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

Ikuo Nakanishi,*a Yoshimi Shoji,a Kei Ohkubo,ab Toshihiko Ozawa,c Ken-ichiro Matsumotoa and Shunichi Fukuzumide

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.
b Institute for Advanced Co-Creation Studies, Open and Transdisciplinary Research Initiatives, Osaka University, 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, SENTAN, Japan Science and Technology Agency (JST), Nagoya, Aichi 468-8502, Japan

E-mail: nakanishi.ikuo@qst.go.jp
Experimental details

Materials
Ascorbic acid (L(+)-ascorbic acid, AscH$_2$) and phosphate buffer solution (0.1 M, pH 7.0) were purchased from Fujifilm Wako Pure Chemical Ind. Ltd., Japan. 2-phenyl-4,4,5,5-tetramethylimidazoline-1-oxy 3-oxide (PTIO•) was commercially obtained from Tokyo Chemical Industry Co., Ltd., Japan. D$_2$O was purchased from Nacalai Tesque, Inc., Japan. The water used in this study was freshly prepared with a Milli-Q system (Millipore Direct-Q UV3). The deuterated phosphate buffer solution was prepared by dissolving phosphate buffer powder (Fujifilm Wako Pure Chemical Ind. Ltd.) to D$_2$O and the pD was adjusted by adding 5 N hydrochloric acid (Fujifilm Wako Pure Chemical Ind. Ltd.). The pD values was calculated by adding 0.4 to the corresponding pH value measured by a HORIBA D-51 pH meter.$^1$

Spectral and kinetic measurements
UV-vis spectra were recorded on an Agilent 8453 photodiode array spectrophotometer. The rates of the scavenging reaction of PTIO• in a phosphate buffer solution (0.05 M, pH 7.0) by AscH$_2$ were determined by monitoring the absorbance change at 560 nm due to PTIO• ($\varepsilon = 1.2 \times 10^3$ M$^{-1}$ cm$^{-1}$) after mixing of PTIO• in water (Milli-Q) with a phosphate buffer solution (0.1 M, pH 7.0) containing AscH$_2$ at a volumetric ratio of 1:1 using a stopped-flow technique on a UNISOKU RSP-1000-02NM spectrophotometer. The pseudo-first-order rate constants ($k_{obs}$) were determined by a least-squares curve fit using an Apple MacBook Pro personal computer. The first-order plots of ln($A - A_{\infty}$) vs. time ($A$ and $A_{\infty}$ are denoted as the absorbance at the reaction time and the final absorbance, respectively) were linear until three or more half-lives with the correlation coefficient $\rho > 0.999$.

References