Electrochemistry of Hypericin Incorporated into a Cationic Lipid Film on a Graphite Electrode in an Aqueous Medium

Takamasa Sagara,* Narimasa Kaba, and Naotoshi Nakashima
Department of Applied Chemistry, Nagasaki University, 1-14 Bunkyo, Nagasaki 852-8521

(Received July 21, 1999; CL-990638)

Quasi-reversible two-consecutive one-electron reduction processes of hypericin (Hyp) in an aqueous medium were achieved by incorporating Hyp into a cast film of a synthetic cationic lipid, dioctadecyldimethylammonium bromide ($2C_{18}N^+Br^-$), coated on a basal-plane pyrolytic graphite (BPG) electrode. Importance of the electrostatic interaction between Hyp anions and the cationic sites in the matrix was suggested.

Hypericin (Hyp: 4,5,7,4',5',7'-hexahydroxy-2,2'-dimethyl-*meso*-naphthodianthrone), a naturally occurring photo-sensitive dye, has attractive photochemical and pharmaceutical activities.[1-9] Hyp is sparingly soluble in water in the absence of a solubilizing reagent such as β-cyclodextrin.[3,4] If aqueous electrochemistry of Hyp could be readily attained at an electrode surface, expected is the use of this dye as an active center of a photo-induced electron or proton transfer system in an aqueous environment. Burel *et al.* have attempted to bring Hyp into an aqueous medium without loss of the electroactivity by encapsulating Hyp in a phospholipid liposome.[7] However, well-resolved voltammogram has been obtained merely at a Hg electrode, and only the first reduction step has appeared chemically-reversible.

We have recently found that, when incorporating [C_{60}] fullerene into a cast film of a synthetic ammonium lipid, multiple reduction steps to C_{60} anions can be clearly observed in an aqueous medium.[10,11] Binding of the electrogenerated C_{60} anions to the ammonium ion makes the formal potential shift to less negative.[12] If such a binding interaction with cationic site is not specific for C_{60} anions but general for π-conjugated anionic dyes, incorporation of Hyp into a cationic lipid may facilitate the electrochemistry of Hyp.

We hereby describe the achievement of quasi-reversible, two-consecutive reduction of Hyp in an aqueous solution at a BPG electrode covered with a cast film of $2C_{18}N^+Br^-$ using the results of voltammetric and electroreflectance (ER) measurements.

A drop of the solution of Hyp (Carl Roth Co.) + $2C_{18}N^+Br^-$ (molar ratio, 1/40) in chloroform containing 1% ethanol was placed on a BPG electrode surface freshly prepared by a peeling-off procedure and was allowed to air-dry. The BPG electrode modified with a cast film of Hyp + $2C_{18}N^+Br^-$ showed a stable cyclic voltammogram (CV) in deaerated 0.01 M KCl aqueous solution (M = mol dm^{-3}) with two redox waves (Figure 1). Differential pulse voltammograms (DPVs) ensured the existence of two waves. CV curves with various negative end potentials confirmed that peaks pc1 and pa1 are coupled as well as peaks pc2 and pa2. The midpoint potentials, E_1 = -837 mV for pc1-pa1 couple and E_2 = -976 mV for pc2-pa2 couple, were consistent with DPV data. The CV peak current values for all four peaks were proportional to v^m in the range of 20-200 mV s^{-1}, where v is the sweep rate. The value of m had sample-to-sample variability but ranged within 0.63-0.73. This connotes that Hyp molecules both in the film interior and in contact with the BPG surface are electroactive and that the current is controlled, in part, by diffusional process. In contrast, the CV curve for a BPG electrode with a deposit of solely Hyp exhibited only a trace of a short-lived irreversible cathodic peak at -1100 mV. Thus, incorporation of Hyp into the $2C_{18}N^+Br^-$ matrix facilitates the reduction of Hyp. As an average of the measurements using four different BPG electrodes covered with Hyp + $2C_{18}N^+Br^-$ film, obtained formal potentials are $E_1^{0'}$ = -851 ± 11 mV and $E_2^{0'}$ = -970 ± 13 mV in 0.01 M KCl solution.

Transmission absorption spectrum of a Hyp + $2C_{18}N^+Br^-$ film (Figure 2-A) showed a feature of Hyp monomer, indicating that aggregation of Hyp is prevented in the cast film of $2C_{18}N^+Br^-$ even in an aqueous medium.[13]

The ER spectra (Figure 2-B) represent the difference absorption spectra between oxidized and reduced forms of reacting Hyp. The details of ER spectroscopic measurements were described elsewhere.[14] As far as the imaginary part is concerned, the positive- and negative-going peaks are due, respectively, to the absorption by oxidized and reduced forms. Therefore, the oxidized form at E_1 exhibits absorption maxima (λ_{max}) at 606, 558 and 484 nm. Although λ_{max} at 606 and 558 nm are at longer wavelengths than those in Figure 2-A, the ER spectral profiles of positive-going bands at E_1 are similar to those in Figure 2-A. The reduced form at E_1 exhibited λ_{max} at 744 nm and around 585 nm. It is known that one-electron reduced form of Hyp$^-$, *i.e.* Hyp$^{\bullet 2-}$, possesses an absorption maximum at 741 nm at an *n*-octadecanethiol ($C_{18}SH$)-monolayer-modified Au electrode in acetonitrile (AN),[8] at 740 nm in tetrahydrofuran,[6] and at 735 nm in AN.[9] Therefore, the 744 nm ER band is assigned to Hyp$^{\bullet 2-}$. Summing up, the redox reaction at E_1 is written as $\mathrm{Hyp^- + e^- \rightleftarrows Hyp^{\bullet 2-}}$ (1). We hereby assume that oxidized form of Hyp in the matrix is

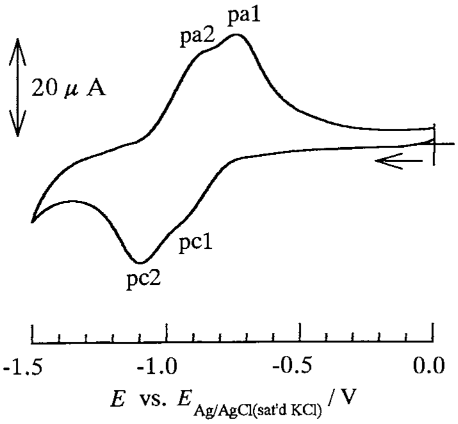

Figure 1. Cyclic voltammogram (100 mV s^{-1}) of a BPG electrode (0.36 cm^2) covered with a cast film of Hyp + $2C_{18}N^+Br^-$ in 0.01 M KCl solution at 23 °C. A 10 μl portion of 1.1 mM Hyp + 44 mM $2C_{18}N^+Br^-$ chloroform solution was coated to prepare the cast film. Ag|AgCl reference electrode in saturated KCl solution was used.

singly deprotonated to Hyp^- and that the proton transfer is not associated with the electron transfer.[15] In order for the protonation state to be clarified, we are currently underway of the detailed pH dependence measurement.

The different ER spectral profile between solid and broken lines in Figures 2-B indicates that a different redox process from the reaction (1) undergoes at E_2. The positive-going band around 596 nm at E_1 corresponds to the negative-going band around 585 nm at E_2, which is responsible to the absorption of $\mathrm{Hyp}^{\bullet 2-}$. The broken line has negative-going bands at 485 and 760 nm, in accordance to the absorption bands of Hyp^{3-} in AN.[8] Therefore, the redox reaction at E_2 can be written as $\mathrm{Hyp}^{\bullet 2-} + e^- \rightleftharpoons \mathrm{Hyp}^{3-}$ (2).

The above interpretation was further confirmed by the use of ER voltammograms (ERV). For example, the imaginary part of the ERV at 485 nm showed a positive-going peak at E_1 and a negative-going peak at E_2, consistent with Figures 1 and 2.

We previously reported the formal potentials of Hyp in AN at a $\mathrm{C_{18}SH}$-modified Au electrode.[8] Those values *vs.* ferrocene|ferrocenium couple in AN can be calibrated to the values *vs.* Ag|AgCl reference electrode in saturated KCl solution by adding 351 mV.[16] Thus, the formal potentials of Hyp in AN are $E_1^{0'} = -1090$ mV and $E_2^{0'} = -1390$ mV *vs.* Ag|AgCl electrode in saturated KCl solution. The values of $E_1^{0'}$ and $E_2^{0'}$ in $\mathrm{2C_{18}N^+Br^-}$ matrix are, respectively, 240 and 420 mV less negative than those in AN. These positive shifts are large enough to let the formal potentials come into the aqueous electrochemical window. The measurements of CV curves of a BPG electrode covered with Hyp + $\mathrm{2C_{18}N^+Br^-}$ at various KCl concentrations (c_KCl) revealed that the binding of Hyp anions to ammonium cationic sites in the matrix plays a key role. The plots of E_1 and E_2 against the logarithm of c_KCl gave straight lines with an identical slope of $\mathrm{d}E_1/\mathrm{dlog}(c_\mathrm{KCl}/\mathrm{M}) = \mathrm{d}E_2/\mathrm{dlog}(c_\mathrm{KCl}/\mathrm{M}) = -43$ mV in the range of c_KCl from 5 mM to 1 M. This fact leads us to a conclusion that binding of Hyp anions to the cationic sites in the film is responsible to the positive shift of the formal potentials and that the binding competes with the binding of Cl^-. Additionally, both the first and second reduction steps produce more negatively-charged reduced forms than oxidized counterparts, supporting the assignment of the redox processes expressed by (1) and (2) with no involvement of proton transfer. The value of the slope indicates also that the apparent number of electrons involved in the redox equilibrium is 0.73, probably reflecting the distribution in the states of Hyp molecules in the matrix.

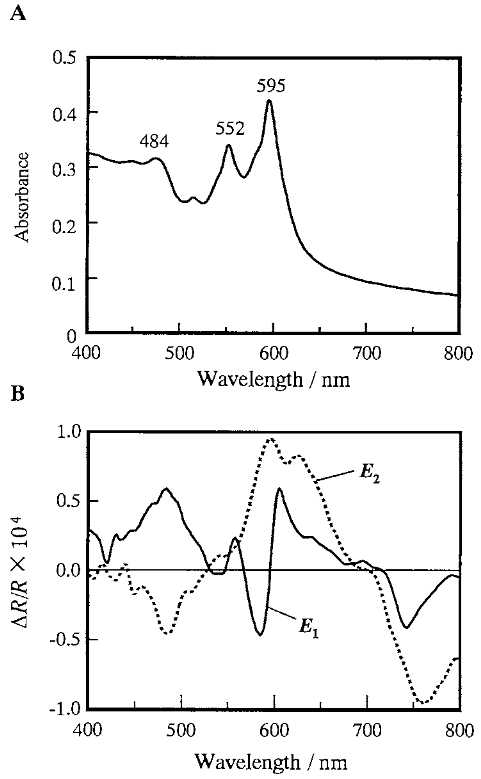

Figure 2. A: UV-vis transmission absorption spectrum of a cast film of Hyp + $\mathrm{2C_{18}N^+Br^-}$ coated on an octadecyltriethoxysilane-treated hydrophobic glass plate in 0.01 M KCl solution. B: ER spectra (imaginary part) of a BPG electrode covered with a cast film of Hyp + $\mathrm{2C_{18}N^+Br^-}$ in 0.01 M KCl solution at E_1 (solid line) and E_2 (broken line). Preparation of the cast film was the same as that in Figure 1. Potential-modulation, 30 $\mathrm{mV_{rms}}$-amplitude at 14 Hz.

In conclusion, incorporation of Hyp in the cationic matrix enables us to observe the two-consecutive one-electron transfer processes of Hyp in an aqueous solution, which cannot be observed at a bare electrode. The binding of Hyp anions to cationic sites in the matrix makes the formal potential shift to less negative and, as a result, facilitates the redox reaction.

This work is financially supported in part by the grant-in-aids from the Ministry of Education, Science, Sports and Culture of Japan to TS and NN.

References and Notes

1 N. Duran and P.-S. Song, *Photochem. Photobiol.*, **43**, 677 (1986).
2 J. Lenard, A. Rabson, and R. Vanderoef, *Proc. Natl. Acad. Sci. U.S.A.*, **90**, 158 (1993).
3 F. Gai, M. J. Fehr, and J. W. Petrich, *J. Phys. Chem.*, **98**, 5784 (1994).
4 Z. Diwu, *Photochem. Photobiol.*, **61**, 529 (1995).
5 B. L. Burel and P. Jardon, *J. Chim. Phys.*, **93**, 300 (1996).
6 F. Gerson, G. Gescheidt, P. Häring, Y. Mazur, D. Freeman, H. Spreitzer, and J. Daub, *J. Am. Chem. Soc.*, **117**, 11861 (1995).
7 L. Burel, P. Jardon, and J.-C. Lepretre, *New J. Chem.*, **21**, 399 (1997).
8 T. Sagara, H. Kawamura, K. Ezoe, and N. Nakashima, *J. Electroanal. Chem.*, **445**, 171 (1998).
9 A. P. Darmanyan, W. Jenks, D. Eloy, and P. Jardon, *J. Phys. Chem. B*, **103**, 3323 (1999).
10 N. Nakashima, T. Kuriyama, T. Tokunaga, H. Murakami, and T. Sagara, *Chem. Lett.*, **1998**, 633.
11 N. Nakashima, T. Tokunaga, Y. Nonaka, T. Nakanishi, H. Murakami, and T. Sagara, *Angew. Chem., Int. Ed.*, **37**, 2671 (1998).
12 T. Nakanishi, H. Murakami, T. Sagara, and N. Nakashima, *J. Phys. Chem. B*, **103**, 304 (1999).
13 An increase in water content in an organic solvent such as ethanol brings about the appearance of the absorption bands of dimer or aggregates of Hyp.[5]
14 T. Sagara, *Recent Res. Dev. Phys. Chem.*, **2**, 159 (1998).
15 The protonation state of the oxidized form in reaction (1) in a neutral aqueous phase is still under debate. Various Hyp|Hyp^- $\mathrm{p}K_\mathrm{a}$ values ranging 2 to 12 have been reported as summarized by Darmanyan *et al.*[9]
16 This conventional calibration cannot rigorously eliminate the contribution from the liquid junction potential.[17]
17 A. J. Bard and L. R. Faulkner, "Electrochemical Methods," John Wiley & Sons, New York (1980), p. 701.