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Solvent-induced hydrogen tunnelling in ascorbate proton-coupled electron transfers

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ABSTRACT

The over-the-barrier proton-coupled electron transfer interaction of an ascorbate monoanion with a hexacyanoferrate(III) ion in water entered into a tunnelling regime in water–1,4-dioxane, water–ethanol and water–acetonitrile mixed solvents.

Quantum-mechanical hydrogen tunnelling is of remarkable significance for chemistry and biochemistry.[1–19] The role of hydrogen tunnelling in such fundamental chemical reactions as hydrogen transfers is well documented[3,4,17–19] and enzymatic C–H activation has been shown to take place via tunnelling.[2–10,13,14,16] Many non-enzymatic H-transfer reactions, including those where there is N–H, S–H and O–H activation[17,18] also involve hydrogen tunnelling. The tunnelling in a condensed phase has sometimes revealed somewhat 'exotic' features. Some examples are the appearance of 'colossal' kinetic isotope effects (KIEs) ranging up to $k_H/k_D = 455$ at room temperature[18a] (in the case of N–H–O transfer) in water–acetonitrile solution, and the rearrangement of matrix-isolated hydroxymethylene at 11 K into formaldehyde[19] which involves H-transfer from oxygen to carbon by pure tunnelling through a large energy barrier.

Proton-coupled electron transfer (PCET) reactions play a key role in a wide range of biological, biochemical and chemical systems,[20–34] including those related to artificial photosynthesis and solar fuels,[23–27] and nanostructures and interfaces.[33,34] Many processes in biology would not be possible without the coupling of proton and electron motion.[22,24] From a theoretical viewpoint, both sequential electron transfer followed by proton transfer (ET/PT) or vice versa (PT/ET), and the concerted transfer of these particles could be viewed within a unified theoretical framework[35] of PCET reactions. Hydrogen atom transfer (HAT) reactions, in which the electron and proton transfer simultaneously between the same donor and acceptor[21,22,35] are also included in the framework. However, the concerted transfer of a proton and an electron where there is a single chemical reaction step, direct coupling of the electron and proton in the transfer is the elementary characteristic of an authentic PCET reaction.

We report here the observation of solvent-induced tunnelling in the oxidation of an ascorbate monoanion with hexacyanoferrate(III) ions[36–38] (Scheme 1). This PCET reaction[37] in 'pure' water does not exhibit hydrogen tunnelling.[37] Very unexpectedly, on the addition of only ~1 M of an organic co-solvent, the reaction exhibited hydrogen tunnelling as demonstrated by the markedly changed isotopic ratios of the Arrhenius pre-factors that are well beyond the semiclassical limits of 0.5–1.4 for the A_H/A_D ratio in a hydrogen transfer process,[1,3,4,39–41] as well as the isotopic differences in the enthalpies of activation ($\Delta\Delta_r H^{\ddagger}$) between D_2O and H_2O, which are greater than the semiclassical value of 5.1 kJ/mol for the difference between zero-point energies $E_0^D - E_0^H$ for the dissociation of an O–H bond and are indicative of hydrogen tunnelling in the reaction.[1,3,4,38–40] (Table 1). To the best of our knowledge, the observation of solvent-induced tunnelling is unprecedented. Our findings are as follows:

(i) The oxidation of ascorbate monoanions with hexacyanoferrate(III) ions (Scheme 1) in various water–organic co-solvent mixtures involves a PCET process. This is true for the water–acetonitrile, water–1,4-dioxane, water–ethanol and water–acetone solvents used in this Letter (see Table 1 and Supplementary data (SI)). Thermochemical analysis of the corresponding consecutive (PT/ET or ET/PT) reactions (see also the Supplementary data)[21,38]

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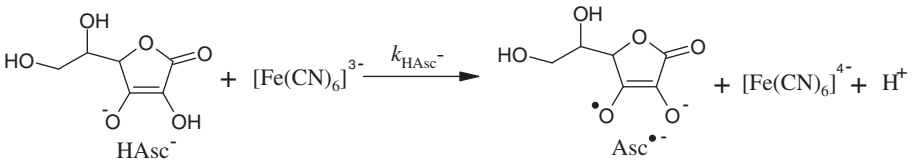

Scheme 1. $HAsc^-$ = ascorbate monoanion, $Asc^{\bullet-}$ = ascorbyl radical anion.[38]

Table 1
Activation parameters and kinetic isotope effects in the reaction shown in Scheme 1 in various mixed solvents[38]

Solvent[a,b,c,d]	k_{HAsc^-}/M^{-1} s^{-1}	KIE	$\Delta H^{\ddagger}$/kJ mol^{-1}	$\Delta S^{\ddagger}$/J K^{-1} mol^{-1}	$\Delta\Delta H^{\ddagger}$[d]/kJ mol^{-1}	A_H/A_D
H_2O–MeCN (1:1 v/v)	18.9 (0.1)	8.25 (0.09)	24.8 (0.3)	−137.3 (1.0)	6.9 (0.4)	0.49 (0.09)
H_2O–MeCN (1:1 v/v), 0.1 M Na–acetate	141.5 (0.8)	6.55 (0.05)				
H_2O–MeCN (1:1 v/v), 0.1 M NaCl	149.5 (1.9)	6.70 (0.27)				
H_2O–1,4-dioxane (1:1 v/v)[b]	8.2 (0.1)	8.37 (0.16)	37.6 (0.2)	−99.0 (0.7)	12.7 (0.4)	0.046 (0.008)
H_2O–1,4-dioxane (1:1 v/v), 0.3 M Na–acetate	282.9 (4.0)					
H_2O–1,4-dioxane (1:1 v/v), 0.3 M NaCl	274.9 (2.3)					
H_2O–EtOH (1:1 v/v)[c]	11.3 (0.1)	7.90 (0.22)	26.4 (0.2)	−136.0 (0.7)	11.9 (0.4)	0.065 (0.010)
H_2O–acetone (1:1 v/v)	6.7 (0.1)	8.59 (0.13)				
H_2O–MeCN (0.75:0.25 v/v)	22.8 (0.1)	7.81 (0.17)				
H_2O–MeCN (0.95:0.05 v/v)	52.2 (1.0)	5.61 (0.16)	19.7 (0.1)	−146.0 (0.3)	7.1 (0.4)	0.32 (0.04)
H_2O–1,4-dioxane (0.95:0.05 v/v)	47.2 (0.1)	5.35 (0.06)				
H_2O–EtOH (0.95:0.05 v/v)	53.6 (0.1)	5.42 (0.02)				
H_2O–acetone (0.95:0.05 v/v)	46.2(0.8)	5.33 (0.17)				
H_2O[e]	71.0 (1.0)	4.60 (0.06)	19.1 (0.2)	−142.7 (0.7)	3.9 (0.4)	0.97 (0.15)

[a] At 298 K, unless otherwise noted. Rate constants were determined as described in the Supplementary data.[38] I = 0.0023, unless otherwise noted.
[b] At 293 K.
[c] I = 0.0015.
[d] $\Delta\Delta H^{\ddagger}$ (D,H).
[e] Data taken from Ref. 37.

shows that the free energy barrier (Δ_rG^O) for initial PT in the sequential PT/ET in, for example, water–acetonitrile (1:1) would be at least 72.5 kJ/mol or even 76.5 kJ/mol. This would be at least 7 kJ/mol 'up-hill' with respect to the observed[38] activation barrier of 65.8 kJ/mol in the reaction in this solvent, at an ionic strength I = 0.0023. As the activation barrier cannot be smaller than the ground-state barrier, that is, $\Delta G^{\ddagger} \geqslant \Delta_rG^O$, this consecutive pathway should be ruled out. The corresponding free energy barrier for the initial ET in the sequential ET/PT would be significantly greater[38] than 31 kJ/mol, the free energy barrier in water, but this sequential pathway should be ruled out since a kinetic isotope effect, (k_H/k_D = 8.2) was observed in the reaction (Table 1; the kinetics were determined spectrophotometrically by monitoring the decrease of absorbance of hexacyanoferrate(III) ions[38]). That is, the observed kinetic isotope effect (KIE) clearly indicates the involvement of proton transfer in the rate-controlling step, whilst in the initial ET the intermediate protonated ascorbyl radical would be formed; this process, of course, should not involve any proton transfer. Additionally, in the hypothetical sequential ET/PT process, the subsequent proton transfer from the protonated ascorbyl radical should be fast (the pK_a of this radical is −0.45[42]) and should not be rate-controlling. Taken together, the obtained evidence strongly suggests a concerted, proton-coupled electron transfer in the reaction. Similar considerations apply in the cases of the other solvents used in this study (see Supplementary data[38]).

(ii) The Arrhenius pre-factor A_H/A_D which is 0.97 in the reaction in water,[37] a typical value for an over-the-barrier reaction, changed to A_H/A_D = 0.49 in water–acetonitrile (1:1 v/v), to A_H/A_D = 0.065 in water–ethanol (1:1 v/v) and to A_H/A_D = 0.046 in water–1,4-dioxane (1:1 v/v), that is, well beyond the semiclassical limits of 0.5–1.4 for the A_H/A_D ratio in a (over-the-barrier) hydrogen transfer process (Table 1). Moreover, on the addition of only 5% v/v of acetonitrile to the water solution, the reaction immediately entered into a tunnelling regime. Very surprisingly, an isotopic ratio of A_H/A_D = 0.32 in water–acetonitrile (0.95:0.05 v/v) was observed. These findings are consistent with hydrogen tunnelling being an important reaction channel at relatively high organic co-solvent content, at least when 1,4-dioxane, ethanol or acetonitrile (and likely acetone) are used, but also suggest entering into a tunnelling regime even at ca. 1 M of the added organic co-solvent, at least in the case of water–acetonitrile. We do not have the A_H/A_D value for the water–acetone systems,[43] but it seems reasonable to suppose, taking into account that the observed changes of the KIEs on going from water to water–acetone mixtures are very similar to the corresponding changes in the other solvents used, that the basic physical picture in this case could be similar to the cases of the other three organic co-solvents.

(iii) The kinetic isotope effect between the ascorbate monoanion and its 2-OD derivative in the reaction changes significantly on the addition of only 5% of the organic co-solvent into the water reaction solution; whilst k_H/k_D = 4.60 in 'pure' water solution,[37] values of k_H/k_D = 5.61 (water–acetonitrile 0.95:0.05 v/v), k_H/k_D = 5.42 (water–ethanol 0.95:0.05 v/v), k_H/k_D = 5.35 (water–1,4-dioxane 0.95:0.05 v/v) and k_H/k_D = 5.33 (water–acetone 0.95:0.05 v/v) were observed[44] (Table 1). Moreover, sizeable changes of the KIE have been observed in the 1:1 v/v water–organic co-solvent systems; here, k_H/k_D = 8.24 (water–acetonitrile 1:1), k_H/k_D = 7.90 (water–ethanol 1:1), k_H/k_D = 8.37 (water–1,4-dioxane 1:1, at 20 °C) and k_H/k_D = 8.59 (water–acetone 1:1). This observation is consistent with an increase in the donor–acceptor distance for the transfer of a proton in a PCET[14,45] process; the donor–acceptor distance in this reaction should also depend on the separation of the redox partners, the hexacyanoferrate(III) anion and the ascorbate anion, due to electrostatic interactions (repulsive forces) between the negatively charged ions (see below).

(iv) The water molecule should be part of the activated complex in the reaction as is evident from the proton inventory[46–48] observed in the reaction in water–acetonitrile 1:1. Inspection of the proton inventory revealed[49] a concave curved dependence of k_{obs} versus x_{D2O}. This observation should be consistent with more than

one proton being involved in the transition state,[46–49] since proton transfer from the ascorbate 2-OH group directly to hexacyanoferrate (followed by subsequent fast transfer of the proton to the bulk under the conditions employed) would produce a linear proton inventory.[46–48] Therefore, the observed concave curved proton inventory should be consistent with a molecule of water being involved in the proton transfer during the reaction.

(v) Neither the observed rate constant nor the observed KIE in the reaction were changed in the presence of 0.1 M of acetate ions,[50] a potential proton acceptor in 1:1 acetonitrile–water system, and in the presence of 0.3 M acetate in water–1,4-dioxane (1:1) (Table 1).[38] This observation suggests that acetate ions do not act as proton acceptors during the reaction. It would appear highly unexpected that 0.3 M acetate (being anionic yet 6.5 pK_a units more basic than water) does not compete with the water for the transferring proton in the reaction if the proton transfers to the bulk water; this unexpected observation suggests that the proton transfer from the 2-OH of ascorbate to a molecule(s) of water could be more energetically favourable than the proton transfer to an acetate ion. As unidirectional[21,51,52] PCET can generally be more energetically favourable than bidirectional PCET, the absence of catalysis in the presence of acetate ions implies a configuration where the water molecule can be found between the redox partners.[53] Taken together with the other evidence obtained, the role of the configuration seems to be critical to the tunnelling phenomena observed in this reaction.

In principle, the observed phenomena can be explained using the Bell tunnelling correction model.[1,3] However, an explanation that involves dynamical aspects in optimizing the inter-nuclear distance for nuclear tunnelling, and the full range of the isotopic differences between the Arrhenius pre-factors from $A_H/A_D \gg 1$ (and the temperature independent KIEs that have been observed in numerous enzymatic reactions) to $A_D/A_H \gg 1$ values and temperature dependent KIEs has been supplied by invoking a Marcus-like tunnelling model.[3–15,55–57] Initially, within the framework of the model, hydrogen tunnelling is considered to be environmentally coupled with the protein dynamics, that is, it is assumed that protein/substrate motions can modulate hydrogen tunnelling.[3–15,55] Furthermore, Johannissen et al.[58] showed that a modification of the model can be also applied to a system that does not involve a protein environment, whilst Pudney et al.[14] have emphasized that compressive modes (the donor–acceptor distance fluctuations coincident and coupled with the reaction coordinate, 'promoting modes') may be a feature of both solution and enzyme systems and may also lead to an increase of the KIE when the donor–acceptor distance decreases (due to the increase of the force constant for a compressive mode).

In our view, an initial elucidation of the observed solvent-induced tunnelling in the reaction could also be traced within the above framework of the Marcus-like tunnelling model. As mentioned, the absence of catalysis by the acetate ion and the observed proton inventory pointed to a transition configuration consisting of the redox partners and a molecule of water, suitable for the unidirectional PCET process. Some insight into the dynamical aspects may come from a consideration of the factors that could influence the initial donor/acceptor separation in the configuration. It seems reasonable to assume the donor–acceptor distance (between the ascorbate 2-OH and the water molecule in the structure) will, among others, depend on the solvent polarity (as the separation of the negative charged redox partners due to repulsive interactions should also depend on the solvent polarity). The expectation is that the initial donor/acceptor separation would increase in the transition structure with decreasing solvent polarity. Indeed, the observed isotope effects on the Arrhenius pre-factor, $A_H/A_D = 0.49$ in the more polar water–acetonitrile solvent is greater than $A_H/A_D = 0.065$ in the less polar water–ethanol solvent, and $A_H/A_D = 0.046$ in the water–1,4-dioxane solvent, which is less polar than the water–ethanol solvent, suggesting a decrease of the A_H/A_D with an expected increase in the initial donor/acceptor separation,[14,45] reminiscent of the case of variation of the ratio of Arrhenius pre-factors versus the donor–acceptor distance obtained in the study of hydrogen tunnelling in soybean lipoxygenase-1.[7–9] At first sight, the observation of the greatly increased, but very similar KIEs on going from water to the mixed solvents (Table 1) could contradict the expectation of an increase in the KIE with the above-inferred increase of the donor–acceptor distance in the reaction. However, as demonstrated by Pudney et al.,[14] the KIE can increase as the donor–acceptor distance decreases because the force constant for a compressive mode can be increased. The result will be the relative invariance of the KIE as was obtained by Pudney et al.[14] in the H-tunnelling reaction of morphinone reductase, and is what in fact we observed in the case of ascorbate oxidation. In addition, the observed strong temperature dependence of the KIE (Table 1 and Fig. 1) also points to the role of the compressive modes coincident with the reaction coordinate, needed for efficient tunnelling.[3–15,59]

Generally, the expectation is that barrier height is critically important as well as barrier width in determining the probability of tunnelling in a chemical reaction.[1,14,15,17] There is an obvious increase in the reaction barrier (see Table 1) in the case of the 1:1 mixed solvents (the barrier in water is 62.3 kJ[37]). However, we also observed the entering into a tunnelling regime in the reaction in the case (0.95:0.05 v/v water–acetonitrile system) where the increase in the reaction barrier was only ~1 kJ, which could be quite puzzling if dynamical aspects were not be taken into account. Perhaps, the observation we obtained, that only 1 M of the co-solvent can have such a dynamic effect, is the most intriguing result in this Letter. In our view, the dynamics of the compressive modes coincident with the reaction coordinate could be coupled with the solvent environment dynamics, which in turn should be strongly determined by the 'integrity' of the solvent shell around the reactive configuration consisting of the redox pair and the water molecule. Hence, involvement of the molecule of acetonitrile might have a significant effect on the dynamical features of the solvent shell, and subsequently on the dynamics of the coupled compressive modes coincident with the reaction coordinate for H-transfer.

In addition, we have reported recently that ascorbate is involved in hydrogen tunnelling[61] in the reaction with the 2,2,6,6-

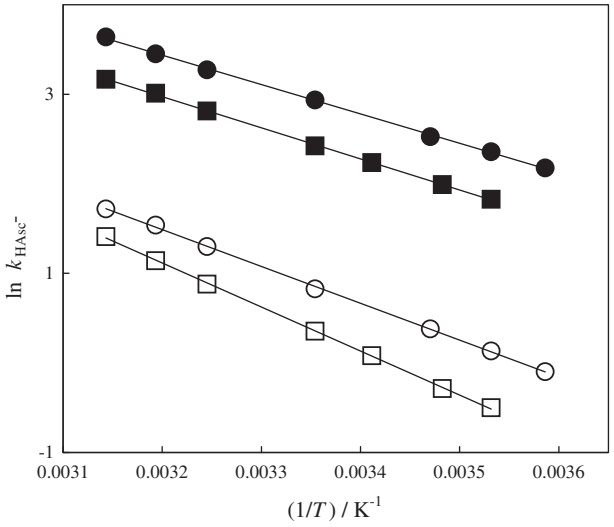

Figure 1. Arrhenius plots for the reaction of ascorbate and hexacyanoferrate(III) ions in MeCN–H_2O (●) and MeCN–D_2O (○) 1:1 v/v mixtures at ionic strength $I = 0.0023$, and in EtOH–H_2O (■) and EtOD–D_2O (□) 1:1 v/v mixtures at ionic strength $I = 0.0015$.

tetramethylpiperidin-1-oxyl (TEMPO) radical in water, possibly suggesting that ascorbate also uses tunnelling in the PCET processes in biological systems. The present results support the inference in so far as the mixed solvents used in this Letter could be more similar to a biological environment than 'pure' water alone.

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Supplementary data

Supplementary data (experimental data: Kinetics and UV spectra) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.01.142.

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50. The rate constant for the reaction is changed (for the reasons we have discussed elsewhere on the addition of the cation/salt, Na^+(NaCl) in this instance, in water–acetonitrile (see Table 1). However, the observed rate constant remained the same when Na-acetate (instead of NaCl) was present in the reaction solution.
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53. The following reasons support the proposal: (i) Any involvement of acetate as a proton acceptor in the PCET process must lead to a change of the observed rate constants in the reaction. The observed rate constants are, however, exactly the same in the presence of 0.3 M acetate and in the absence of acetate ions. (ii) It would be highly unexpected that acetate (being an anion, yet is 6.5 pK_a units more basic than water) does not compete with the water for transferring the proton in the reaction if the proton transfers to the bulk water. (iii) The observed proton inventory indicates the water molecule should be part of the activated complex in the reaction. (iv) The water molecule acting as a proton acceptor may therefore be found between the redox partners. This configuration can be appropriate for the unidirectional PCET. The advantage of the unidirectional[21,51,52] PCET arises from a lowering of the activation barrier due to smaller reorganizational energy λ, as compared with a bidirectional PCET;[21,51,52] the activated complex could resemble a solvent-shared[54] ion pair (taking into regard repulsion between negatively charged redox partners). The ascorbate 2-OH is necessary oriented towards the hexacyanoferrate ion as any other orientation would lead to an unfavourable bidirectional PCET. (v) A consequence of the (potential) involvement of acetate ions between the redox partners (instead of the water molecule) would be greater separation between the redox partners which should diminish the (electronic) couplings between them, and thus decrease the rate of the PCET reaction, since the rate of PCET reactions depend strongly on the couplings. These points could explain why acetate does not compete with water for the ascorbate 2-OH proton in the PCET process.
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55. Knapp et al.[56] adapted the model presented by Kuznetsov and Ulstrup[57] giving the expression:

$$k_{\text{tunn}} = \sum_{\upsilon} P_{\upsilon} \sum_{w} \frac{1}{2\pi} |V_{el}|^2 \sqrt{4\pi^3/\lambda RT\hbar^2} \exp^{-(\Delta G^{\circ} + E_{vib} + \lambda)^2/(4\lambda RT)} \times (\text{F.C.}term)_{\upsilon,w}$$

where k_{tunn} is the rate constant related to tunnelling. The first term describes electronic coupling and is isotope-independent; the second is an environmental energy term relating the reorganizational energy, λ, and the driving force of the reaction, $\Delta_r G^{O}$. E_{vib} is the vibrational energy difference between product and reactant. The contribution of hydrogen stretching to the rate due to a vibration-level specific Frank–Condon nuclear overlap along the hydrogen coordinate is contained in the F.C. term, which determines the tunnelling probability of hydrogen ($\hbar$, R and T are Planck's constant divided by 2π, the gas constant and absolute temperature, respectively). This model could explain the whole range of the phenomena observed, that is, the full range of the observed isotopic differences between the Arrhenius pre-factors from $A_H/A_D \gg 1$ to $A_D/A_H \gg 1$ as well as the temperature independent KIEs. A very important feature of this model takes into account motions along the

H-transfer coordinate (compressive modes, promoting modes) that could lead to a distance sampling (gating), and consequently to an increased rate when the initial distance between the H donor and acceptor is too long to support efficient tunnelling. The basis for this expectation is the strong distance dependence of proton coupling, hence, it is assumed that environmental oscillations can alter the donor–acceptor distance and the hydrogen overlap.

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59. Edwards et al.[60] have pointed out that the temperature dependence of KIEs could increase as the frequency of the compressive mode decreases (this frequency could decrease as the donor–acceptor distance increases[7,8,14]) which is expected here, for instance, in the case of the water–1,4-dioxane system, where a stronger temperature dependence of the KIE was observed than in the case of water–acetonitrile.
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