DOI: 10.1002/anie.200700157

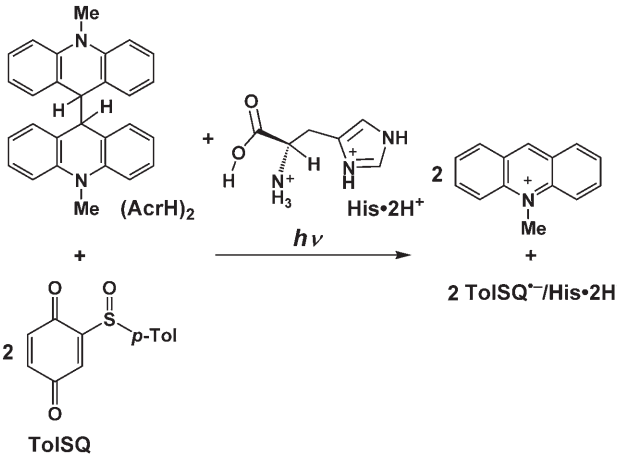
Hydrogen-Bonded Complex

Direct EPR Detection of a Hydrogen-Bonded Complex between a Semiquinone Radical Anion and a Protonated Amino Acid, and Electron Transfer Driven by Hydrogen Bonding**

*Junpei Yuasa, Shunsuke Yamada, and Shunichi Fukuzumi**

In the photosynthetic reaction center (RC), two quinones denoted Q_A and Q_B act as electron acceptors.[1] The RC catalyzes light-induced two-electron reduction of Q_B with two protons to yield hydroquinone Q_BH_2, which is released from the RC and replaced by another quinone molecule from the pool.[2] Although the first electron transfer, $Q_A^{\bullet-} \rightarrow Q_B$, does not involve direct protonation of $Q_B^{\bullet-}$,[3,4] the electron transfer (ET) is coupled with protonation of a nearby amino acid residue through hydrogen-bond formation with $Q_B^{\bullet-}$.[5–7] Specific hydrogen bonds with nearby amino acid residues enable the quinone to perform a specific function, and hence differences in the nature of the hydrogen-bonding interactions of Q_A and Q_B have been suggested to result in their differing functions.[8] Since hydrogen bonds are largely electrostatic in nature,[9] protonation of amino acids that leads to a change in fractional charge on one of the components in a hydrogen bond will remarkably affect the strength of hydrogen bonding. Such a change in hydrogen-bonding strength would have a significant effect on the one-electron reduction potential of the quinone.[10–13] Direct EPR detection of a hydrogen-bonded complex between a semiquinone radical anion and a protonated amino acid would afford valuable insight into the hydrogen-bond strength and electronic structure of the hydrogen-bonded complex. However, protonation of singly reduced species of carbonyl compounds acting as strong bases is generally too fast to detect any intermediate in organic solvent in the presence of proton donors.[14]

We report herein the first successful EPR detection of a hydrogen-bonded complex between a semiquinone radical anion and protonated histidine ($His{\cdot}2H^+$)[15] using 1-(*p*-tolylsulfinyl)-2,5-benzoquinone (TolSQ). Effects of hydrogen bonding between $TolSQ^{\bullet-}$ and $His{\cdot}2H^+$ on the one-electron reduction potential of TolSQ and the rate of ET reduction were examined to reveal how the ET rate is controlled by hydrogen bonding.



(1)

The EPR spectrum of a hydrogen-bonded complex of a semiquinone radical anion with protonated histidine ($TolSQ^{\bullet-}/His{\cdot}2H^+$) was detected in photoinduced ET from 10,10′-dimethyl-9,9′-biacridine [$(AcrH)_2$] to TolSQ in the presence of $His{\cdot}2HClO_4$ in MeCN at 298 K [Eq. (1); see experimental details in the Supporting Information].[16] Biacridine $(AcrH)_2$ is known to act as a two electron donor to produce two equivalents of the radical anion of an electron acceptor.[17] Since the $TolSQ^{\bullet-}/His{\cdot}2H^+$ complex is unstable, the EPR spectrum of a solution of $(AcrH)_2$ (1.6×10^{-2} M) and TolSQ (4.0×10^{-3} M) in MeCN in the presence of $His{\cdot}2H^+$ (4.0×10^{-3} M) was measured under steady-state photoirradiation (Figure 1a). The EPR spectrum is well reproduced by a simulated spectrum with hfc values of $a(3H) = 0.88$, 5.31, and 6.08 G and superhyperfine splitting due to one nitrogen atom and three equivalent protons [$a(N) = 1.35$ G and $a(3H) = 2.97$ G] of $His{\cdot}2H^+$ (Figure 1b). The complete agreement of the observed EPR spectrum (Figure 1a) with the simulated spectrum (Figure 1b) clearly indicates formation of the $TolSQ^{\bullet-}/His{\cdot}2H^+$ complex. The *g* value (2.0026) and the hfc values [$a(3H) = 0.88$, 5.31, and 6.08 G] of $TolSQ^{\bullet-}/His{\cdot}2H^+$ are drastically changed from those of $TolSQ^{\bullet-}$ in the absence of $His{\cdot}2H^+$, which are $g = 2.0057$ and $a(3H) = 2.00$, 2.20, and 3.35 G.[18,19]

The optimized structure of $TolSQ^{\bullet-}/His{\cdot}2H^+$ was obtained by density functional calculations with the BLYP/6-31G** basis set (see experimental details in the Supporting Information).[20] Hydrogen bonds are found between the C=O oxygen atom of $TolSQ^{\bullet-}$ and the COOH proton as well as the NH_3^+ protons of $His{\cdot}2H^+$, and also between the S=O oxygen atom of $TolSQ^{\bullet-}$ and the NH^+ proton of the imidazole ring of $His{\cdot}2H^+$ (Figure 1c; for hydrogen-bond lengths, see the

---

[*] Dr. J. Yuasa, S. Yamada, Prof. Dr. S. Fukuzumi
Department of Material and Life Science
Graduate School of Engineering
Osaka University and SORST (JST)
Suita, Osaka 565-0871 (Japan)
Fax: (+81) 6-6879-7370
E-mail: fukuzumi@chem.eng.osaka-u.ac.jp

[**] This work was partially supported by Grants-in-Aid (No. 16205020) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

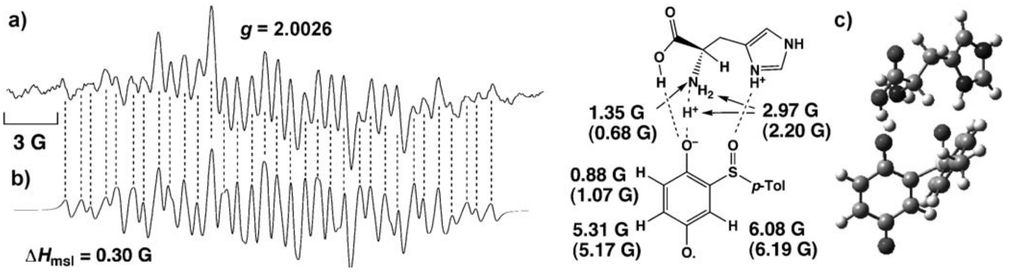


Figure 1. a) EPR spectrum of $TolSQ^{\bullet -}/His{\cdot}2H^+$ produced by photoinduced ET from $(AcrH)_2$ (1.6×10^{-2} M) to TolSQ (4.0×10^{-3} M) in the presence of His (4.0×10^{-3} M) and $HClO_4$ (8.0×10^{-3} M) in deaerated MeCN at 298 K. b) Spectrum simulated with the hfc values of $TolSQ^{\bullet -}/His{\cdot}2H^+$. ΔH_{msl}(maximum slope linewidth) = 30 G. c) Optimized structure of $TolSQ^{\bullet -}/His{\cdot}2H^+$ calculated by DFT at the BLYP/6-31G** level (calculated hfc values are given in parentheses).

Supporting Information).[21] Such multiple hydrogen bonding between $TolSQ^{\bullet -}$ and $His{\cdot}2H^+$ may stabilize $TolSQ^{\bullet -}/His{\cdot}2H^+$. The hfc values calculated with the optimized structure are given in parentheses in Figure 1c. The superhyperfine coupling due to the hydrogen-bonded NH_3^+ proton of $His{\cdot}2H^+$ is estimated as 6.61 G. The average of the calculated hfc values (2.20 G) due to the three NH_3^+ protons agrees with the observed value.[22] This indicates that the hydrogen-bonded proton is rapidly exchanged among the NH_3^+ protons on the EPR timescale. The existence of superhyperfine coupling due to the hydrogen-bonded protons and nitrogen atoms of NH_3^+ (Figure 1) strongly supports the presence of a strong hydrogen bond.

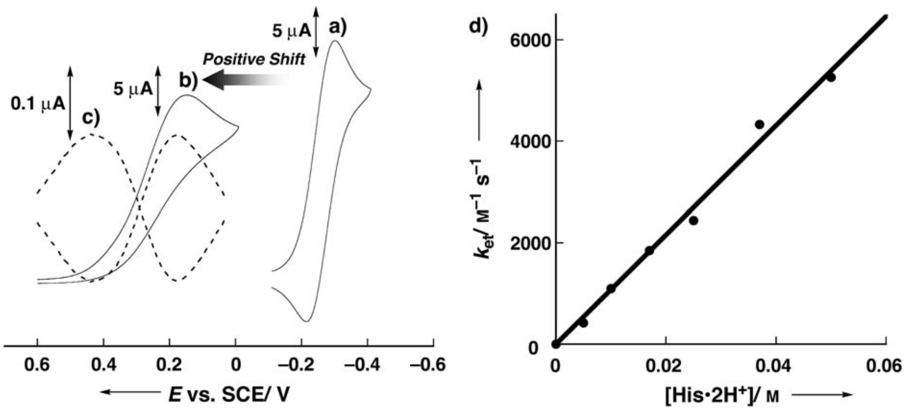


Figure 2. a) Cyclic voltammogram of TolSQ (1.0×10^{-2} M) in the absence of $His{\cdot}2H^+$. b) Cyclic voltammogram and c) second-harmonic alternating-current voltammogram of TolSQ (5.0×10^{-3} M) in the presence of $His{\cdot}2H^+$ (5.0×10^{-2} M) in deaerated MeCN containing tetrabutylammonium perchlorate (TBAP, 0.10 M) with a Pt working electrode at 298 K. d) Dependence of k_{et} on $[His{\cdot}2H^+]$ for ET from Me_2Fc (1.0×10^{-4} M) to TolSQ in the presence of $His{\cdot}2H^+$ in deaerated MeCN at 298 K.

When TolSQ is replaced by *p*-benzoquinone (Q), only the protonated species (semiquinone radical $QH^{\bullet}$) is detected by EPR in photoinduced ET from $(AcrH)_2$ to Q in the presence of $His{\cdot}2H^+$ (see the Supporting Information). Thus, the S=O oxygen atom in TolSQ plays a crucial role in multiple hydrogen bonding between $TolSQ^{\bullet -}$ and $His{\cdot}2H^+$.

The strong hydrogen bonding between $TolSQ^{\bullet -}$ and $His{\cdot}2H^+$ is expected to increase the electron-acceptor ability of TolSQ.[11] The positive shift of the one-electron reduction potential E_{red} of TolSQ in the presence of $His{\cdot}2H^+$ (5.0×10^{-2} M) was verified by electrochemical measurements. The cyclic voltammogram of TolSQ exhibits a reversible redox wave (Figure 2a). In contrast, the cyclic voltammogram of TolSQ in the presence of $His{\cdot}2H^+$ exhibits an irreversible cathodic wave due to the instability of the $TolSQ^{\bullet -}/His{\cdot}2H^+$ complex (Figure 2b). Thus, the E_{red} value of TolSQ in the presence of $His{\cdot}2H^+$ was determined by second-harmonic alternating-current voltammetry (Figure 2c). The E_{red} value of TolSQ [−0.26 V vs saturated calomel electrode (SCE)] is shifted to 0.29 V versus SCE in the presence of 5.0×10^{-2} M of $His{\cdot}2H^+$. In contrast to the strong hydrogen bonding between $TolSQ^{\bullet -}$ and $His{\cdot}2H^+$, there is virtually no interaction between neutral TolSQ and $His{\cdot}2H^+$. In such a case, the positive shift in the one-electron reduction potential of TolSQ in the presence of $His{\cdot}2H^+$ is expressed by Equation (2),

$$E_{red} = E^0_{red} + (2.3\,RT/F)\log\{K_{red}[\mathrm{His}\cdot 2\,\mathrm{H}^+]\} \qquad (2)$$

where E^0_{red} is the reduction potential of TolSQ in the absence of $His{\cdot}2H^+$, and K_{red} the formation constant of the $TolSQ^{\bullet -}/His{\cdot}2H^+$ complex. The K_{red} value was determined as 4.2×10^{10} M^{-1} from the E_{red} value of TolSQ in the presence of 5.0×10^{-2} M of $His{\cdot}2H^+$. Such a large K_{red} value of the $TolSQ^{\bullet -}/His{\cdot}2H^+$ complex clearly indicates strong hydrogen bonding between $TolSQ^{\bullet -}$ and $His{\cdot}2H^+$.

The positive shift of the E_{red} value of TolSQ should enhance ET from an electron donor to TolSQ.[12,13] This was confirmed by ET from 1,1′-dimethylferrocene (Me_2Fc) to TolSQ in the presence of $His{\cdot}2H^+$. No ET from Me_2Fc (E_{ox} = 0.26 V vs SCE) to TolSQ (E_{red} = −0.26 V vs SCE) occurs, because the free-energy change of ET is highly endergonic (ΔG_{et} = 0.52 eV). In the presence of 5.0×10^{-2} M of $His{\cdot}2H^+$, however, efficient ET from Me_2Fc to TolSQ occurs to yield Me_2Fc^+ [Eq. (3)], as expected from the negative free-energy change of electron transfer (ΔG_{et} = −0.03 eV).

$$\mathrm{Me_2Fc + TolSQ + His\cdot 2\,H^+ \xrightarrow{k_{et}} Me_2Fc^+ + TolSQ^{\bullet -}/His\cdot 2\,H^+} \quad (3)$$

The ET rates obeyed pseudo-first-order kinetics in the presence of a large excess TolSQ and His·2H^+ relative to the concentration of Me_2Fc (see the first-order plot in the Supporting Information). The observed pseudo-first-order rate constant k_{obs} increases proportionally with increasing TolSQ concentration. The second-order rate constant k_{et} also increases linearly with [His·2H^+] (Figure 2d).

Since His·2H^+ has no effect on the oxidation potential of Me_2Fc, the free-energy change of ET from Me_2Fc to TolSQ in the presence of His·2H^+ (ΔG_{et}) can be expressed by Equation (4), where ΔG^0_{et} is the free-energy change in the

$$\Delta G_{et} = \Delta G^0_{et} - (2.3\,RT/F)\log\{K_{red}[\mathrm{His\cdot 2\,H^+}]\} \quad (4)$$

absence of His·2H^+. Such a change in ΔG_{et} has previously been reported for metal-ion-promoted ET from Fc to the naphthoquinone (NQ) moiety of a ferrocene–naphthoquinone (Fc–NQ) linked dyad.[23] The dependence of k_{et} of metal-ion-promoted ET on driving force is well evaluated in terms of the Marcus theory of electron transfer[24] when the k_{et} value increases linearly with increasing metal ion concentration.[23] In the case of His·2H^+ too, the k_{et} value increases linearly with [His·2H^+] (Figure 2d).

In summary, we have detected a hydrogen-bonded complex of a semiquinone radical anion with protonated histidine ($TolSQ^{\bullet -}$/His·2H^+) by EPR, which reveals strong hydrogen bonding between $TolSQ^{\bullet -}$ and His·2H^+. This finding provides valuable insight into the specific function of quinones in the photosynthetic RC. Strong hydrogen bonding between semiquinone radical anion and protonated amino acid residues would result in a positive shift in the one-electron reduction potential of quinones and facilitate the ET reduction of quinones in the RC.

Received: January 12, 2007

Keywords: amino acids · electron transfer · EPR spectroscopy · hydrogen bonds · quinones

---

[1] *Functions of Quinones in Energy Conserving Systems* (Ed.: B. I. Trumpower), Academic Press, New York, **1986**.

[2] *The Photosynthetic Bacterial Reaction Center—Structure and Dynamics* (Eds.: J. Breton, H. Vermeglio), Plenum, New York, **1988**.

[3] G. Feher, R. A. Isaacson, M. Y. Okamura, W. Lubitz in *Antennas and Reaction Centers of Photosynthetic Bacteria* (Ed.: M. E. Michel-Beyerle), Springer, Berlin, **1985**, pp. 174–189.

[4] P. J. O'Malley, T. K. Chandrashekar, G. T. Babcock in *Antennas and Reaction Centers of Photosynthetic Bacteria* (Ed.: M. E. Michel-Beyerle), Springer, Berlin, **1985**, pp. 339–344.

[5] E. Takahashi, C. A. Wraight, *Biochemistry* **1992**, *31*, 855–866.

[6] M. L. Paddock, S. H. Rongey, G. Feher, M. Y. Okamura, *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 6602–6606.

[7] P. Ädelroth, M. L. Paddock, L. B. Sagle, G. Feher, M. Y. Okamura, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 13086–13091.

[8] a) P. J. O'Malley, *J. Am. Chem. Soc.* **1998**, *120*, 5093–5097; b) P. J. O'Malley, *J. Phys. Chem. A* **1998**, *102*, 248–253.

[9] K. Morokuma, *Acc. Chem. Res.* **1977**, *10*, 294–300.

[10] H. Ishikita, G. Morra, E.-W. Knapp, *Biochemistry* **2003**, *42*, 3882–3892.

[11] For effects of hydrogen bonding on E_{red} of quinones, see: a) K. Okamoto, K. Ohkubo, K. M. Kadish, S. Fukuzumi, *J. Phys. Chem. A* **2004**, *108*, 10405–10413; b) Y. Ge, R. R. Lilienthal, D. K. Smith, *J. Am. Chem. Soc.* **1996**, *118*, 3976–3977; c) N. A. Macias-Ruvalcaba, N. Okumura, D. H. Evans, *J. Phys. Chem. B* **2006**, *110*, 22043–22047; d) N. Gupta, H. Linschitz, *J. Am. Chem. Soc.* **1997**, *119*, 6384–6391.

[12] For effects of hydrogen bonding on ET reduction of quinones, see: a) S. Fukuzumi, H. Kitaguchi, T. Suenobu, S. Ogo, *Chem. Commun.* **2002**, 1984–1985; b) S. Fukuzumi, K. Okamoto, Y. Yoshida, H. Imahori, Y. Araki, O. Ito, *J. Am. Chem. Soc.* **2003**, *125*, 1007–1013.

[13] For effects of hydrogen bonding on proton-coupled electron transfer (PCET), see: a) I. J. Rhile, T. F. Markle, H. Nagao, A. G. DiPasquale, O. P. Lam, M. A. Lockwood, K. Rotter, J. M. Mayer, *J. Am. Chem. Soc.* **2006**, *128*, 6075–6088; b) I. J. Rhile, J. M. Mayer, *J. Am. Chem. Soc.* **2004**, *126*, 12718–12719; c) A. Niemz, V. M. Rotello, *Acc. Chem. Res.* **1999**, *32*, 44–52; d) M. Gray, A. O. Cuello, G. Cooke, V. M. Rotello, *J. Am. Chem. Soc.* **2003**, *125*, 7882–7888.

[14] For fast proton transfer from proton donor to semiquinone radical anions, see: C. G. Schaefer, K. S. Peters, *J. Am. Chem. Soc.* **1980**, *102*, 7566–7567.

[15] It has been suggested that His-H126 and His-H128 near Q_B facilitate proton transfer into the RC: a) M. L. Paddock, M. S. Graige, G. Feher, M. Y. Okamura, *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6183–6188; b) P. Ädelroth, M. L. Paddock, A. Tehrani, J. T. Beatty, G. Feher, M. Y. Okamura, *Biochemistry* **2001**, *40*, 14538–14546.

[16] Although His is difficult to dissolve in MeCN, it becomes soluble in MeCN in the presence of 2 equiv of $HClO_4$.

[17] a) S. Fukuzumi, T. Kitano, K. Mochida, *J. Am. Chem. Soc.* **1990**, *112*, 3246–3247; b) S. Fukuzumi, Y. Tokuda, *J. Phys. Chem.* **1992**, *96*, 8409–8413.

[18] J. Yuasa, S. Yamada, S. Fukuzumi, *J. Am. Chem. Soc.* **2006**, *128*, 14938–14948.

[19] The smaller *g* value of $TolSQ^{\bullet -}$/His·2H^+ compared to $TolSQ^{\bullet -}$ (2.0057) indicates that the spin density on oxygen nuclei in $TolSQ^{\bullet -}$ is reduced significantly due to strong hydrogen bonding with His·2H^+.

[20] The hfc values of semiquinone radical ($QH^{\bullet}$) calculated by using BLYP methods were in good agreement with experimental data: M. Nonella, *J. Phys. Chem. B* **1997**, *101*, 1235–1246.

[21] The hydrogen-bonded proton of NH_3^+ is not covalently bound to $TolSQ^{\bullet -}$ but electrostatically bound to $TolSQ^{\bullet -}$ through the hydrogen bond. The N–H bond length in NH_3^+ (1.57 Å) is longer than that between the NH_3^+ proton and the C=O oxygen atom of $TolSQ^{\bullet -}$ (1.05 Å) in the optimized structure of $TolSQ^{\bullet -}$/His·2H^+ (see the Supporting Information). This indicates that binding of the N–H bond in NH_3^+ is significantly weakened by formation of strong hydrogen bonds with $TolSQ^{\bullet -}$. The presence of the strong hydrogen bond is supported by the existence of superhyperfine coupling due to the hydrogen-bonded protons and nitrogen atom of NH_3^+ (Figure 1).

[22] The calculated hfc values of $TolSQ^{\bullet -}$/His·2H^+ agree well with the observed hfc values within the errors due to the imperfect density functional method (BLYP/6-31G**).

[23] K. Okamoto, H. Imahori, S. Fukuzumi, *J. Am. Chem. Soc.* **2003**, *125*, 7014–7021.

[24] R. A. Marcus, *Angew. Chem.* **1993**, *105*, 1161–1172; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1111–1121.