


 Cite this: *Chem. Commun.*, 2020, 56, 11505

 Received 31st July 2020,
 Accepted 24th August 2020

DOI: 10.1039/d0cc05214k

rsc.li/chemcomm

A large kinetic isotope effect in the reaction of ascorbic acid with 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO[•]) in aqueous buffer solutions†

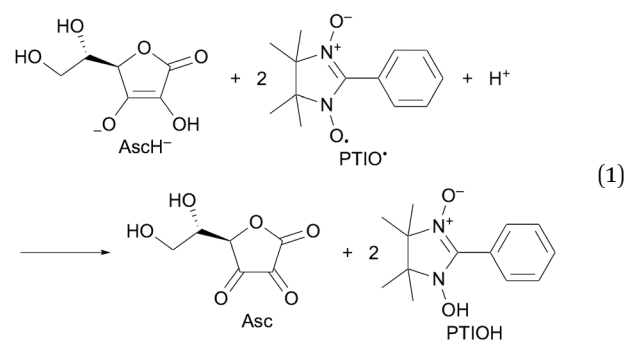
 Ikuo Nakanishi,^a Yoshimi Shoji,^a Kei Ohkubo,^{ab} Toshihiko Ozawa,^c Ken-ichiro Matsumoto^a and Shunichi Fukuzumi^{de}

A large kinetic isotope effect (KIE, k_H/k_D) of 12.8 was observed for the hydrogen-transfer reaction from ascorbic acid to 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide (PTIO[•]) in a phosphate buffer solution (0.05 M, pH/pD 7.0) at 298 K. The isotopic difference in the activation energies (6.8 kJ mol⁻¹) determined from the temperature dependence of the KIE suggests that quantum mechanical tunneling may partly play a role in the reaction, although the isotopic ratio of the Arrhenius prefactor ($A_H/A_D = 0.86$) is within the semiclassical limits.

Quantum mechanical tunneling in hydrogen-transfer reactions^{1–3} in biological redox systems has attracted considerable attention with regard to the quantum mechanical behaviour in biology in recent years.⁴ Uršić *et al.* reported that a large kinetic isotope effect (KIE, k_H/k_D) of 24.2 was observed in water for the hydrogen-transfer reaction from ascorbic acid (AsCH₂), one of the representative water-soluble antioxidants, to 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) radicals.⁵ This is the first report about hydrogen tunneling in a reaction involving AsCH₂. On the other hand, Li has recently reported a new and simple antioxidant assay *in vitro* using 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxyl 3-oxide radicals (PTIO[•]), one of the nitronyl nitroxide radicals, where a hydrogen transfer occurred from antioxidants to PTIO[•].⁶ However, little is known about the kinetics of the reaction between

antioxidants and PTIO[•], as well as the KIE. We report herein the observation of a large kinetic isotope effect for the reaction of AsCH₂ with PTIO[•] in a phosphate buffer. A possibility of the involvement of quantum mechanical tunneling is also discussed based on the temperature dependence of the KIE.

When AsCH₂ was added to the phosphate buffer solution (0.05 M, pH 7.0) of PTIO[•], the bands at 345 and 560 nm decreased immediately with clear isosbestic points at 218, 244, 279 and 313 nm as shown in Fig. 1. Since the pK_a value of AsCH₂ is reported to be 4.1,⁷ AsCH₂ undergoes deprotonation and exists in its anionic form, AsCH⁻, in phosphate buffer solution (0.05 M, pH 7.0). Thus, this spectral change indicates that AsCH⁻ efficiently scavenged PTIO[•] in phosphate buffer. The spectral titration (inset of Fig. 1) shows that the stoichiometry of the reaction is given by eqn (1), where AsCH⁻ reacts with 2PTIO[•].



The decay of the absorbance at 560 nm monitored by a stopped-flow technique obeyed pseudo-first-order kinetics, when the concentration of AsCH₂ ([AsCH₂]) was maintained at more than a 10-fold excess of PTIO[•] concentration (Fig. 2). The pseudo-first-order rate constants (k_{obs}) linearly increased with increasing [AsCH₂] (Fig. 3). From the slope of the linear plot, the second-order rate constant (k_H) for the scavenging reaction of PTIO[•] by AsCH₂ [eqn (2)] was determined in a phosphate buffer

^a Quantitative RedOx Sensing Group, Department of Basic Medical Sciences for Radiation Damages, National Institute of Radiological Sciences (NIRS), Quantum Medical Science Directorate, National Institutes for Quantum and Radiological Science and Technology (QST), Inage-ku, Chiba 263-8555, Japan.

E-mail: nakanishi.ikuo@qst.go.jp; Fax: +81-43-255-6819; Tel: +81-43-206-3131

^b Institute for Advanced Co-Creation Studies, Open and Transdisciplinary Research Initiatives, Osaka University, 2-8 Yamada-oka, Suita, Osaka 565-0871, Japan

^c Nihon Pharmaceutical University, Kitaadachi-gun, Saitama 362-0806, Japan

^d Department of Chemistry and Nano Science, Ewha Womans University,

Seoul 03760, Korea

^e Faculty of Science and Engineering, Meijo University, Nagoya, Aichi 468-8502, Japan

† Electronic supplementary information (ESI) available: Experimental details. See DOI: 10.1039/d0cc05214k



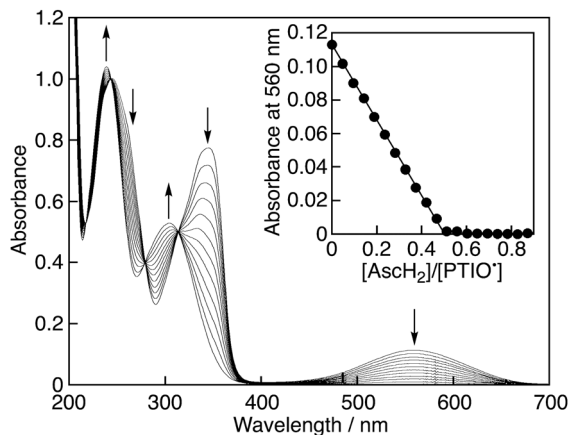
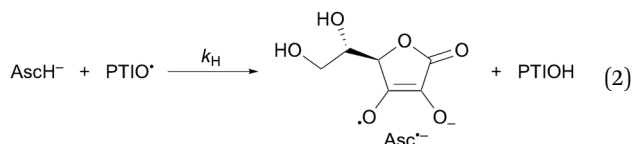


Fig. 1 Spectral change observed upon addition of AscH_2 (0 – 5.2×10^{-5} M, 4.6×10^{-6} M each) to PTIO^* (1.0×10^{-4} M) in phosphate buffer (0.05 M, pH 7.0). Inset: Plot of the absorbance at 560 nm vs. $[\text{AscH}_2]/[\text{PTIO}^*]$.

(0.05 M, pH 7.0) to be $2.4 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$. This value is smaller than that determined for the reaction between AscH_2 and β -cyclodextrin-solubilised 2,2-diphenyl-1-picrylhydrazyl radicals (DPPH^*)⁸ under the same experimental conditions ($k_{\text{H}} = 5.6 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$). Thus, the reactivity of PTIO^* toward AscH_2 is lower than that of DPPH^* .



When H_2O was replaced by D_2O to prepare the phosphate buffer, the exchangeable O–H protons in AscH_2 are replaced by deuterons from D_2O to produce AscD_2 . The second-order rate constant (k_{D}) thus determined for the reaction of AscD_2 with PTIO^* was significantly decreased to be $1.9 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$.

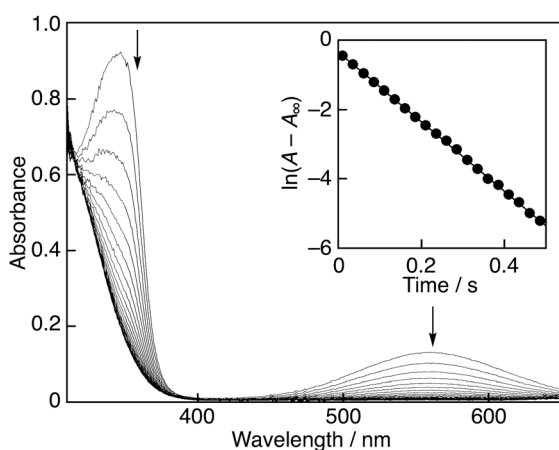


Fig. 2 Spectral change (interval: 25 ms) observed during the reaction of AscH_2 (4.0×10^{-3} M) with PTIO^* (9.4×10^{-5} M) in phosphate buffer (0.05 M, pH 7.0) at 298 K. Inset: The first-order plot of the absorbance at 560 nm.

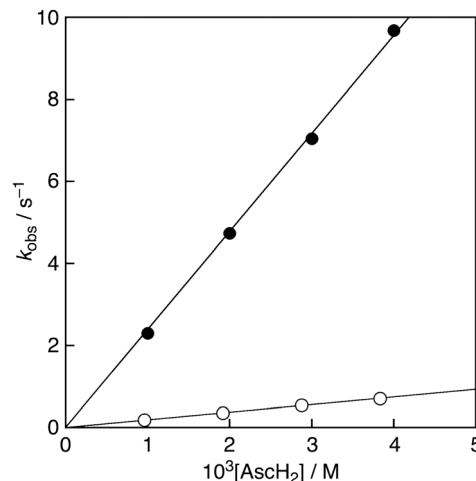


Fig. 3 Plots of pseudo-first-order rate constants (k_{obs}) vs. concentrations of AscH_2 in phosphate buffer (H_2O , 0.05 M, pH 7.0) (closed circles) and in phosphate buffer (D_2O , 0.05 M, pD 7.0) (open circles).

Thus, the KIE ($k_{\text{H}}/k_{\text{D}}$) is calculated to be 12.8. Such a large KIE value has clearly precluded an electron-transfer pathway in the oxidation reaction of AscH_2 by PTIO^* . This value is beyond the maximum expected semiclassical value of 7.9 for the dissociation of the O–H bond.⁹ We also performed the reaction of AscH_2 with PTIO^* in the temperature range from 288 to 308 K and the k_{H} and k_{D} values were determined from the slopes of the linear plots of the k_{obs} values vs. concentrations of AscH_2 or AscD_2 (Table 1).

Furthermore, as the Arrhenius plots are shown in Fig. 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 AscH_2 with PTIO^* in the whole temperature range. From the intercepts of Fig. 4, the isotopic ratio of the Arrhenius prefactor ($A_{\text{H}}/A_{\text{D}} = 0.86$) was obtained. This value can be fitted within the semiclassical limits of 0.7–1.4 for the $A_{\text{H}}/A_{\text{D}}$ value in a hydrogen-transfer process.⁹ The isotopic difference in the activation energies $E_{\text{a}}(\text{D}) - E_{\text{a}}(\text{H})$ was 6.8 kJ mol^{-1} , which is beyond the difference in zero-point energies of 5.1 kJ mol^{-1} .⁹ A large $A_{\text{H}}/A_{\text{D}}$ value ($\gg 1$) in hydrogen transfer of some enzymes has been reported by Klinman *et al.*, proposing a full tunneling mode to explain such observation.³ Furthermore, Uršič *et al.* claimed that quantum mechanical tunneling plays a role in the reaction between AscH_2 and TEMPO in water–dioxane (1 : 1 v/v) based on the large KIE value of 31.1 and $E_{\text{a}}(\text{D}) - E_{\text{a}}(\text{H})$ value of 8.2 kJ mol^{-1} , although the $A_{\text{H}}/A_{\text{D}}$ value is 1.2.⁵ Thus, quantum mechanical tunneling may

Table 1 k_{H} , k_{D} and $k_{\text{H}}/k_{\text{D}}$ values for the reaction of AscH_2 or AscD_2 in phosphate buffer solutions (0.05 M, pH 7.0 or pD 7.0)

T/K	$k_{\text{H}}/\text{M}^{-1} \text{ s}^{-1}$	$k_{\text{D}}/\text{M}^{-1} \text{ s}^{-1}$	$k_{\text{H}}/k_{\text{D}}$
288	1.5×10^3	9.6×10	15.6
293	2.0×10^3	1.5×10^2	13.3
298	2.4×10^3	1.9×10^2	12.8
300	2.8×10^3	1.9×10^2	14.8
303	2.9×10^3	2.2×10^2	13.3
308	3.1×10^3	2.6×10^2	12.2



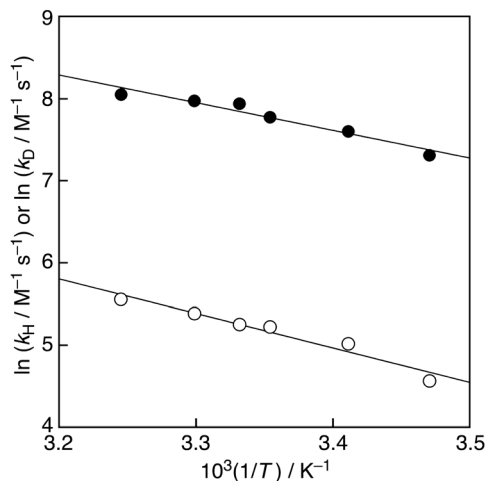


Fig. 4 Arrhenius plots of $\ln k_{\text{H}}$ vs. T^{-1} (closed circles) and $\ln k_{\text{D}}$ vs. T^{-1} (open circles) in phosphate buffer (H_2O , 0.05 M, pH 7.0) and in phosphate buffer (D_2O , 0.05 M, pD 7.0), respectively.

partly play a role in the reaction between AscH_2 and PTIO^{\bullet} in a phosphate buffer solution.

In summary, a large KIE was observed for the hydrogen-transfer reaction from AscH_2 to PTIO^{\bullet} . The temperature dependence of the KIE suggests that quantum mechanical tunneling may partly play a role in the reaction, although the isotopic ratio of the Arrhenius prefactor ($A_{\text{H}}/A_{\text{D}}$) is within the semiclassical limits. Because there are only a few reports about a large KIE in a reaction involving AscH_2 , this study provides valuable

information for the biological redox reactions including ascorbic acid.

This work was partially supported by Grant-in-Aid (No. 18K06620 to I. N., 20H02779, 20H04819, 18H04650, 17H03010 and 16H02268 to K. O.) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We also thank Dr Hiroaki Kotani (University of Tsukuba) for valuable discussions.

Conflicts of interest

There are no conflicts to declare.

Notes and references

- J. Meisner and J. Kästner, *Angew. Chem., Int. Ed.*, 2016, **55**, 5400.
- P. R. Schreiner, *J. Am. Chem. Soc.*, 2017, **139**, 15276.
- (a) J. P. Klinman and A. R. Offenbacher, *Acc. Chem. Res.*, 2018, **51**, 1966; (b) Z. D. Nagel and J. P. Klinman, *Nat. Chem. Biol.*, 2009, **5**, 543.
- J. McFadden and J. Al-Khalili, *Proc. R. Soc. A*, 2018, **474**, 20180674.
- (a) I. Sajenko, V. Pilepić, C. Jakobušić Brala and S. Uršić, *J. Phys. Chem. A*, 2010, **114**, 3423; (b) A. Karković Marković, C. Jakobušić Brala, V. Pilepić and S. Uršić, *Molecules*, 2020, **25**, 1443.
- X. Li, *J. Agric. Food Chem.*, 2017, **65**, 6288.
- (a) C. Creutz, *Inorg. Chem.*, 1981, **20**, 4449; (b) N. H. Williams and J. K. Yandell, *Aust. J. Chem.*, 1982, **35**, 1133.
- (a) I. Nakanishi, K. Ohkubo, K. Imai, M. Kamibayashi, Y. Yoshihashi, K. Matsumoto, K. Fukuhara, K. Terada, S. Itoh, T. Ozawa and S. Fukuzumi, *Chem. Commun.*, 2015, **51**, 8311; (b) I. Nakanishi, K. Ohkubo, M. Kamibayashi, Y. Ogawa, T. Ozawa, K. Matsumoto and S. Fukuzumi, *ChemistrySelect*, 2016, **1**, 3367.
- R. P. Bell, *The Tunnel Effect in Chemistry*, Chapman & Hall, London, 1980.

