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Photoreactions of 1,4-naphthoquinone with lysozyme studied by laser flash photolysis and steady-state analysis

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ABSTRACT

Photoprocesses of 1,4-naphthoquinone (NQ) and its photoreactions with lysozyme in acetonitrile/water (3:1, v/v) solution were studied using 355 nm laser flash photolysis technique combined with electrophoresis and turbidimetric assay. The transient spectra of NQ were observed and the transient species were assigned. The electron transfer process from *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) to NQ triplet state ($^3NQ^*$) was investigated and the rate constant was determined to be $k_{t1} = 2.0 \times 10^{10}\ M^{-1}\ s^{-1}$. It has been found that $^3NQ^*$ can abstract hydrogen atom from lysozyme with a rate constant of $k_{t2} = 2.4 \times 10^{10}\ M^{-1}\ s^{-1}$. Furthermore, the results of steady-state analysis suggested that lysozyme can be damaged by NQ irradiated with UVA light influenced by the concentration of NQ and the gas saturated in the solution. The mechanisms of photosensitized damage of lysozyme were discussed.

1. Introduction

Quinones, found probably in all respiring animal and plant cells, play a major role in photosynthesis and photobiology and have been widely investigated in many scientific fields [1]. Much attention has been paid to photochemistry and photobiology of quinones involving hydrogen atom abstraction and electron transfer [2–18]. A large number of studies on photochemical properties and elementary photoreactions of benzoquinones (BQ), anthraquinones (AQ) and NQ have been carried out by means of flash photolysis [19–31], pulse radiolysis [12–16,32], steady-state [21,24], EPR [9] and chemically induced dynamic electron polarization (CIDEP) [8,33] techniques. It has been known that the reactive states for the photoreduction of both BQ and AQ are the triplets, and the semiquinone radical plays a key role in the photoreduction of quinones especially when H-atom donor is present [10,13,15].

Moreover, quinone–protein interactions are very important. For example, the interactions between ubiquinone and protein play a crucial role in photosynthetic reaction centers [34,35]. It is interesting to study the interactions between protein and other quinones like NQ. NQ exhibits a high quantum yield of intersystem crossing (Φ_{ISC}), e.g. $\Phi_{ISC} = 0.74$ in acetonitrile [2,3]. The $^3NQ^*$ has a maximum absorption at 365 nm with a molar absorption coefficient of 8200 $M^{-1}\ cm^{-1}$ in acetonitrile [3]. The reactive state for hydrogen atom abstraction of photoexcited NQ has been shown to be the triplet by means of CIDEP [36,37]. Photoinduced hydrogen atom abstraction from a suitable donor such as phenol and ascorbate to the triplet state of NQ involves semiquinone radical ($NQH^{\cdot}/NQ^{\cdot-}$) [3,12,14]. Alternatively, dimethyl sulfide, dimethyl sulfoxide, triethylenediamine (DABCO), triethylamine (TEA) and some aromatic amino acids quenched triplet NQ via electron transfer [5,13,16,38]. Previous researches have made a good foundation for the further studies on the photoprocesses of NQ with protein.

In the present study, we attempted to investigate photoprocesses of NQ and its photoreactions with TMPD and lysozyme in acetonitrile/water (3:1, v/v) solution using 355 nm laser flash photolysis (LFP), electrophoresis and turbidimetric assay. The possible mechanism had been discussed and the related quenching rate constants for $^3NQ^*$ were obtained. Irradiation of lysozyme with UVA light in the presence of NQ was performed to study photooxidation of lysozyme induced by NQ. Results implied that the photooxidation mechanism of lysozyme in presence of oxygen involved both type I and type II photodynamic mechanism and major mechanism was type II process. This paper gained a deeper insight into the photoreduction reactions of NQ and lysozyme, which was helpful for the understanding of quinone–protein interactions.

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2. Materials and methods

2.1. Materials

NQ, TMPD and acetonitrile were purchased from Sigma. Lysozyme (LH, molecular weight is 14.6 kDa) was obtained from Fluka. All solutions were prepared freshly with acetonitrile and ultrapure water provided by Millipore purification system.

2.2. Instrumentation and experimental conditions

Nd: YAG laser provides 355 nm pulses with a duration of 5 ns and the maximum energy of 80 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. The transmitted light entered a monochromator equipped with an R955 photomultiplier. The output signal from the HP54510B digital oscillograph was transferred to a personal computer for study. The LFP setup has been previously described [39]. All experiments were performed in acetonitrile/water (3:1, v/v) solutions. Samples were bubbled with high-purity N_2 or O_2 for at least 20 min before photolysis.

Lysozyme was dissolved in acetonitrile/water (3:1, v/v) solution and irradiated with UVA light (320–380 nm) in the presence of NQ at room temperature. Solutions for irradiation (0.2 mL) were placed in quartz cuvettes and bubbled with N_2, air or O_2 continuously during the irradiation. Light from a 500 W Xe lamp passing through a cut-off filter produced an irradiance of 5 mW/cm^2 of UVA light at the cuvette surface.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed by the Laemmli method [40] using a vertical electrophoresis unit (Bio-Rad Mini-PROTEAN 3 Cell). Briefly, a stacking gel of 3% acrylamide, a resolving gel of 15% acrylamide, and a running buffer of 25 mM Tris, 192 mM glycine, 0.1% SDS and pH 8.3 were used. The irradiated solutions containing 5.86 mg/mL of proteins were loaded onto the gel. Prestained molecular weight standard from Sigma was used. Electrophoresis was carried out in two steps: at 80 V for 20 min and 120 V for 1.5 h at room temperature. Gels were stained with Coomassie Brilliant Blue G-250 solution and destained in a solution containing methanol and acetic acid. The gels were subsequently scanned with a Quantity one scanner (Bio-Rad).

The activity of lysozyme was measured by turbidimetric assay which are based on a clearing [41] phenomenon involving a sensitive organism, Micrococcus iysodeikticus. A UV–visible spectrophotometer (U-3010) was used to measure the change of transmittance (T) of the suspension at 530 nm. The lysozyme obtained from Sigma without any treatment is used as standard. We can obtain the relative activity of lysozyme from Eq. (1) (T_S, T_B, and T_{STD} represents of the transmittance of sample, blank and standard, respectively).

$$\text{Relative activity}\% = [(T_S - T_B)/(T_{STD} - T_B)]\% \quad (1)$$

3. Results and discussion

3.1. Transient absorption spectra of NQ in acetonitrile/water (3:1, v/v) solution

Transient absorption spectra of NQ have been widely studied in mixed solution [4,17,28]. Our aim is to investigate its behavior in acetonitrile/water (3:1, v/v) solution using a 355 nm laser as the excitation source. Fig. 1 shows the transient absorption spectra of N_2-saturated acetonitrile/water (3:1, v/v) solution containing NQ. At 0.1, 0.5 and 1 μs after laser pulse, the spectra showed a strong absorption band with maximum at 360 nm, while at 7 μs the band disappeared and a new absorption band with maximum at 395 nm was observed

Efficient population of $^3NQ^*$ (reaction I) is known to be $\Phi_{ISC} = 0.74$ in acetonitrile [2,3]. In our system, the main transient around 360 nm can be assigned to $^3NQ^*$ based on the fact that it is formed within the pulse and quenched by oxygen (k_q=1.5 × 10^{10} L mol^{-1} s^{-1}) (Inset of Fig. 1). The long-lived species with absorption maximum at 395 nm could also be scavenged by O_2 and should be assigned to the radical anion of NQ ($NQ^{\cdot-}$) produced by self-quenching reaction (reaction III) [4,16,42], which could happen in acetonitrile/water (3:1, v/v) solution but could not happen in acetonitrile solution due to the higher polarity of acetonitrile/water (3:1, v/v) solution. The absorption of $NQ^{\cdot-}$ in water has been assigned to be at 390 nm by means of pulse radiol-

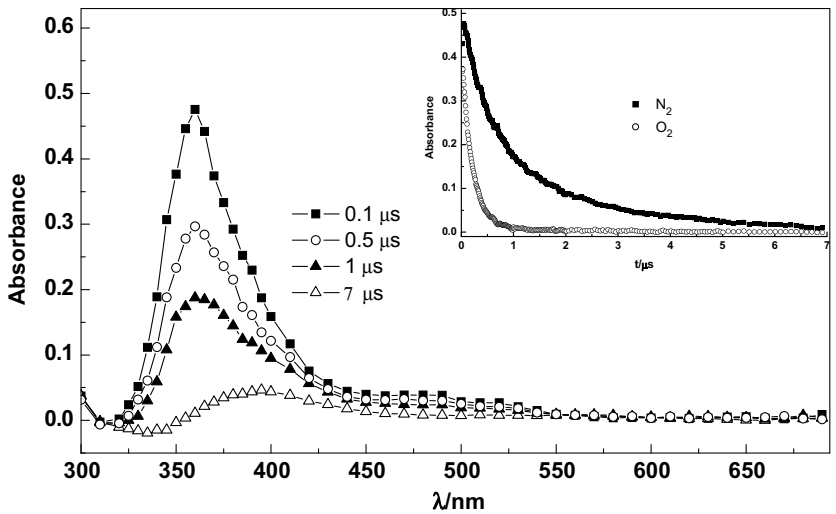

Fig. 1. Transient absorption spectra of N_2-saturated acetonitrile/water (3:1, v/v) solution of 0.3 mM NQ recorded at: (■) 0.1 μs; (○) 0.5 μs; (▲) 1 μs; (△) 7 μs after 355 nm laser excitation. Inset: transient absorption decay of 0.3 mM NQ in acetonitrile/water (3:1, v/v) solution saturated with N_2 (■) and O_2 (○) monitored at 360 nm from 355 nm LFP.