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PCET in the oxidation of ascorbate. Dramatic change of the kinetic isotope effect on the change in solvent polarity

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Abstract—The first step in the oxidation of ascorbate with substituted nitrosobenzenes is a proton-coupled electron transfer (PCET) reaction and the observed kinetic isotope effects in the reaction, k_{H_2O}/k_{D_2O}, change dramatically with a change in solvent polarity, in line with known theoretical predictions.

Proton-coupled electron transfers (PCET) are processes where proton motion can affect electron transfer.[1–13] Owing to its exceptional importance in a wide range of chemical, biochemical, and biological systems, this phenomenon has been greatly studied, both experimentally[2,4–7] and theoretically.[8–13] An important example of how PCET works in natural systems is presented by the well known case of oxygen production by the photosystem II oxygen-evolving complex (PSII OEC).[1,4] It is the fundamental feature of PCET reactions that the concerted transfer of a proton and an electron constitute a single chemical reaction step and the direct coupling of the electron and proton in the transfer is the most elementary characteristic of a PCET event.[1,2] A special class of PCET reaction is termed as hydrogen atom transfer (HAT). Here, transfer of an electron and a proton occurs along a roughly common path; the proton is transferred together with one of its bonding electrons.[1,2]

Transfer of an electron and a proton (net hydrogen atom transfer) from ascorbate ($HAsc^-$) is important in the chemistry and biochemistry of this ubiquitous, simple biomolecule. Examples include ascorbate peroxidase,[14a] cytochrome b_{561},[14b] a secretory-vesicle redox chain,[14c] the biologically important interaction of ascorbate with peroxynitrite,[14d] the recycling of α-tocopheroxyl radicals by ascorbate,[14e] and ascorbate-induced release of nitric oxide from *N*-nitrosated tryptophan.[14f] Concerted proton–electron transfer has been inferred for some systems[14b,c,e,g] on the basis of thermochemical arguments and observed kinetic isotope effects (KIE). Here, we present evidence in support that the first step[22] in the oxidation of ascorbate with nitrosobenzenes (Scheme 1) is a PCET reaction, and the observed kinetic deuterium isotope effects (KIE) in the reaction depend dramatically on the solvent polarity. The latter observation is among the first experimental confirmations of theoretical predictions[10c,d] on the dependence of KIE on solvent polarity in PCET processes. The evidence is as follows:

(i) The observed free energy of activation $\Delta G^{\ddagger}$ for the first step of the reaction of ascorbate with nitrosobenzene (Scheme 1) in water is 53.3 kJ/mol. Applying the usual square scheme[1,2] to describe the thermochemistry of different pathways of the reaction reveals that as the pK_a of the ascorbate 2-OH group is[15] 11.79 and the pK_a of the nitroso group of nitrosobenzene is[16] −9.5, the expected barrier for the initial proton transfer (PT) from ascorbate to the nitroso group in a stepwise PT/ET process would be ca. 121.5 kJ mol^{-1}, which is 68 kJ larger than the observed $\Delta G^{\ddagger}$ of the reaction. Furthermore, since the reduction potential for the couple $HAsc^{\bullet}/HAsc^-$ is[15a,c] 774 mV, the corresponding barrier for initial electron transfer (ET) from ascorbate to the nitroso group should be 73.8 kJ mol^{-1}, ca. 21 kJ more than the observed $\Delta G^{\ddagger}$. Therefore, concerted electron and proton transfer seems to be the likely mechanism to avoid higher energy intermediates during the process.

Keywords: Proton-coupled electron transfer; Kinetic isotope effects; Ascorbate.

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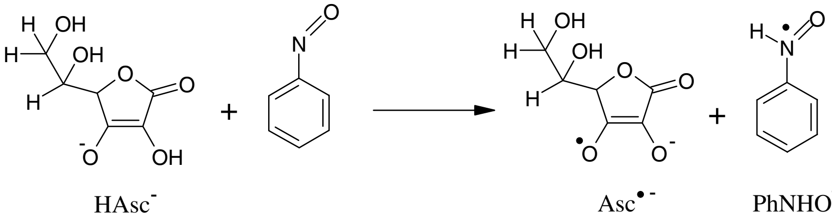

Scheme 1.

Table 1. Kinetic isotope effects k_{H_2O}/k_{D_2O} in the reaction of ascorbate with substituted nitrosobenzenes in water and in water–dioxane at 298 K[a]

	p-CH$_3$	p-H	p-Cl	p-Br	m-Cl
Water	2.63 (0.17)	2.43 (0.07)	2.30 (0.10)	2.07 (0.14)	2.20 (0.11)
Water–dioxane[b]	9.42 (0.11)	8.51 (0.08)	8.70 (0.26)	8.40 (0.28)	6.40 (0.05)

[a] All the data are related[22] to the process defined by Scheme 1.

[b] In 1:1 v/v water–dioxane and 1:1 v/v D_2O–dioxane.

(ii) Kinetic isotope effects k_{H_2O}/k_{D_2O} ranging from 2.63 (p-CH$_3$ nitrosobenzene) to 2.20 (m-Cl nitrosobenzene) were observed (Table 1) in the reaction (Scheme 1). If there is a rate-limiting electron transfer step in the reaction, only significantly smaller secondary isotope effects would be expected.[17] The kinetic evidence[22] clearly indicates that ascorbate anions and the nitroso compound are the only reactants in the process, and the above thermochemical analysis strongly suggests a concerted process. Hence, the observed isotope effects should be consistent with a PCET process. The observed KIE for the reaction in water are not large. PCET processes are considered to occur by tunneling[1,8–10,12,13a] (see also below) but KIE (referred to as the H/D kinetic isotope effects) in PCET reactions are not necessarily large, as demonstrated both experimentally[5a,6] and theoretically.[9,10c,d,13a] Thus, for example, the observed KIE in the range 1.2–2.1 are not uncommon[5a,6] for a PCET reaction.

(iii) The observed difference in the enthalpies of activation in H_2O and D_2O, $\Delta H^{\ddagger}_{H_2O} = 8.9\ (0.3)\ \mathrm{kJ\ mol^{-1}}$ and $\Delta H^{\ddagger}_{D_2O} = 31.6\ (1.7)\ \mathrm{kJ\ mol^{-1}}$, respectively, are substantially larger than the expected semiclassical values calculated from the observed KIE. This observation indicates that tunneling[18] is likely in the proton transfer. The same is true with regard to the corresponding observed entropies of activation, $\Delta S^{\ddagger}_{H_2O} = -148.8\ (3.5)\ \mathrm{J\ K^{-1}\ mol^{-1}}$, and $\Delta S^{\ddagger}_{D_2O} = -80.0\ (3.8)\ \mathrm{J\ K^{-1}\ mol^{-1}}$, respectively, which suggest a ratio of isotopic Arrhenius pre-exponential factors substantially different from the semiclassical expectation of unity. The observation of tunneling is as expected[1,8–10,12,13a] for a PCET process. Eyring and co-workers[19a] have observed closely similar isotopic differences in the enthalpies and entropies of activation in the ferrate(VI) oxidation of aqueous phenol and the evidence clearly suggests a PCET process. The k_{H_2O}/k_{D_2O} value of 2.4 observed is very similar to the KIE observed in our reaction in water.

(iv) The Hammett plot of $\log k$ versus σ parameters for the reaction gave a slope (ρ) of 1.64, consistent with a charge redistribution and the presence of a partial negative charge on the acceptor in the transition state. Analogous Hammett correlations are not uncommon in PCET reactions. For example, in the oxidation of *meta*-substituted phenols by oxochromium(IV) ions[19b] the observed slope (ρ) is −1.72, and the observed k_{H_2O}/k_{D_2O} is 14.7, a result indicative of tunneling in the PCET reaction.

(v) A dramatic change of the KIE in the reaction on going from water to water–dioxane[†] solvent was observed (Table 1). To our knowledge, this observation presents the first experimental confirmation of the theoretical predictions on the influence of solvent polarity on the magnitude of KIE in PCET reactions.[10c,d] According to this theoretical explanation, a complex interplay between several factors, including the localization of, and the distance of the reactant and product proton vibrational wave function and the electron donor–acceptor distance (which influences electron–proton electrostatic interactions) and the solvent polarity control the magnitude of the corresponding KIE. In general, the KIE in a PCET process increases as the probability of the PCET mechanism increases. In particular, the KIE increases as the proton donor–acceptor distance increases and the electron donor–acceptor distance decreases and as solvent polarity decreases in a PCET process. Here, since the observed Hammett $\log k/\sigma$ parameters correlation suggests some charge reorganization/separation in the activation process, the observed change of KIE could be interpreted as a consequence of a decrease in the electron donor–acceptor distance in the PCET process[‡] due to lowering of the solvent dielectric constant. This should not contradict the fact that the proton donor–acceptor distance also possibly

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[†] The rate of reaction in 50:50 water–dioxane is reduced from 2900 $\mathrm{mol^{-1}\ s^{-1}}$ in water to 1800 $\mathrm{mol^{-1}\ s^{-1}}$ in water–dioxane.

[‡] A referee has suggested that it is not clear that the electron donor–acceptor distance in the PCET process would decrease, and that it is not necessary for this distance to be decreased for the observed change of KIE. He has argued that the analysis in Ref. 10c indicates that, even with a constant electron donor–acceptor distance, the KIE is expected to increase as the solvent polarity decreases because of changes in the curvatures and relative energies of the free energy curves along the collective solvent coordinate.

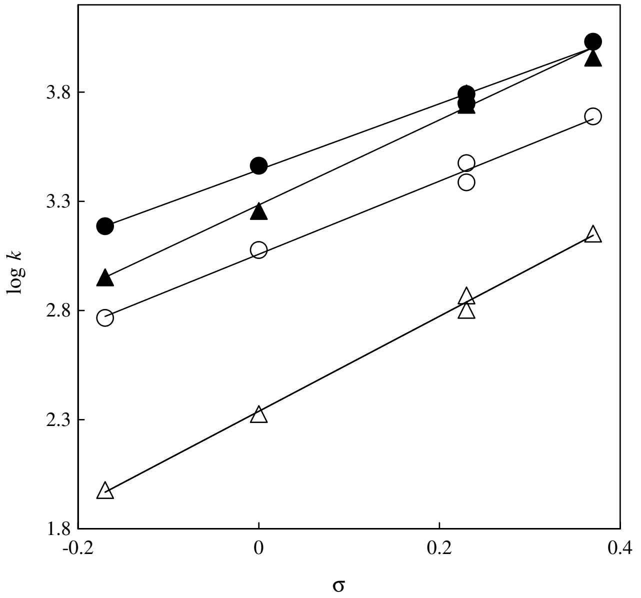

Figure 1. Hammett plots for the reaction of ascorbate with substituted nitrosobenzenes at 298 K in water and in water–dioxane. Solid circles and open circles, in water and D_2O ($\rho_{H_2O} = 1.51$, $r^2 = 0.993$; $\rho_{D_2O} = 1.67$, $r^2 = 0.991$); solid triangles and open triangles, in 1:1 v/v water dioxane and D_2O–dioxane ($\rho_{H_2O\text{–Dioxane}} = 1.95$, $r^2 = 0.990$; $\rho_{D_2O\text{–Dioxane}} = 2.18$, $r^2 = 0.997$), respectively. Rate constants were obtained as described in the text.[22]

decreases; according to these theoretical predictions,[10c,d] KIE are largest for intermediate proton donor–acceptor distances due to the opposed effects of the ratio of the hydrogen-to-deuterium couplings (these increase as the proton donor–acceptor distance becomes larger, thus increasing the KIE) and the probability of PCET (which increases as the proton donor–acceptor distance decreases, which can lead to a larger KIE[10c,d]).

(vi) The observed variation of the KIE with different substituents on the nitrosobenzene (Fig. 1, Table 1) may serve as further evidence in support of the above noted theoretical predictions.[10c,d] Accordingly, it would be expected[10c,d] that the KIE become larger as the reaction becomes more endothermic (or less exothermic, in the normal Marcus regime). Our experimental results show that the observed KIE could increase as the reaction becomes more endothermic, assuming however, that the entropy of activation remained essentially unchanged[§] on changing a substituent. This is also illustrated by the difference between the slopes (ρ values) of the Hammett $\log k$ versus σ correlations for the examined reaction in H_2O and D_2O ($\rho_{H_2O} = 1.51$, $\rho_{D_2O} = 1.67$, $\Delta\rho = 0.16$) and H_2O–dioxane and D_2O–dioxane ($\rho_{H_2O\text{–Dioxane}} = 1.95$, $\rho_{D_2O\text{–Dioxane}} = 2.18$, $\Delta\rho = 0.23$). The differences are relatively small but statistically significant for both systems.

There is a question[¶] whether the reaction is a HAT process, or a PCET where the electron and proton are transferred between different sets of orbitals.[1,2,20a,b] Several lines of evidence can be interpreted to be more in support of PCET than a HAT process. The rate of the reaction depends markedly on the solvent polarity (see Fig. 1) while only small variations of reaction rates on the solvent polarity are expected for HAT reactions.[21a] The observed entropy of activation in the reaction, $\Delta S^\ddagger = -148.8\ \mathrm{J\,K^{-1}\,mol^{-1}}$ can be compared, for example, with the value of $\Delta S^\ddagger = -144\ \mathrm{J\,K^{-1}\,mol^{-1}}$ in the PCET reaction of phenols with oxochromium(IV) ions[19b] and the values for numerous *formal* HAT reactions for which DiLabio and Ingold have proposed[20b] to occur by PCET. In the HAT reaction[21b,c] of $PhCH_2^{\cdot}$ with $PhCH_3$, $\Delta S^\ddagger \approx -50\ \mathrm{J\,K^{-1}\,mol^{-1}}$. Furthermore, in the PCET and ET self-exchange reactions of iron-bi-imidazoline complexes,[21c] $\Delta S^\ddagger(\mathrm{PCET}) = -109\ \mathrm{J\,K^{-1}\,mol^{-1}}$ and $\Delta S^\ddagger(\mathrm{ET}) = -105\ \mathrm{J\,K^{-1}\,mol^{-1}}$, while in the self-exchange reaction between $Fe(H_2O)_6^{2+}$ and $Fe(H_2O)_6^{3+}$, $\Delta S^\ddagger(\mathrm{ET}) = -105\ \mathrm{J\,K^{-1}\,mol^{-1}}$ and similar values were observed for other self-exchange ET reactions[21c] of iron(II)/iron(III) complexes. The change in charge distribution is larger for ET than for the corresponding PCET[11a] (except when the electron and proton in a PCET are transferred in opposite directions)[11b] and the similarities in the magnitude of the entropies of activation in the ET and the PCET reactions may support the expectation that the entropy change in the latter is largely caused by charge redistribution originating from the motion of the electron in transfer. It should be added that the proton in a PCET is transferred over a much smaller distance than the electron[1,8–12] thus requiring correspondingly less solvent reorganization. In a HAT process, accompanied transfer of a proton and an electron is expected to cause less charge redistribution[9] and consequently, less solvent reorganization[21c] which would be reflected in a smaller entropy of activation and a smaller influence of solvent polarity on the rate of reaction.

In conclusion, the results obtained should be relevant for improved understanding and interpretation of kinetic isotope effects in PCET processes in biochemical/biological systems. Finally, after Mayer,[20a] and DiLabio and Ingold[20b] demonstrated theoretically that the formal HAT reaction can in fact be a PCET process, and that some PCET reactions could involve a cyclic transition state,[20b] the question arises whether a multi-center, cyclic PCET might occur in the reaction studied here. This is currently being investigated in our laboratory.

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[§] The expectation that the changes of entropy of activation due to the changes of the substituent on the electron/proton acceptor could be relatively unimportant relies on an assumption that the solvation in the corresponding transition state would differ only slightly within the series of substrates differing by these uncharged substituents.

[¶] According to the referee's opinion, the distinction between HAT and PCET is not rigorous because the electron and the proton behave quantum mechanically. A point of interest in this study is, however, if the electron and the proton are transferred between the different sites (possibly via a cyclic transition state).

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22. All the rate constants (the rate parameters were calculated from the experimental pseudo-first-order rate constants obtained spectrophotometrically[14g] by following the disappearance of the absorbance of the nitroso compound at 306 nm.) presented are related solely to the process (Scheme 1). The reverse reaction (the recombination of $Asc^{\bullet -}$ and $PhNHO^{\bullet}$, k_{-1})

$$HAsc^- + PhNO \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} Asc^{\bullet -} + PhNHO^{\bullet} \qquad (1)$$

should be insignificant under the conditions applied. This is probably due to a 10^3-fold increase in the rate of disproportionation of an ascorbyl radical to ascorbate and dehydroascorbic acid on going from the neutral to the acidic range (see for example Ref. 15b, pp 78–79, and references cited therein), since the disproportionation competes with the reverse reaction. The net chemical reaction is[14g]

$$HAsc^- + PhNO + H^+ \rightarrow DHA + PhNHOH \qquad (2)$$

(DHA = dehydroascorbic acid, PhNHOH = phenylhydroxylamine), as confirmed spectroscopically and by product analysis, according to previous findings.[14g] The reaction goes to completion, under all the conditions employed. The findings relevant to the above are (i) the kinetics are second order overall and first order with respect to both ascorbate and the nitroso compound. This is true in acidic[14g] as well in the neutral range; (ii) in the acidic range, the kinetics follow the rate law[14g]

$$k_{obs} = \left[(k_{HA^-} - k_{H_2A})\left(\frac{K_a}{K_a + [H^+]}\right) + K_{H_2A}\right][H_2A]_o$$

where k_{HA^-} and k_{H_2A} are the second-order rate constants for the reactions of the nitroso compound with ascorbate and ascorbic acid, respectively, K_a is the first dissociation constant of ascorbic acid, and since k_{HA^-} is[14g] 10^3 greater than k_{H_2A}, the latter can, to a good approximation, be neglected at H^+ concentrations greater than 10^{-3} mol dm^{-3} ($\geqslant$98% ascorbate reaction); (iii) the second-order rate constants (k_1, 2) calculated from the observed pseudo-first-order rate constants using the above rate law are

invariably the same in the acidic range from 0.1 to 0.0002 mol dm^{-3} of H^{+}; (iv) the ratio k_{obs}/[HAsc^{-}] decreases steadily from 2.9×10^{3} at 0.0002 mol dm^{-3} of H^{+} to 5.5×10^{2} at pH 6; (v) the obtained ESR spectra confirmed the formation of ascorbyl and phenylnitroxyl radicals in the reaction, in line with early[14h] and the recent ESR studies (for PhNHO$^{\bullet}$ see also Ref. 14i; for Asc$^{\bullet-}$ see Refs. 15a, pp 123–126 and 15b, Chapter 4).