

Communication

Tunneling in the Hydrogen-Transfer Reaction from a Vitamin E Analog to an Inclusion Complex of 2,2-Diphenyl-1-picrylhydrazyl Radical with β -Cyclodextrin in an Aqueous Buffer Solution at Ambient Temperature

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Abstract: Recently, increasing attention has been paid to quantum mechanical behavior in biology. In this study, we investigated the involvement of quantum mechanical tunneling in the hydrogen-transfer reaction from Trolox, a water-soluble analog of vitamin E (α -tocopherol), to 2,2-diphenyl-1-picrylhydrazyl radical (DPPH \cdot) in a phosphate buffer solution (0.05 M, pH 7.0). DPPH \cdot was used as a reactivity model of reactive oxygen species and solubilized in water using β -cyclodextrin (β -CD). The second-order rate constants, k_H and k_D , in 0.05 M phosphate buffer solutions prepared with H $_2$ O (pH 7.0) and D $_2$ O (pD 7.0), respectively, were determined for the reaction between Trolox and DPPH \cdot , using a stopped-flow technique at various temperatures (283–303 K). Large kinetic isotope effects (KIE, k_H/k_D) were observed for the hydrogen-transfer reaction from Trolox to the β -CD-solubilized DPPH \cdot in the whole temperature range. The isotopic ratio of the Arrhenius prefactor ($A_H/A_D = 0.003$), as well as the isotopic difference in the activation energies (19 kJ mol $^{-1}$), indicated that quantum mechanical tunneling plays a role in the reaction.

Keywords: antioxidant; hydrogen transfer; kinetic isotope effect; tunneling

1. Introduction

Hydrogen-transfer reactions are cornerstones of the radical-scavenging reactions of antioxidants, such as vitamins C (ascorbic acid) and E (α -tocopherol), flavonoids, and so on, where hydrogen atoms (or protons and electrons) are transferred from antioxidants to oxygen radicals as an initial step. On the other hand, increasing attention has been paid to quantum mechanical behavior in biology in recent years [1], such as hydrogen tunneling [2–5]. Mukai et al. reported a large kinetic isotope effect (KIE, k_H/k_D) of 22.5, observed in the hydrogen-transfer reaction from α -tocopherol to aroxyl radical in ethanol, demonstrating that quantum mechanical tunneling plays a role in this reaction [6]. 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH \cdot) is a stable radical and has been used as a reactivity model of reactive oxygen species, to investigate the radical-scavenging reactivity of antioxidants, as well as their mechanism, for more than 60 years [7–9]. However, to the best of our knowledge, there has no reports about tunneling in a reaction involving DPPH \cdot . Furthermore, the insolubility of DPPH \cdot in water has precluded its use in aqueous solutions, especially in concentrated buffer solutions. We have recently succeeded in solubilizing DPPH \cdot in water by forming an inclusion complex with β -cyclodextrin (β -CD) [10,11]. This

enables us to investigate DPPH[•]-scavenging reactivity and the mechanism of antioxidants in aqueous buffer solutions [10–13]. We report herein the first observation of the temperature dependence of large primary kinetic isotope effects for the reaction of Trolox, a water-soluble analog of α -tocopherol, with β -CD-solubilized DPPH[•] (DPPH[•]/ β -CD) in a phosphate buffer (Figure 1), indicating that quantum mechanical tunneling plays a role in this reaction.

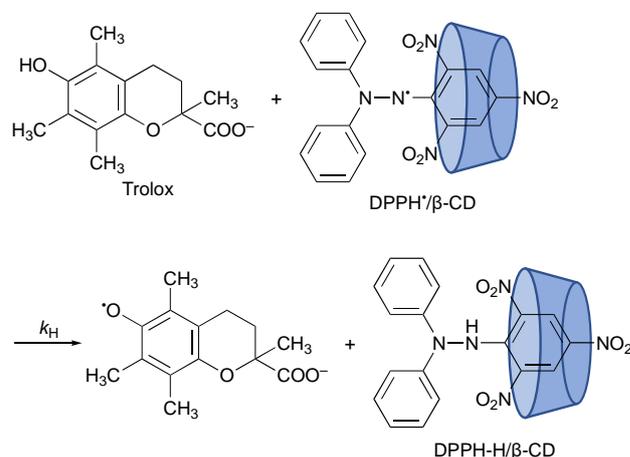


Figure 1. Hydrogen transfer from Trolox to DPPH[•]/ β -CD.

2. Materials and Methods

2.1. Materials

Trolox and β -CD was commercially obtained from Tokyo Chemical Industry Co., Ltd., Japan. DPPH[•] and phosphate buffer solution (0.1 M, pH 7.0) were purchased from Fujifilm Wako Pure Chemical Ind. Ltd., Osaka, Japan. D₂O was commercially obtained from Nacalai Tesque, Inc., Kyoto, Japan. A Milli-Q system (Millipore Direct-Q UV3) (Merck Millipore, Burlington, MA, USA) was used to freshly prepare the water used in this study. DPPH[•] was solubilized in water by β -CD, according to the procedure described in the literature [10]. The deuterated phosphate buffer solution was prepared by dissolving phosphate buffer powder (Fujifilm Wako Pure Chemical Ind. Ltd., Osaka, Japan) into D₂O and the pD was adjusted by adding 5 N hydrochloric acid (Fujifilm Wako Pure Chemical Ind. Ltd., Osaka, Japan). The pD values were calculated by adding 0.4 to the corresponding pH values measured using a HORIBA D-51 pH meter (Horiba, Ltd., Kyoto, Japan) [14].

2.2. Spectral and Kinetic Measurements

An Agilent 8453 photodiode array spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) was used to record the UV-vis spectra. The scavenging rates of DPPH[•]/ β -CD by Trolox in a phosphate buffer solution (0.05 M, pH 7.0) by Trolox were followed by monitoring the absorbance change at 527 nm due to DPPH[•] ($\epsilon = 1.1 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) after the mixing of DPPH[•] in water (Milli-Q) with a phosphate buffer solution (0.1 M, pH 7.0) containing Trolox at a volumetric ratio of 1:1 using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer (UNISOKU Co., Ltd., Osaka, Japan), which was thermostated with a Thermo Scientific NESLAB RTE-7 Circulating Bath (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Pseudo-first-order rate constants (k_{obs}) were obtained by a least-square curve fit, using an Apple MacBook Pro personal computer (Apple Inc., Cupertino, CA, USA). The first-order plots of $\ln(\text{Abs} - \text{Abs}_{\infty})$ vs. time (Abs and Abs_{∞} are the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives, with a correlation coefficient $\rho > 0.999$. In each case, it was confirmed that the k_{obs} values derived from at least three independent measurements agreed within an experimental error of $\pm 5\%$.

3. Results and Discussion

Upon mixing of a phosphate buffer solution (0.1 M, pH 7.0) of Trolox with DPPH[•]/β-CD in water (Milli-Q) at a volumetric ratio of 1:1 on a stopped-flow spectrophotometer, the absorption band at 537 nm due to DPPH[•] decreased immediately, with clear isosbestic point at 424 nm, as shown in Figure 2. This spectral change indicates that Trolox efficiently scavenged DPPH[•] in the phosphate buffer. Since the p*K*_a value of the carboxylic group of Trolox is known to be 3.89 [15], the carboxylic group was completely deprotonated at pH 7.0 (Figure 1). Thus, the hydrogen transfer occurred from the phenolic OH group in Trolox to DPPH[•]. The decay of the absorbance at 527 nm, which was monitored using a stopped-flow technique, obeyed pseudo-first-order kinetics, when the concentration of Trolox ([Trolox]) was maintained at more than a 10-fold excess of DPPH[•] concentration (inset of Figure 2). The pseudo-first-order rate constants (*k*_{obs}) increased linearly with increasing [Trolox] (Figure 3). The second-order rate constant (*k*_H) in Equation (1) was obtained from the slope of the plot Equation (2) for the hydrogen transfer from Trolox to DPPH[•] (Figure 1) in a phosphate buffer solution (0.05 M, pH 7.0) to $1.4 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$.

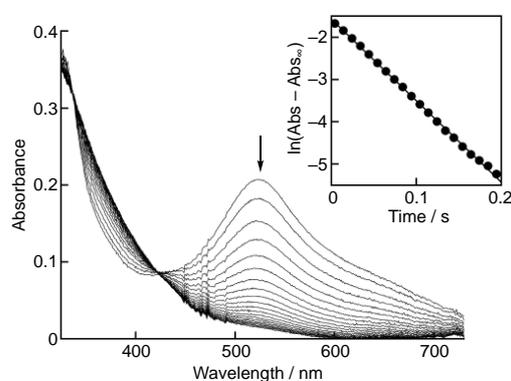


Figure 2. Spectral change (interval: 10 ms) observed during the reaction of Trolox ($1.4 \times 10^{-3} \text{ M}$) with DPPH[•]/β-CD ($1.9 \times 10^{-5} \text{ M}$) in phosphate buffer (0.05 M, pH 7.0) at 298 K. Inset: the first-order plot of the absorbance at 527 nm.

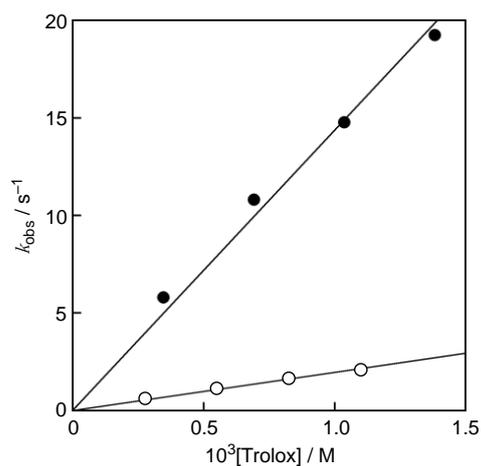


Figure 3. Plots of pseudo-first-order rate constants (*k*_{obs}) vs. concentrations of Trolox in phosphate buffer (H₂O, 0.05 M, pH 7.0) (closed circles) and in phosphate buffer (D₂O, 0.05 M, pD 7.0) (open circles).

When D₂O was used instead of H₂O to prepare the phosphate buffer, the phenolic O–H proton in Trolox was replaced by deuterium from D₂O. The second-order rate constant (*k*_D) determined for the reaction of Trolox with DPPH[•]/β-CD was much smaller

($2.0 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$) than the k_{H} value. Thus, the KIE ($k_{\text{H}}/k_{\text{D}}$) was calculated to be 7.4, which is slightly smaller than the semi-classical isotope effect for O–H bonds (7.9) [16].

$$-d[\text{DPPH}^\bullet]/dt = k_{\text{H}}[\text{Trolox}][\text{DPPH}^\bullet] \quad (1)$$

$$k_{\text{obs}} ([\text{Trolox}] > 10[\text{DPPH}^\bullet]) = k_{\text{H}}[\text{Trolox}] \quad (2)$$

The reaction of Trolox with $\text{DPPH}^\bullet/\beta\text{-CD}$ was also carried out in temperature range from 283 to 303 K. Table 1 lists the k_{H} and k_{D} values determined from the slopes of the linear plots of the k_{obs} vs. the Trolox concentrations.

Table 1. k_{H} , k_{D} , and $k_{\text{H}}/k_{\text{D}}$ values for the reaction of Trolox with $\text{DPPH}^\bullet/\beta\text{-CD}$ in phosphate buffer solutions (0.05 M, pH 7.0, or pD 7.0).

T/K	$k_{\text{H}}/\text{M}^{-1} \text{ s}^{-1}$	$k_{\text{D}}/\text{M}^{-1} \text{ s}^{-1}$	$k_{\text{H}}/k_{\text{D}}$
283	3.0×10^3	2.9×10^2	11
288	4.3×10^3	5.3×10^2	8.2
293	7.8×10^3	1.2×10^3	6.6
298	1.4×10^4	2.0×10^3	7.4
303	1.5×10^4	2.7×10^3	5.7

Furthermore, as seen in the Arrhenius plots based on the Arrhenius equation Equation (3) ($E_{\text{a}}(\text{H})$, $E_{\text{a}}(\text{D})$: activation energy, A_{H} , A_{D} : Arrhenius prefactor, R : gas constant and T : temperature in K) shown in Figure 4, linear correlations of $\ln k_{\text{H}}$ vs. T^{-1} and $\ln k_{\text{D}}$ vs. T^{-1} were observed in the reaction of Trolox with $\text{DPPH}^\bullet/\beta\text{-CD}$ in the whole temperature range. From the intercepts and slopes of the linear plots in Figure 4, the Arrhenius prefactors and activation energies were obtained as $A_{\text{H}} = 1.5 \times 10^{15} \text{ M}^{-1} \text{ s}^{-1}$, $A_{\text{D}} = 5.2 \times 10^{17} \text{ M}^{-1} \text{ s}^{-1}$, $E_{\text{a}}(\text{H}) = 63 \text{ kJ mol}^{-1}$, and $E_{\text{a}}(\text{D}) = 82 \text{ kJ mol}^{-1}$, respectively. The isotopic ratio of ratio, $A_{\text{H}}/A_{\text{D}}$, was obtained as 0.003, which is beyond the semiclassical limits of 0.4–1.4 [16]. The isotopic difference, $E_{\text{a}}(\text{D}) - E_{\text{a}}(\text{H})$, (19 kJ mol^{-1}) was significantly greater than the difference in zero-point energies of 5.1 kJ mol^{-1} [16]. These results indicate that quantum mechanical tunneling plays a role in the hydrogen-transfer reaction from Trolox to $\text{DPPH}^\bullet/\beta\text{-CD}$ in a phosphate buffer [17–20].

$$\ln k_{\text{H}} = -E_{\text{a}}(\text{H})/(RT) + \ln A_{\text{H}} \text{ or } \ln k_{\text{D}} = -E_{\text{a}}(\text{D})/(RT) + \ln A_{\text{D}} \quad (3)$$

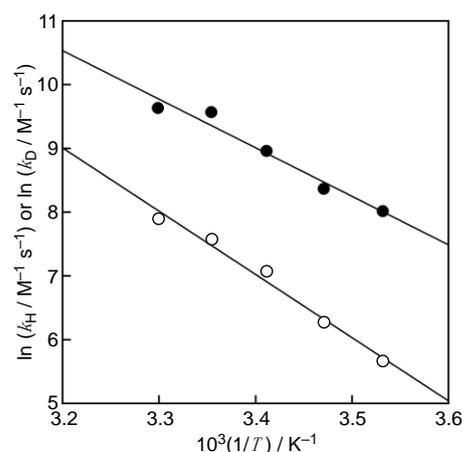


Figure 4. Arrhenius plots of $\ln k_{\text{H}}$ vs. T^{-1} (closed circles) and $\ln k_{\text{D}}$ vs. T^{-1} (open circles) in phosphate buffer (H_2O , 0.05 M, pH 7.0) and in phosphate buffer (D_2O , 0.05 M, pD 7.0), respectively.

4. Conclusions

The solubilization of DPPH^\bullet in water by $\beta\text{-CD}$ enabled us to investigate the kinetics of hydrogen-transfer reactions involving DPPH^\bullet in aqueous media. The large KIE, as well as

the temperature dependence of the KIE observed for the hydrogen-transfer reaction from Trolox to β -CD-solubilized DPPH \bullet , indicates that quantum mechanical tunneling played a role in the reaction. To the best of our knowledge, this is the first report about quantum mechanical tunneling in a reaction of DPPH \bullet in aqueous media at ambient temperature.

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