How vitamin E scavenges DPPH radicals in polar protic media

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Although three mechanisms, *i.e.*, one-step H-atom transfer (HAT), sequential proton-loss–electron-transfer (SPLET) and stepwise electron-transfer–proton-transfer (ET–PT), have been proposed to elucidate the 1,1-diphenyl-2-picrylhydrazyl radical (DPPH•) scavenging of 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), a vitamin E model, in methanol, our theoretical analysis indicates that the former two processes are more probable than the latter one.

Due to the important roles played by free radicals in various pathological processes in humans, the scavenging of radicals by antioxidants has stimulated continuing interest.[1] To screen or design novel antioxidants efficiently, one has to understand radical-scavenging mechanisms in detail. It has been recognized that most antioxidants (RXH) neutralize radicals (*e.g.*, RO•) by donating H-atoms through three pathways: (i) one-step H-atom transfer (HAT) (eqn (1));[2,3] (ii) stepwise electron-transfer–proton-transfer (ET–PT) (eqn (2));[4] (iii) sequential proton-loss–electron-transfer (SPLET) (eqn (3)), provided RXH can deprotonate.[5,6]

$$\mathrm{RXH + RO^{\bullet} \rightarrow RX^{\bullet} + ROH} \quad (1)$$

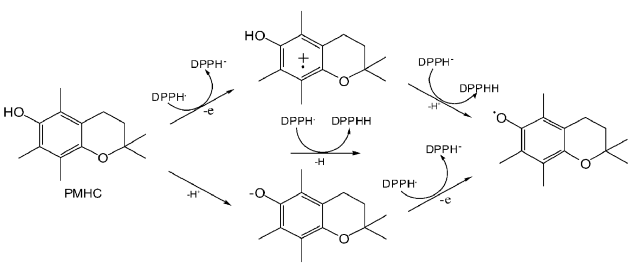
$$\mathrm{RXH + RO^{\bullet} \rightarrow RXH^{\bullet +} + RO^{-} \rightarrow RX^{\bullet} + ROH} \quad (2)$$

$$\mathrm{RXH \rightarrow RX^{-} + H^{+};\ RX^{-} + RO^{\bullet} \rightarrow RX^{\bullet} + RO^{-};\ RO^{-} + H^{+} \rightarrow ROH} \quad (3)$$

Although the three pathways give the same final products, they behave quite distinctly in different solvents. Roughly speaking, as the first pathway does not involve charge separation, it is preferred in non-polar solvents, whereas the second and the third are favored in polar media, due to the charge separation processes. In addition, the radical-scavenging mechanisms also depend on the characteristics of the radical.[7] For radicals with high electron affinity, ET–PT and SPLET are preferred, while for radicals with high H-atom affinity, HAT is favored. As a result, the same antioxidant may exhibit distinct behaviors in scavenging different radicals and/or in different environments,[7] which results in frequent debates on the action mechanisms of antioxidants. For instance, although there has been a consensus on how vitamin E (one of the most popular non-enzymatic bio-relevant antioxidants) scavenges radicals in non-polar solvents, *i.e.*, a HAT process,[8] there is some dispute as to how vitamin E scavenges radicals in polar media. Nakanishi *et al.*[9] claimed that a vitamin E model, 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC), scavenges 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH•) in methanol through a stepwise ET–PT mechanism (Scheme 1). However, Musialik and Litwinienko[10] argued that SPLET and HAT rather than ET–PT are responsible for DPPH• scavenging of PMHC in the same solvent (Scheme 1).

In a recent effort to elucidate the structure–activity relationships (SARs) of some flavonoid antioxidants as galvinoxyl radical scavengers in polar solvents, we revealed that the SARs cannot be explained unless the phenolate anions are considered.[11] This indicates the critical role of SPLET in radical scavenging of phenolic antioxidants in polar systems. As the ionization potential (IP) of PMHC ($\sim$40 kcal mol^{-1} lower than that of phenol in the gas phase)[12] is comparable with IPs of flavonoids (30–40 kcal mol^{-1} lower than that of phenol in the gas phase)[13] and DPPH• is more electron-deficient than the phenoxyl radical, we speculate that SPLET rather than ET–PT is more likely involved in the DPPH• scavenging of PMHC in polar protic media.

To determine which pathway is more possible, the homolytic O–H bond dissociation enthalpy (BDE) of the parent PMHC (which is defined as $H_r + H_h - H_p$, in which H_r is the enthalpy of the PMHC radical generated through donating a H-atom, H_h is the enthalpy of a H-atom, $-0.497\,65$ hartree, and H_p is the enthalpy of the parent molecule) and the adiabatic IPs (defined as $E_d - E_i$, where E_d is the energy of the molecule generated by donating an electron and E_i is the energy of the initial molecule) of the parent PMHC and its anion were calculated by a combined density functional theory (DFT) method, labeled as (RO)B3LYP/6-311+G(2d,2p)//AM1/AM1, which means that the semiempirical method AM1[14] was used to optimize the molecular geometries and determine the vibrational frequencies and then single-point electronic energies were calculated by the (RO)B3LYP functional[15] at the 6-311+G(2d,2p) level. Solvent effects were taken into consideration on the single-point level by employing the self-consistent reaction field (SCRF) method with a



Scheme 1 Three mechanisms, stepwise electron-transfer–proton-transfer (above), one step H-atom transfer (middle) and sequential proton-loss–electron-transfer (below), proposed to elucidate the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging of 2,2,5,7,8-pentamethyl-6-hydroxychroman (PMHC) in methanol.

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polarizable continuum model (PCM).[16] The effectiveness of this method has been demonstrated by previous studies.[11,12,17]

The homolytic O–H BDE of PMHC was calculated to be 77.9 kcal mol^{-1},[18] 5.31 kcal mol^{-1} lower than the absolute value of the H-atom affinity of DPPH$^{\bullet}$ (-83.2 kcal mol^{-1}, which is defined as $H_n - H_r - H_h$, in which H_n is the enthalpy of neutralized DPPH$^{\bullet}$, H_r is the enthalpy of DPPH$^{\bullet}$ and H_h is the enthalpy of the H-atom, -0.49765 hartree),[19] suggesting that HAT is not prohibited in the scavenging of DPPH$^{\bullet}$ by PMHC in methanol. The adiabatic IPs of PMHC and its anion in methanol were calculated to be 122.6 kcal mol^{-1} and 88.5 kcal mol^{-1}, respectively.[20] In comparison with the adiabatic electron affinity (EA, which is defined as $E_a - E_i$, where E_a is the energy of the molecule generated by accepting an electron and E_i is the energy of the initial molecule) of DPPH$^{\bullet}$ in methanol, -95.2 kcal mol^{-1},[12] it can be inferred that the electron transfer between a PMHC anion and DPPH$^{\bullet}$ is thermodynamically permitted (because IP + EA is negative, -6.7 kcal mol^{-1}), whereas that between the parent PMHC and DPPH$^{\bullet}$ is forbidden (because IP + EA is positive, 27.4 kcal mol^{-1}). Therefore, HAT and SPLET rather than ET–PT are supported by the present calculations.[21]

In addition, to explore the mechanisms of vitamin E to scavenge bio-relevant radicals, the H-atom affinities and adiabatic EAs of the alkoxyl radical (MeO$^{\bullet}$) (-107.8 kcal mol^{-1} and -120.1 kcal mol^{-1}, respectively) and the peroxyl radical (MeOO$^{\bullet}$) (-94.4 kcal mol^{-1} and -89.3 kcal mol^{-1}, respectively) in methanol were also estimated, which suggests that HAT and SPLET are more preferred than ET–PT to neutralize these radicals by vitamin E (especially α-tocopherol) in polar protic media.

Last but not least, in biological systems, vitamin E components are located in cellular membranes, a heterogeneous environment, which means that their long lipid tail is buried in the lipid phase, and thus the phenolic head is able to reach the aqueous phase and to deprotonate. As a result, HAT and/or SPLET are very likely to play a dominant role in scavenging *in vivo* radicals by vitamin E. However, it is challenging to determine the respective contributions of HAT and SPLET to the *in vivo* radical scavenging of antioxidants.[22]

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References

1 (*a*) *Natural Antioxidants: Chemistry, Health Effects, and Applications*, ed. F. Shahidi, The American Oil Chemists' Society, Champaign, IL, 1997; (*b*) C. E. Thomas, in *Handbook of Synthetic Antioxidants*., ed. L. Packer and E. Cadenas, Marcel Dekker Inc., New York, 1997, pp. 1–52; (*c*) H.-Y. Zhang, *Curr. Comput.-Aided Drug Des*., 2005, **1**, 257–273.
2 (*a*) G. W. Burton, T. Doba, E. J. Gabe, L. Hughes, F. L. Lee, L. Prasad and K. U. Ingold, *J. Am. Chem. Soc*., 1985, **107**, 7053–7065; (*b*) M. I. de Heer, P. Mulder, H. G. Korth, K. U. Ingold and J. N. Lusztyk, *J. Am. Chem. Soc*., 2000, **122**, 2355–2360.
3 This pathway is also called electron-coupled proton transfer or proton-coupled electron transfer: J. M. Mayer and I. J. Rhile, *Biochim. Biophys. Acta*, 2004, **1655**, 51–58.
4 (*a*) S. V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic and M. G. Simic, *J. Am. Chem. Soc*., 1994, **116**, 4846–4851; (*b*) S. V. Jovanovic, S. Steenken, Y. Hara and M. G. Simic, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2497–2504.
5 (*a*) G. Litwinienko and K. U. Ingold, *J. Org. Chem*., 2003, **68**, 3433–3438; (*b*) M. C. Foti, C. Daquino and C. Geraci, *J. Org. Chem*., 2004, **69**, 2309–2314; (*c*) G. Litwinienko and K. U. Ingold, *J. Org. Chem*., 2004, **69**, 5888–5896.
6 L. Kong, Z.-L. Sun, L.-F. Wang, H.-Y. Zhang and S.-D. Yao, *Helv. Chim. Acta*, 2004, **87**, 511–515.
7 L. Shen, H.-Y. Zhang and H.-F. Ji, *Org. Lett*., 2005, **7**, 243–246.
8 G. W. Burton and K. U. Ingold, *Acc. Chem. Res*., 1986, **19**, 194–201.
9 I. Nakanishi, T. Kawashima, K. Ohkubo, H. Kanazawa, K. Inami, M. Mochizuki, K. Fukuhara, H. Okuda, T. Ozawa, S. Itoh, S. Fukuzumi and N. Ikota, *Org. Biomol. Chem*., 2005, **3**, 626–629.
10 M. Musialik and G. Litwinienko, *Org. Lett*., 2005, **7**, 4951–4954.
11 L.-F. Wang and H.-Y. Zhang, *Bioorg. Chem*., 2005, **33**, 108–115.
12 H.-F. Ji, G.-Y. Tang and H.-Y. Zhang, *QSAR Comb. Sci*., 2005, **24**, 826–830.
13 L.-F. Wang, Z.-L. Sun and H.-Y. Zhang, *J. Shandong Univ. Tech. (Sci. & Tech. Ed.)*, 2003, **17**, 13–17.
14 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc*., 1985, **107**, 3902–3909.
15 (*a*) C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785–789; (*b*) A. D. Becke, *J. Chem. Phys*., 1993, **98**, 1372–1377.
16 (*a*) S. Miertus, E. Scrocco and J. Tomasi, *Chem. Phys*., 1981, **55**, 117–129; (*b*) S. Miertus and J. Tomasi, *Chem. Phys*., 1982, **65**, 239–256; (*c*) M. Cossi, V. Barone and J. Cammi, *Chem. Phys. Lett*., 1996, **255**, 327–335.
17 (*a*) J. S. Wright, E. R. Johnson and G. A. DiLabio, *J. Am. Chem. Soc*., 2001, **123**, 1173–1183; (*b*) L. Kong, L.-F. Wang and H.-Y. Zhang, *J. Mol. Struct. (Theochem)*, 2005, **716**, 27–31; (*c*) H.-F. Ji, H.-Y. Zhang and L. Shen, *Nat. Prod. Commun*., 2006, **1**, 229–235.
18 This value is ~2 kcal mol^{-1} higher than that calculated in gas phase (~75 kcal mol^{-1}),[12,17a] supporting the notion that solvent effects on homolytic BDEs are rather weak.[3b].
19 This value is comparable to the experimental value (~-80 kcal mol^{-1}) determined in benzene:[19b] L. R. Mahoney, G. D. Mendenhall and K. U. Ingold, *J. Am. Chem. Soc*., 1973, **95**, 8610–8614.
20 According to the calculation results, the O–H proton dissociation enthalpy (PDE, which is defined as $H_d + H_{proton} - H_p$, in which H_d is the enthalpy of the molecule derived from proton dissociation, H_{proton} is the enthalpy of the proton, 0.00236 hartree, and H_p is the enthalpy of the parent molecule) of PMHC in methanol was estimated as 302 kcal mol^{-1}, which is in line with the lower proton dissociation ability of α-tocopherol (pK_a = 11.92)[10] than quercetin, a typical flavonoid (whose first pK_a (for 7-OH) is 7.03,[11] O–H PDE = 292 kcal mol^{-1} for 7-OH in methanol).
21 The calculated vertical IPs of PMHC and the derived anion (121.9 kcal mol^{-1} and 87.7 kcal mol^{-1}, respectively) and the vertical EA of DPPH$^{\bullet}$ (-94.2 kcal mol^{-1}) lead to the same conclusion .
22 It should be kept in mind that the relative importance of HAT and SPLET is not only determined by microenvironmental features but also governed by radical characteristics.

