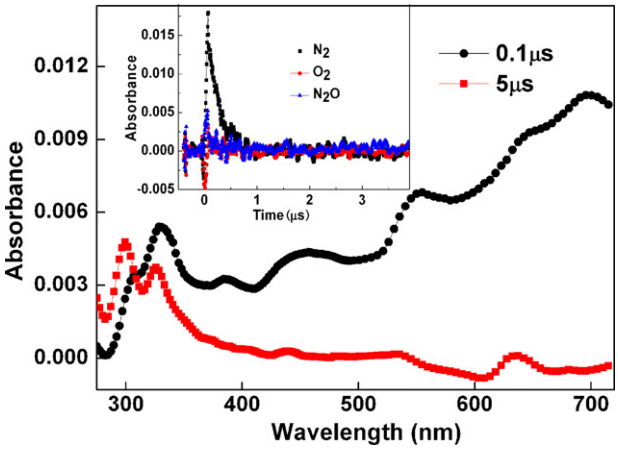

Fig. 2. Transient absorption spectra obtained from 266 nm LFP of N_2-saturated 5 mM SPRC aqueous solution recorded at: (●) 0.1 μs, (■) 5 μs after laser pulse. Inset: kinetic decay curve observed at 700 nm saturated with (■) N_2, (●) O_2, and (▲) N_2O with t-BuOH, respectively.

$$e_{aq}^- + N_2O + H_2O \rightarrow {}^{\bullet}OH + OH^- + N_2 \quad (3)$$

$$^{\bullet}OH + \text{t-BuOH} \rightarrow \text{t-Bu}^{\bullet}OH + OH^- \quad (4)$$

In order to determine whether the photo-ionization of SPRC by 266 nm laser pulse is caused by a mono-photon or a bi-photon process, the yield of e_{aq}^- was calculated from OD_0 (optical density value recorded at 0 time after laser pulse) at 700 nm by changing intensity of laser pulse (I_L) [28]. For SPRC the yield of e_{aq}^- was found to increase with the square of the incident laser intensity (I_L) (Fig. 3). A $\log(OD_0)$–$\log(I_L)$ plot yields slope of 1.8 (inset of Fig. 3). This clearly indicates that photo-ionization of SPRC proceeds in a two-photon process.

3.2. Reaction of SPRC with e_{aq}^-

Here, the decay of e_{aq}^- at 700 nm followed the pseudo-first-order kinetic process and was obviously accelerated with the increase of SPRC concentration (Fig. 4). This is ascribed predominantly to their reactions with the substrate molecules to form its radical anion ($SPRC^{\bullet -}$) (Eq. (5)). Since electrons are readily solvated in polar solvents, back electron transfer to the radical cation is not important [29,30]. The rate constant of reaction between SPRC and e_{aq}^- was determined to be $(5.32 \pm 0.10) \times 10^8\ M^{-1}\ s^{-1}$ by monitoring the observed pseudo-first-order decay rate constant (k_{obs}) at

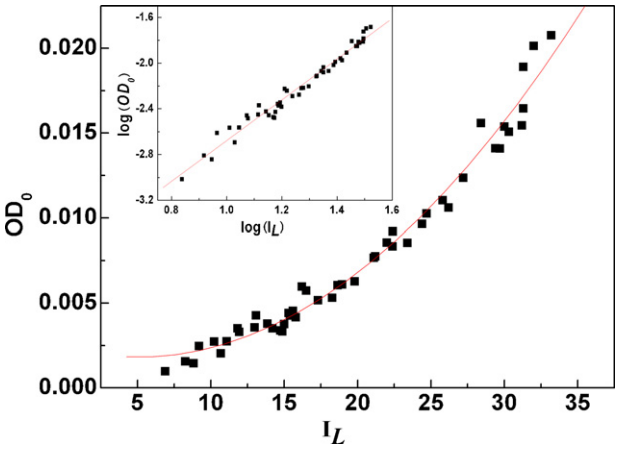

Fig. 3. Dependence of OD_0 at 700 nm on laser intensity (I_L) immediately after 266 nm LFP of N_2-saturated 5 mM SPRC aqueous solution pH = 6.7. Inset: plot of $\log(OD_0)$ vs $\log(I_L)$.

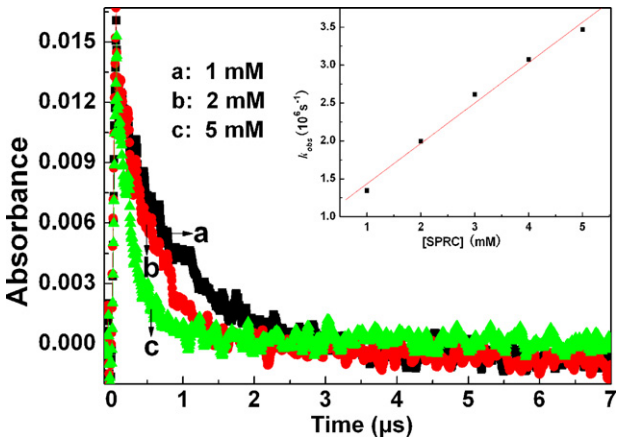

Fig. 4. Kinetic decay curve observed at 700 nm obtained from 266 nm LFP of N_2-saturated aqueous solution pH = 6.7 containing different concentrations of SPRC: (a) 1 mM, (b) 2 mM and (c) 5 mM. Inset: plot of the observed pseudo-first-order decay rate constant (k_{obs}) at 700 nm vs SPRC concentration. The straight line represents a linear fit to the experimental data.

700 nm versus the SPRC concentration (inset of Fig. 4).

$$e_{aq}^- + \text{SPRC} \rightarrow \text{SPRC}^{\bullet -} \quad (5)$$

3.3. Reaction of SPRC with SPRC$^{\bullet -}$

The decay of e_{aq}^- is followed by the formation of an absorbance around 300 nm (Fig. 2). However, the buildup of 300 nm is not synchronous with the decay of e_{aq}^- at 700 nm (Fig. 5A). It suggested that the generated new transient species with absorption at 300 nm could not be ascribed to SPRC$^{\bullet -}$. The addition of e_{aq}^- scavengers, such as O_2 and N_2O with t-BuOH, could eliminate the buildup trace at 300 nm (Fig. 5B), which implied that e_{aq}^- is indispensable for the buildup. Considering that SPRC$^{\bullet -}$ is the main initial product of the reaction of e_{aq}^-, the new species may result from the decay of SPRC$^{\bullet -}$. Then the kinetic analysis showed that the buildup process followed the pseudo-first-order kinetic process and the generation rate increased with a rate linearly proportional to the concentration of SPRC (Fig. 5C). Therefore, it could be inferred that SPRC involved in the formation of 300 nm. The new species could be ascribed to the product of the reaction of SPRC with SPRC$^{\bullet -}$ ((2SPRC)$^{\bullet -}$) (Eq. (6)). From a dependence of the observed generation rate (k_{obs}) on SPRC concentration (1–5 mM), the bimolecular rate can be estimated to be $(1.14 \pm 0.05) \times 10^8\ M^{-1}\ s^{-1}$ (Fig. 5D).

$$\text{SPRC}^{\bullet -} + \text{SPRC} \rightarrow (\text{2SPRC})^{\bullet -} \quad (6)$$

3.4. Identification and the characterization of SPRC$^{\bullet}$(–H)

SPRC was photo-ionized by 266 nm laser pulse irradiation to produce e_{aq}^- and the radical cation of SPRC (SPRC$^{\bullet +}$) or deprotonated radical cation of SPRC (SPRC$^{\bullet}$(–H)). In order to confirm the absorption of SPRC$^{\bullet +}$/SPRC$^{\bullet}$(–H), transient absorption spectra was obtained from 266 nm LFP of O_2-saturated 5 mM SPRC aqueous solution (Fig. 6). In this condition, O_2 can exclude the absorption of e_{aq}^- and products resulting from e_{aq}^-, or the potential triplet state of SPRC. The absorption band at 330 nm should be ascribed to SPRC$^{\bullet +}$/SPRC$^{\bullet}$(–H).

The transient absorption at 330 nm was investigated by changing pH value of the O_2-saturated aqueous solution containing 5 mM SPRC (Fig. 7). According to the titration curve, the pKa value of SPRC$^{\bullet +}$ is obtained from the inflection point of the curve [31], which is 3.89. Given the pH value of SPRC solution is 6.7, SPRC$^{\bullet +}$ would

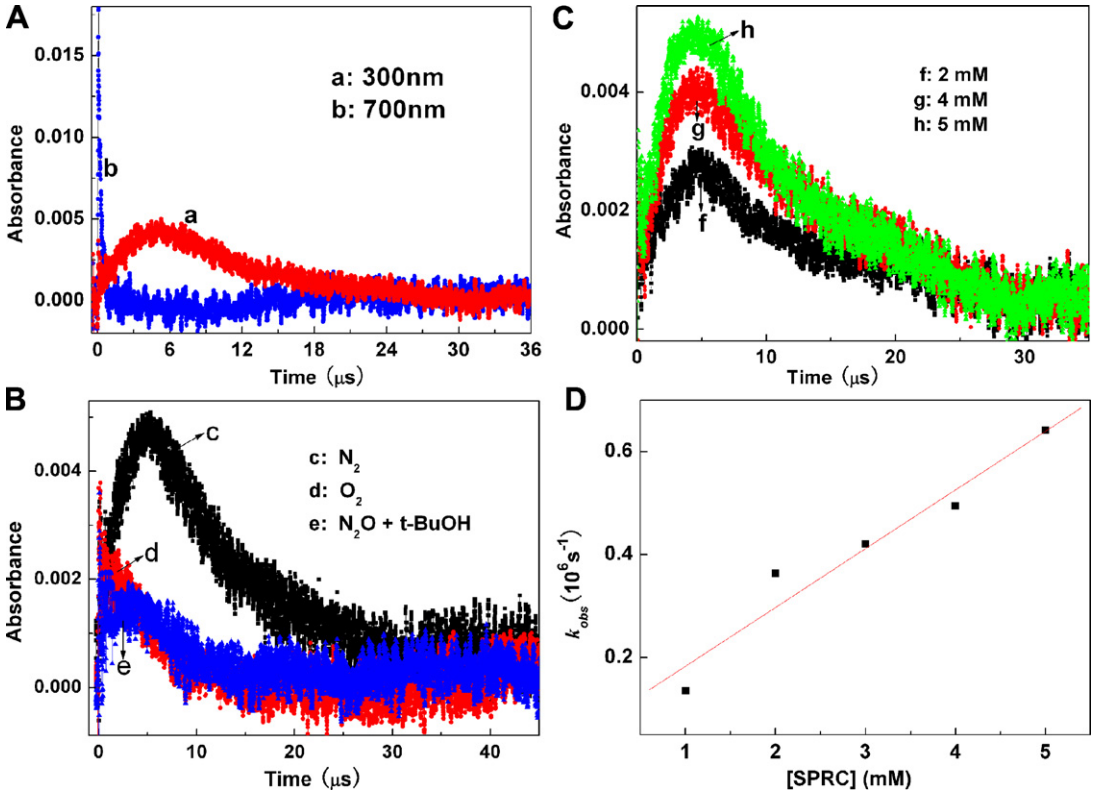

Fig. 5. (A) Kinetic decay curve obtained from 266 nm LFP of N_2-saturated 5 mM SPRC aqueous solution observed at (a) 300 nm and (b) 700 nm, respectively. (B) Kinetic decay curve observed at 300 nm obtained from 266 nm LFP of 5 mM SPRC aqueous solution saturated with (c) N_2 or (d) O_2 or (e) N_2O with t-BuOH, respectively. (C) The buildup trace observed at 300 nm obtained from 266 nm LFP of N_2-saturated aqueous solution containing different concentrations of SPRC: (f) 2 mM, (g) 4 mM and (h) 5 mM. (D) Plot of the observed generation rate constant k_{obs} at 300 nm vs SPRC concentration. The straight line represents a linear fit to the experimental data.

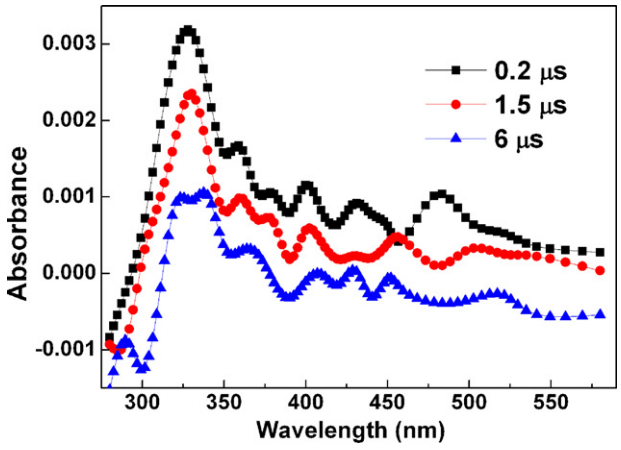

Fig. 6. Transient absorption spectra obtained from 266 nm LFP of O_2-saturated 5 mM SPRC aqueous solution pH 6.7 recorded at: (■) 0.2 μs, (●) 1.5 μs, and (▲) 6 μs after laser pulse.

change into SPRC•(–H) via deprotonation (Eq. (7)) and the absorption at 330 nm was attributed to SPRC•(–H).

$$SPRC^{\bullet+} \rightarrow SPRC^{\bullet}(-H) + H^{+} \quad (7)$$

3.5. Scavenging $SO_4^{\bullet-}$ by SPRC

SPRC may have antioxidant activity like other sulfur-containing amino acids. For the sake of gaining insight into the antioxidant activity of SPRC and further confirming the assignment of SPRC•(–H), the reaction of quenching $SO_4^{\bullet-}$ by SPRC was investigated. Concentration of $K_2S_2O_8$ (0.05 M OD = 0.524) was designed to assure that $K_2S_2O_8$ absorbed the main energy of laser pulse and the absorption of SPRC (1 mM OD = 0.0016) could be neglected. Fig. 8 shows that after the laser pulse, $SO_4^{\bullet-}$ with typical absorption around 450 nm was generated [32,33]. The decay of $SO_4^{\bullet-}$ was followed by the formation of new transient species with absorption around 330 nm. Transient absorption spectra showed the buildup trace of 330 nm was overlapped by the decay of $SO_4^{\bullet-}$. Here kinetic decay curve at 480 nm was chosen to represent the decay of $SO_4^{\bullet-}$ in order to avoid the disturbance of new transient species. According to the subtraction method [31], we have derived the pure growth trace at 330 nm which is synchronic with the decay of $SO_4^{\bullet-}$ at 480 nm (inset of Fig. 8). $SO_4^{\bullet-}$ can be generated easily by 266 nm LFP of $S_2O_8^{2-}$ (Eqs. (8) and (9)). As a one-electron oxidant, $SO_4^{\bullet-}$ can oxide SPRC into SPRC$^{\bullet+}$ which turns into SPRC•(–H) via deprotonation (Eq. 7). Therefore, the new transient species could be assigned as SPRC•(–H). From a plot of the observed pseudo-first-order decay rate constant (k_{obs}) at 480 nm versus the SPRC concentration, the rate constant can be determined to be $(2.57 \pm 0.01) \times 10^9\ M^{-1}s^{-1}$.

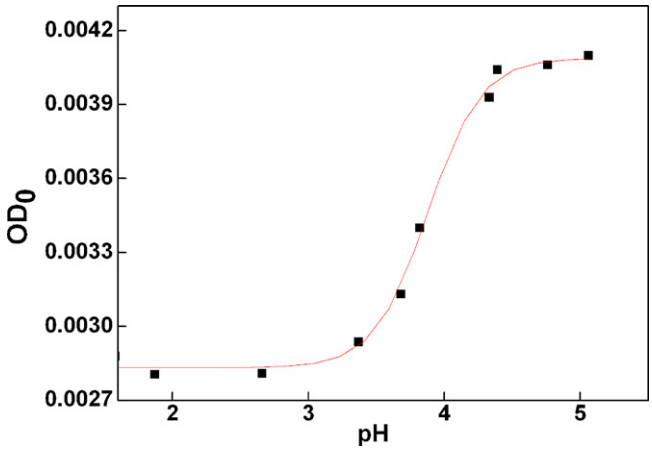

Fig. 7. Absorbance at 330 nm as a function of pH dependence, recorded immediately after the 266 nm laser pulse.

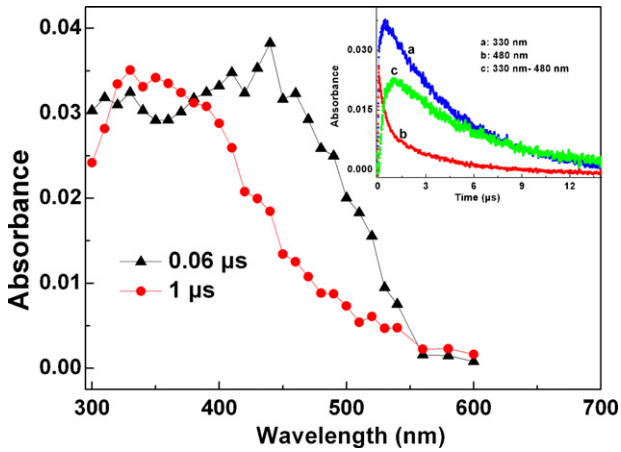

Fig. 8. Transient absorption spectra obtained from 266 nm LFP of N_2-saturated aqueous solution pH 4.8 containing 1 mM SPRC and 0.05 M $K_2S_2O_8$ recorded at 0.06 μs (▲) and 1 μs (●) after laser pulse. Inset: kinetic decay curves at (a) 330 nm; (b) 480 nm; (c) the buildup trace of 330 nm obtained by subtracting trace (b) from trace (a).

$$S_2O_8^{2-} \xrightarrow{hv} 2SO_4^{\bullet-} \quad (8)$$

$$SPRC + SO_4^{\bullet-} \rightarrow SPRC^{\bullet}(-H) + SO_4^{2-} + H^{+} \quad (9)$$

The rate constant shows that SPRC is very active to the strong oxidative radicals $SO_4^{\bullet-}$. Many heart diseases, such as myocardial ischemia-reperfusion injury and myocardial fibrogenesis, are associated with the generation of oxy-radical adducts (superoxide anion radical, $O_2^{\bullet-}$ and hydroxyl radicals, •OH) or nitrogen oxides (peroxynitrite $NO_3^{\bullet-}$) [34–36]. Just like $SO_4^{\bullet-}$, •OH and $NO_3^{\bullet-}$ are also strong oxidative radicals. Considering the reaction of SPRC with $SO_4^{\bullet-}$, it can be inferred that •OH and $NO_3^{\bullet-}$ may be also easily eliminated by SPRC. SPRC was proposed to show cardioprotective effects partly mediated by the CSE/H_2S pathway [23]. According to the ability of SPRC to eliminate strong oxidative radicals, another possible mechanism can be proposed that SPRC may show cardioprotective effects via directly scavenging the strong oxidative radicals (such as OH• and $NO_3^{\bullet-}$) generated in heart.

4. Conclusion

Photo-ionization of SPRC was investigated by 266 nm laser flash photolysis. The transient products of photo-ionization of SPRC were identified as e_{aq}^-, SPRC$^{\bullet-}$, (2SPRC)$^{\bullet-}$ and SPRC•(–H). The reactions of SPRC with e_{aq}^-, SPRC$^{\bullet-}$ and $SO_4^{\bullet-}$ were investigated. The results show that SPRC is a very active drug which cannot only be reduced by e_{aq}^- into SPRC$^{\bullet-}$ but also be oxidized by oxidative radical $SO_4^{\bullet-}$ into SPRC•(–H). e_{aq}^- can be easily generated by exposure of organism to sunlight and strong oxidative radicals (such as OH• and $NO_3^{\bullet-}$) are common active species in pathological tissues and organs. After absorbed by human body, SPRC inevitably reacts with these active species to produce new transient species, and thereby alleviates related diseases. Therefore, this study may be helpful to elucidate the pharmacology of SPRC.

Acknowledgements

The work was supported by the 973 project of the Ministry of Science and Technology (No. 2010CB912604 and 2010CB933901), Genetically modified organisms breeding major projects (No.

2009ZX08011-032B), and Shanghai key laboratory of cell signaling and diseases (Grant no 09DZ2260100).

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