

An Efficient Fluorescence Sensor for Superoxide with an Acridinium Ion-Linked Porphyrin Triad

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S Supporting Information

ABSTRACT: Addition of potassium superoxide with 18-crown-6 ether ($\text{KO}_2^{\bullet-}$ –18-crown-6) to a toluene solution of an acridinium ion-linked porphyrin triad ($\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$) resulted in a remarkable enhancement of the fluorescence intensity. Thus, $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ acts as an efficient fluorescence sensor for superoxide. Electron transfer from $\text{KO}_2^{\bullet-}$ –18-crown-6 to the Acr^+ moiety to produce the two-electron-reduced species ($\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$) results in inhibition of the fluorescence quenching via photoinduced electron transfer, as revealed by laser flash photolysis measurements.

Fluorescence sensors have been widely developed to visualize not only metal cations¹ but also anions² and reactive oxygen species (ROS)³ in vivo or in vitro. The sensor molecules can recognize redox-inactive metal cations and anions and emit specific fluorescence upon binding of these targets.^{4,5} In the case of ROS, superoxide ($\text{O}_2^{\bullet-}$) is the first species to be produced in the respiratory chain by an electron-transfer reduction of oxygen.³ The detection of $\text{O}_2^{\bullet-}$ is confounded by the lack of a sensitive and specific method. Development of new fluorescence probes, where a probe molecule begins to fluoresce at low concentrations of ROS, would expand the scope of the availability of fluorescence techniques for routine analysis of ROS.⁶ In such a case, high sensitivity toward $\text{O}_2^{\bullet-}$ would be required in fluorescence sensors.

On the other hand, photoinduced electron transfer (PET) is one of the common processes for quenching the fluorescence in fluorescence sensors.^{1b,2c,7} The efficiency of the PET process depends on the redox potentials of the electron-donor moiety (D) and the electron-acceptor moiety (A) in D–A-linked molecules together with the excitation energy of the fluorescent moiety. When D–A-linked molecules are reduced by $\text{O}_2^{\bullet-}$, the fluorescence intensity would be affected by $\text{O}_2^{\bullet-}$. However, there has been no report on the response of such a D–A-linked fluorescence sensor to a simple external signal such as electron transfer with $\text{O}_2^{\bullet-}$.

We report herein a quantitative fluorescence sensor for $\text{O}_2^{\bullet-}$ based on an acridinium ion (Acr^+)-linked porphyrin triad ($\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$) (Figure 1a). The acridinium ion used as an electron acceptor in this work has been utilized for a long-lived ET state because the λ value for electron self-exchange between

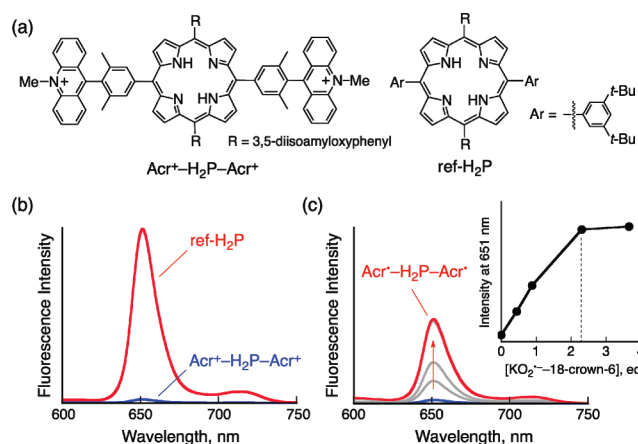
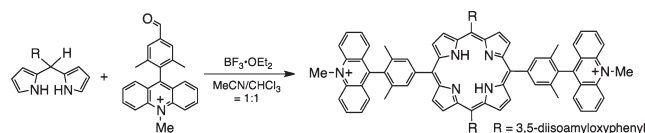


Figure 1. (a) Structures of the acridinium ion-linked porphyrin triad ($\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$) and the reference compound ($\text{ref-H}_2\text{P}$). (b) Fluorescence spectra of $\text{ref-H}_2\text{P}$ and $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ observed in a deaerated toluene solution at 298 K (excitation wavelength $\lambda_{\text{ex}} = 512$ nm). (c) Fluorescence spectra observed in the titration of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ with $\text{KO}_2^{\bullet-}$ –18-crown-6 in a deaerated toluene at 298 K ($[\text{KO}_2^{\bullet-}$ –18-crown-6] = 0–7.0 μM ; $\lambda_{\text{ex}} = 512$ nm). Inset: plot of fluorescence intensity at 651 nm vs $[\text{KO}_2^{\bullet-}$ –18-crown-6].

Scheme 1



Acr^+ and the corresponding one-electron-reduced radical ($\text{Acr}^{\bullet-}$) is the smallest (0.3 eV).^{8,9}

$\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ was synthesized by condensation of 5-(3,5-diisopropoxyphenyl)dipyromethane with the corresponding aldehyde (Scheme 1) and characterized by ¹H NMR analysis [see the Experimental Section in the Supporting Information (SI)]. The UV–vis spectrum of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ exhibited absorption bands at 361 nm due to the Acr^+ moiety and 418 and 512 nm due to the H_2P moiety (see Figure S1 in the SI).

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The fluorescence spectrum of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ is shown in Figure 1b, where the H_2P moiety was exclusively excited at $\lambda_{\text{ex}} = 512$ nm. The fluorescence intensity of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ was significantly quenched in comparison with that of the reference compound (ref- H_2P). The one-electron oxidation potential of the H_2P moiety ($E_{\text{ox}} = 0.97$ V vs SCE) and the one-electron reduction potential of the Acr^+ moiety ($E_{\text{red}} = -0.54$ V)⁸ in acetonitrile (MeCN) were determined by cyclic voltammetry (see Figure S2 in the SI). Upon addition of potassium superoxide ($\text{KO}_2^{\bullet-}$; $E_{\text{ox}} = -0.68$ V) with 18-crown-6 ether ($\text{KO}_2^{\bullet-}$ -18-crown-6)¹⁰ to a toluene solution of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ (2.0 μM), the fluorescence intensity at $\lambda_{\text{em}} = 651$ nm was remarkably enhanced (Figure 1c), giving a fluorescence spectrum similar to that of ref- H_2P . The Figure 1c inset shows a plot of the fluorescence intensity at 651 nm versus $[\text{KO}_2^{\bullet-}$ -18-crown-6]. The stoichiometry of $[\text{KO}_2^{\bullet-}$ -18-crown-6] with respect to $[\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+]$ was determined to be 2:1 from the fluorescence spectral titration. This indicates that $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ is reduced by 2 equiv of $\text{KO}_2^{\bullet-}$ -18-crown-6 to produce the two-electron-reduced species ($\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$), which shows the fluorescence of the H_2P moiety. Thus, $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ acts as an effective off/on fluorescence sensor for a small concentration of $\text{O}_2^{\bullet-}$ less than micromolar order. Such a significant enhancement of the fluorescence intensity was also observed when tetramethylsemiquinone radical anion ($\text{Me}_4\text{Q}^{\bullet-}$; $E_{\text{ox}} = -0.88$ V) was employed in toluene instead of $\text{KO}_2^{\bullet-}$ -18-crown-6 (see Figure S3 in the SI). This indicates that the electron-transfer reduction of the Acr^+ moieties in $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ results in the formation of $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$, which exhibits the fluorescence of the H_2P moiety in toluene. When a polar solvent such as acetonitrile was employed instead of toluene, no fluorescence of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ was observed in the absence or presence of $\text{KO}_2^{\bullet-}$ -18-crown-6. Thus, the use of a nonpolar solvent such as toluene is required for the detection of $\text{O}_2^{\bullet-}$ or $\text{Me}_4\text{Q}^{\bullet-}$ with $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$.

The formation of $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ was confirmed by electron paramagnetic resonance (EPR) measurements, as shown in Figure 2a. The hyperfine splitting constants of $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ were similar to those reported for the acridinyl radical.⁸ The EPR intensity increased linearly up to the addition of 2 equiv of $\text{KO}_2^{\bullet-}$ -18-crown-6 to produce $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ (Figure 2b,c). This indicates that there is no interaction between the two $\text{Acr}^{\bullet-}$ moieties in $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ because of the long distance between the two spins.

In order to understand why the fluorescence of the H_2P moiety was recovered when the Acr^+ moiety was reduced, we compared transient absorption spectra observed upon photoexcitation of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ and $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$. Femtosecond laser flash photolysis of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ in deaerated toluene with $\lambda_{\text{ex}} = 430$ nm revealed a transient absorption spectrum with peaks at $\lambda = 480$ and 620 nm due to the singlet excited state of the H_2P moiety in $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ ($\text{Acr}^+-^1\text{H}_2\text{P}^+-\text{Acr}^+$) (Figure 3a), in agreement with the spectrum of $^1\text{ref-H}_2\text{P}^*$ (Figure S4 in the SI). The decay time profile at $\lambda = 620$ nm exhibited monoexponential decay with a lifetime of 200 ps (red line in Figure 3b), whereas $^1\text{ref-H}_2\text{P}^*$ exhibited little decay in this time range (Figure S4 in the SI). Such fast decay of the absorbance at $\lambda = 620$ nm due to $^1\text{H}_2\text{P}^*$ is ascribed to electron transfer from the $^1\text{H}_2\text{P}^*$ moiety to the Acr^+ moiety to form the electron-transfer state ($\text{Acr}^+-\text{H}_2\text{P}^{*+}-\text{Acr}^{\bullet-}$). From the fluorescence quenching in Figure 1b, the rate constant (k_{et}) for electron transfer from the $^1\text{H}_2\text{P}^*$ moiety to the Acr^+ moiety was estimated to be $2.0 \times 10^9 \text{ s}^{-1}$ by comparison of the intensity of

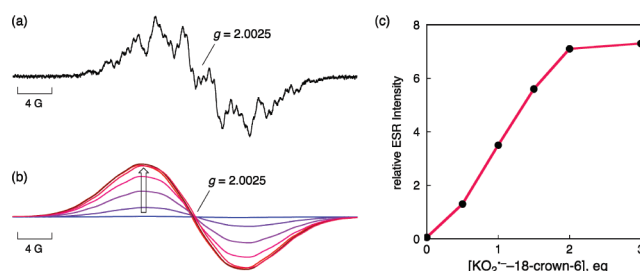


Figure 2. (a) EPR spectra of $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ produced by the addition of $\text{KO}_2^{\bullet-}$ -18-crown-6 (1.5 mM) to a deaerated toluene solution of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ (0.50 mM) with a modulation width of 0.10 G. (b) EPR spectral change by addition of $\text{KO}_2^{\bullet-}$ -18-crown-6 with a modulation width of 10 G. (c) Plot of the ESR intensity of $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ vs $[\text{KO}_2^{\bullet-}$ -18-crown-6].

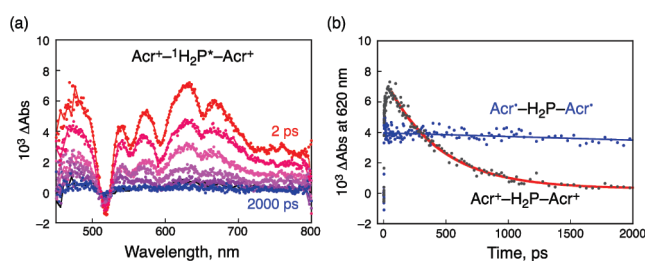


Figure 3. (a) Transient absorption spectra of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ (1.0 μM) in deaerated toluene at 298 K taken after femtosecond laser excitation at $\lambda_{\text{ex}} = 430$ nm. (b) Decay time profiles at $\lambda = 620$ nm for $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ and for $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$ produced by the addition of $\text{KO}_2^{\bullet-}$ -18-crown-6 (2.0 μM).

$^1\text{H}_2\text{P}^*$ in $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ with that of $^1\text{ref-H}_2\text{P}^*$ ($\tau = 10.1$ ns);¹¹ this agrees with the k_{et} value of $2.0 \times 10^9 \text{ s}^{-1}$ determined from the lifetime of $^1\text{H}_2\text{P}^*$ in Figure 3b (red line). The free energy change for electron transfer from $^1\text{H}_2\text{P}^*$ to Acr^+ (ΔG_{et}) in MeCN was determined to be -0.38 eV on the basis of the one-electron oxidation potential of H_2P ($E_{\text{ox}} = 0.97$ V vs SCE), the energy of the singlet excited state (1.89 eV),¹² and the one-electron reduction potential of the Acr^+ moiety ($E_{\text{red}} = -0.54$ V vs SCE).⁸ The electron transfer from $^1\text{H}_2\text{P}^*$ to Acr^+ in toluene is also energetically feasible judging from the negative ΔG_{et} value (-0.14 eV), which was evaluated using an empirical equation for the estimation of ΔG_{et} in a solvent of known dielectric constant ($\epsilon = 2.38$ for toluene) based on the ΔG_{et} value in MeCN.¹³ However, the transient absorption band due to the electron-transfer state was not observed during the decay of the transient absorption due to $^1\text{H}_2\text{P}^*$. This suggests that back electron transfer from the $\text{Acr}^{\bullet-}$ moiety to the H_2P^{*+} moiety is much faster than the forward electron transfer.

In sharp contrast to the fast decay of $^1\text{H}_2\text{P}^*$ in $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$, little decay of $^1\text{H}_2\text{P}^*$ was observed when $\text{KO}_2^{\bullet-}$ -18-crown-6 (2 equiv) was added to a deaerated toluene solution of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ (blue line in Figure 3b). Because the Acr^+ moiety of $\text{Acr}^+-\text{H}_2\text{P}-\text{Acr}^+$ is reduced by 2 equiv of $\text{O}_2^{\bullet-}$ to produce $\text{Acr}^{\bullet-}-\text{H}_2\text{P}-\text{Acr}^{\bullet-}$, electron transfer from $^1\text{H}_2\text{P}^*$ to $\text{Acr}^{\bullet-}$ becomes energetically unfeasible. On the other hand, electron transfer from the $\text{Acr}^{\bullet-}$ moiety to the $^1\text{H}_2\text{P}^*$ moiety is energetically feasible judging from the negative ΔG_{et} value (-0.94 eV).¹⁴ However, the spin state of the electron-transfer

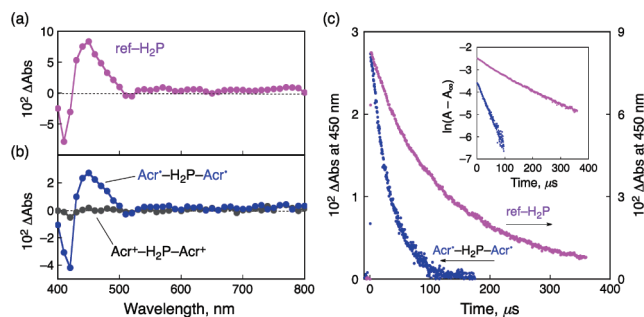


Figure 4. (a) Transient absorption spectrum of ref-H₂P (1.0 μM). (b) Transient absorption spectra of Acr⁺-H₂P-Acr⁺ (1.0 μM) and Acr⁺-H₂P-Acr⁺ produced by the addition of KO₂⁻-18-crown-6 (5.0 μM) in deaerated toluene at 298 K taken 2.0 μs after laser excitation at λ_{ex} = 430 nm. (c) Decay time profiles at λ = 450 nm for ³ref-H₂P* and Acr⁺-³H₂P*-Acr⁺.

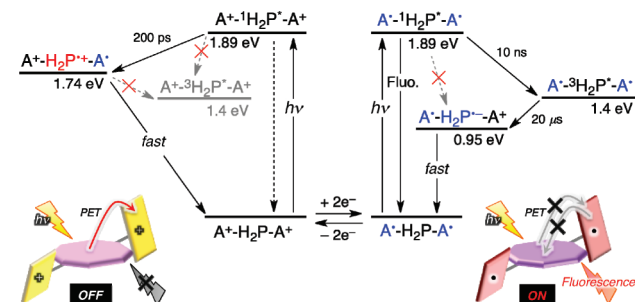
product may be different. The spin state of Acr[•]-H₂P-Acr[•] is two independent doublets (see above), whereas the spin state of the electron-transfer product (Acr⁺-H₂P^{•-}-Acr[•]) is either a singlet or a triplet because the H₂P^{•-} moiety can interact with the Acr[•] moiety. Thus, electron transfer from the Acr[•] moiety to the ¹H₂P* moiety in Acr[•]-H₂P-Acr[•] to produce Acr⁺-H₂P^{•-}-Acr[•] (or Acr[•]-H₂P^{•-}-Acr⁺) is spin-forbidden. The endergonic electron transfer from the Acr[•] moiety to the ¹H₂P* moiety is also spin-forbidden. This may be the reason why no quenching of ¹H₂P* was observed in Acr[•]-H₂P-Acr[•].

The electron-transfer quenching of the fluorescence of ¹H₂P* by the Acr⁺ moiety was also confirmed by nanosecond laser flash photolysis measurements. No transient absorption due to the triplet excited state of the H₂P moiety was observed in Acr⁺-H₂P-Acr⁺ because of electron transfer from ¹H₂P* to Acr⁺, which is faster than the intersystem crossing from ¹H₂P* to ³H₂P* (black line in Figure 4). In the case of Acr[•]-H₂P-Acr[•] produced by the two-electron reduction of Acr⁺-H₂P-Acr⁺ with 2 equiv of KO₂⁻-18-crown-6, however, the transient absorption band (λ_{max} = 450 nm) was clearly observed, as in the case of ref-H₂P (blue line in Figure 4b vs the reference spectrum in Figure 4a). The triplet lifetime of Acr[•]-³H₂P*-Acr[•] is shorter than that of ref-H₂P (Figure 4c), probably because of electron transfer from the Acr[•] moiety to the ³H₂P* moiety followed by fast back electron transfer.

The energy diagrams for the photodynamics of Acr⁺-H₂P-Acr⁺ and Acr[•]-H₂P-Acr[•] are summarized in Scheme 2. The singlet excited state (¹H₂P*) produced upon photoexcitation of Acr⁺-H₂P-Acr⁺ is quenched by spin-allowed electron transfer from the ¹H₂P* moiety to the Acr⁺ moiety followed by fast back electron transfer from the Acr[•] moiety to the H₂P^{•+} moiety, and therefore, it exhibits little fluorescence. Once the two Acr⁺ moieties are reduced by O₂^{•-} to produce Acr[•]-H₂P-Acr[•], the ¹H₂P* moiety is not quenched in toluene by either endergonic electron transfer from the ¹H₂P* moiety to the Acr[•] moiety or spin-forbidden electron transfer from the Acr[•] moiety to the ¹H₂P* moiety, so it exhibits much stronger fluorescence than Acr⁺-H₂P-Acr⁺.

In a polar solvent such as MeCN, the formation of Acr[•]-H₂P-Acr[•] was also confirmed by EPR measurements (Figure S5 in the SI). In sharp contrast to the case in toluene, no fluorescence from the singlet excited state in Acr[•]-H₂P-Acr[•] (Acr[•]-¹H₂P*-Acr[•]) was observed in MeCN because of fast electron transfer from the Acr[•] moiety to the ¹H₂P* moiety.¹⁵

Scheme 2



In conclusion, we have developed an efficient fluorescence sensor for detection of O₂^{•-} using an acridinium ion-linked porphyrin triad (Acr⁺-H₂P-Acr⁺) that is responsive to electron-transfer reduction of the Acr⁺ moiety. The present study provides a new strategy for fluorescence sensors that are responsive to one-electron reductants such as O₂^{•-}.

■ ASSOCIATED CONTENT

S Supporting Information. Experimental procedures and Figures S1–S5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(14) From the E_{ox} value of Acr^{\bullet} (-0.54 V), the E_{red} value of H_2P (-1.26 V), and the energy of the singlet excited state of H_2P (1.89 eV), the ΔG_{et} value in MeCN was determined to be 1.17 eV. This was converted to the value in toluene using an empirical equation for the estimation of ΔG_{et} in a solvent of known dielectric constant ($\epsilon = 2.38$ for toluene).¹³ For the E_{red} value of H_2P , see: Schuster, D. I.; Li, K.; Guldi, D. M.; Palkar, A.; Echegoyen, L.; Stanisky, C.; Cross, R. J.; Niemi, M.; Tkachenko, N. V.; Lemmetyinen, H. *J. Am. Chem. Soc.* **2007**, 129, 15973.

(15) Electron transfer from the Acr^{\bullet} moiety to the $^1\text{H}_2\text{P}^{\bullet}$ moiety may occur rapidly prior to the spin–spin interaction between the Acr^{\bullet} and $\text{H}_2\text{P}^{\bullet}$ moieties because of the larger driving force for electron transfer in MeCN.