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 ($KO_2^{\bullet-}$—18-crown-6) to a toluene solution of an acridinium ion-linked porphyrin triad (Acr^+—H_2P—Acr^+) resulted in a remarkable enhancement of the fluorescence intensity. Thus, Acr^+—H_2P—Acr^+ acts as an efficient fluorescence sensor for superoxide. Electron transfer from $KO_2^{\bullet-}$—18-crown-6 to the Acr^+ moiety to produce the two-electron-reduced species ($Acr^{\bullet}$—H_2P—$Acr^{\bullet}$) 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 cations[1] but also anions[2] 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 ($O_2^{\bullet-}$) is the first species to be produced in the respiratory chain by an electron-transfer reduction of oxygen.[3] The detection of $O_2^{\bullet-}$ 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 $O_2^{\bullet-}$ 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 $O_2^{\bullet-}$, the fluorescence intensity would be affected by $O_2^{\bullet-}$. 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 $O_2^{\bullet-}$.

We report herein a quantitative fluorescence sensor for $O_2^{\bullet-}$ based on an acridinium ion (Acr^+)-linked porphyrin triad (Acr^+—H_2P—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
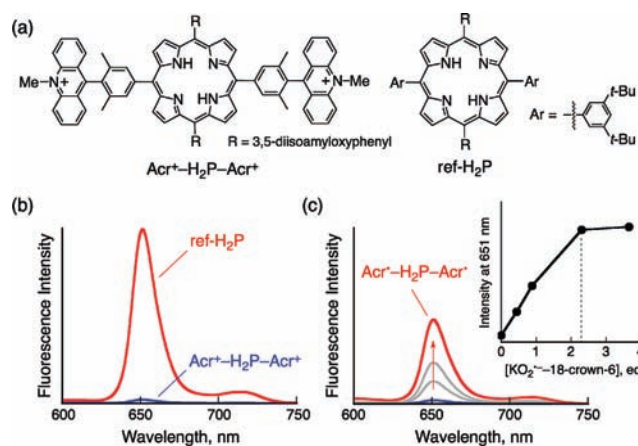

Figure 1. (a) Structures of the acridinium ion-linked porphyrin triad (Acr^+—H_2P—Acr^+) and the reference compound (ref-H_2P). (b) Fluorescence spectra of ref-H_2P and Acr^+—H_2P—Acr^+ observed in a deaerated toluene solution at 298 K (excitation wavelength λ_{ex} = 512 nm). (c) Fluorescence spectra observed in the titration of Acr^+—H_2P—Acr^+ (2.0 μM) with $KO_2^{\bullet-}$—18-crown-6 in a deaerated toluene at 298 K ([$KO_2^{\bullet-}$—18-crown-6] = 0–7.0 μM; λ_{ex} = 512 nm). Inset: plot of fluorescence intensity at 651 nm vs [$KO_2^{\bullet-}$—18-crown-6].

Scheme 1
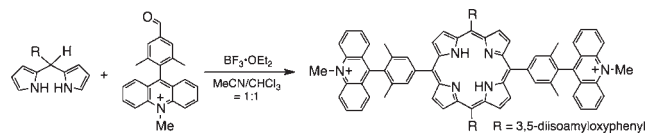
Acr^+ and the corresponding one-electron-reduced radical ($Acr^{\bullet}$) is the smallest (0.3 eV).[8,9]

Acr^+—H_2P—Acr^+ was synthesized by condensation of 5-(3,5-diisoamyloxyphenyl)dipyrromethane with the corresponding aldehyde (Scheme 1) and characterized by ^{1}H NMR analysis [see the Experimental Section in the Supporting Information (SI)]. The UV–vis spectrum of Acr^+—H_2P—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).

The fluorescence spectrum of $Acr^+-H_2P-Acr^+$ is shown in Figure 1b, where the H_2P moiety was exclusively excited at $\lambda_{ex} = 512$ nm. The fluorescence intensity of $Acr^+-H_2P-Acr^+$ was significantly quenched in comparison with that of the reference compound (ref-H_2P). The one-electron oxidation potential of the H_2P moiety ($E_{ox} = 0.97$ V vs SCE) and the one-electron reduction potential of the Acr^+ moiety ($E_{red} = -0.54$ V)[8] in acetonitrile (MeCN) were determined by cyclic voltammetry (see Figure S2 in the SI). Upon addition of potassium superoxide ($KO_2^{\bullet-}$; $E_{ox} = -0.68$ V) with 18-crown-6 ether ($KO_2^{\bullet-}-18$-crown-6)[10] to a toluene solution of $Acr^+-H_2P-Acr^+$ (2.0 μM), the fluorescence intensity at $\lambda_{em} = 651$ 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 $[KO_2^{\bullet-}-18$-crown-6$]$. The stoichiometry of $[KO_2^{\bullet-}-18$-crown-6$]$ with respect to $[Acr^+-H_2P-Acr^+]$ was determined to be 2:1 from the fluorescence spectral titration. This indicates that $Acr^+-H_2P-Acr^+$ is reduced by 2 equiv of $KO_2^{\bullet-}-18$-crown-6 to produce the two-electron-reduced species ($Acr^{\bullet}-H_2P-Acr^{\bullet}$), which shows the fluorescence of the H_2P moiety. Thus, $Acr^+-H_2P-Acr^+$ acts as an effective off/on fluorescence sensor for a small concentration of $O_2^{\bullet-}$ less than micromolar order. Such a significant enhancement of the fluorescence intensity was also observed when tetramethylsemiquinone radical anion ($Me_4Q^{\bullet-}$; $E_{ox} = -0.88$ V) was employed in toluene instead of $KO_2^{\bullet-}-18$-crown-6 (see Figure S3 in the SI). This indicates that the electron-transfer reduction of the Acr^+ moieties in $Acr^+-H_2P-Acr^+$ results in the formation of $Acr^{\bullet}-H_2P-Acr^{\bullet}$, 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 $Acr^+-H_2P-Acr^+$ was observed in the absence or presence of $KO_2^{\bullet-}-18$-crown-6. Thus, the use of a nonpolar solvent such as toluene is required for the detection of $O_2^{\bullet-}$ or $Me_4Q^{\bullet-}$ with $Acr^+-H_2P-Acr^+$.

The formation of $Acr^{\bullet}-H_2P-Acr^{\bullet}$ was confirmed by electron paramagnetic resonance (EPR) measurements, as shown in Figure 2a. The hyperfine splitting constants of $Acr^{\bullet}-H_2P-Acr^{\bullet}$ were similar to those reported for the acridinyl radical.[8] The EPR intensity increased linearly up to the addition of 2 equiv of $KO_2^{\bullet-}-18$-crown-6 to produce $Acr^{\bullet}-H_2P-Acr^{\bullet}$ (Figure 2b,c). This indicates that there is no interaction between the two $Acr^{\bullet}$ moieties in $Acr^{\bullet}-H_2P-Acr^{\bullet}$ 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 $Acr^+-H_2P-Acr^+$ and $Acr^{\bullet}-H_2P-Acr^{\bullet}$. Femtosecond laser flash photolysis of $Acr^+-H_2P-Acr^+$ in deaerated toluene with $\lambda_{ex} = 430$ 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 $Acr^+-H_2P-Acr^+$ ($Acr^+-{}^1H_2P^*-Acr^+$) (Figure 3a), in agreement with the spectrum of ^{1}ref-H_2P^* (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}ref-H_2P^* exhibited little decay in this time range (Figure S4 in the SI). Such fast decay of the absorbance at $\lambda = 620$ nm due to ${}^1H_2P^*$ is ascribed to electron transfer from the ${}^1H_2P^*$ moiety to the Acr^+ moiety to form the electron-transfer state ($Acr^+-H_2P^{\bullet+}-Acr^{\bullet}$). From the fluorescence quenching in Figure 1b, the rate constant (k_{et}) for electron transfer from the ${}^1H_2P^*$ moiety to the Acr^+ moiety was estimated to be 2.0×10^9 s^{-1} by comparison of the intensity of
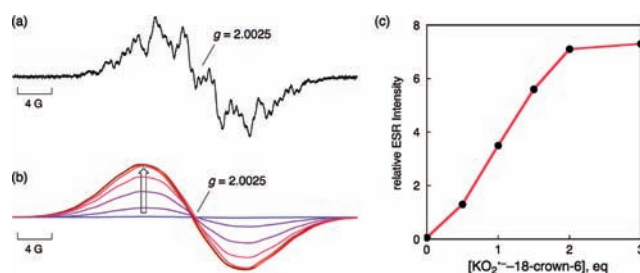

Figure 2. (a) EPR spectra of $Acr^{\bullet}-H_2P-Acr^{\bullet}$ produced by the addition of $KO_2^{\bullet-}-18$-crown-6 (1.5 mM) to a deaerated toluene solution of $Acr^+-H_2P-Acr^+$ (0.50 mM) with a modulation width of 0.10 G. (b) EPR spectral change by addition of $KO_2^{\bullet-}-18$-crown-6 with a modulation width of 10 G. (c) Plot of the ESR intensity of $Acr^{\bullet}-H_2P-Acr^{\bullet}$ vs $[KO_2^{\bullet-}-18$-crown-6$]$.

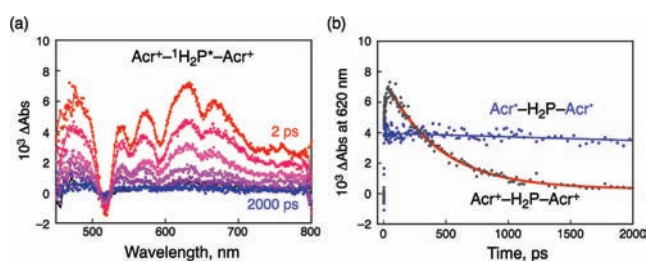**Figure 3.** (a) Transient absorption spectra of $Acr^+-H_2P-Acr^+$ (1.0 μM) in deaerated toluene at 298 K taken after femtosecond laser excitation at $\lambda_{ex} = 430$ nm. (b) Decay time profiles at $\lambda = 620$ nm for $Acr^+-H_2P-Acr^+$ and for $Acr^{\bullet}-H_2P-Acr^{\bullet}$ produced by the addition of $KO_2^{\bullet-}-18$-crown-6 (2.0 μM).

${}^1H_2P^*$ in $Acr^+-H_2P-Acr^+$ with that of ^{1}ref-H_2P^* ($\tau = 10.1$ ns);[11] this agrees with the k_{et} value of 2.0×10^9 s^{-1} determined from the lifetime of ${}^1H_2P^*$ in Figure 3b (red line). The free energy change for electron transfer from ${}^1H_2P^*$ 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 ($E_{ox} = 0.97$ V vs SCE), the energy of the singlet excited state (1.89 eV),[12] and the one-electron reduction potential of the Acr^+ moiety ($E_{red} = -0.54$ V vs SCE).[8] The electron transfer from ${}^1H_2P^*$ 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 ($\varepsilon = 2.38$ 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 ${}^1H_2P^*$. This suggests that back electron transfer from the $Acr^{\bullet}$ moiety to the $H_2P^{\bullet+}$ moiety is much faster than the forward electron transfer.

In sharp contrast to the fast decay of ${}^1H_2P^*$ in $Acr^+-H_2P-Acr^+$, little decay of ${}^1H_2P^*$ was observed when $KO_2^{\bullet-}-18$-crown-6 (2 equiv) was added to a deaerated toluene solution of $Acr^+-H_2P-Acr^+$ (blue line in Figure 3b). Because the Acr^+ moiety of $Acr^+-H_2P-Acr^+$ is reduced by 2 equiv of $O_2^{\bullet-}$ to produce $Acr^{\bullet}-H_2P-Acr^{\bullet}$, electron transfer from ${}^1H_2P^*$ to $Acr^{\bullet}$ becomes energetically unfeasible. On the other hand, electron transfer from the $Acr^{\bullet}$ moiety to the ${}^1H_2P^*$ moiety is energetically feasible judging from the negative ΔG_{et} value (-0.94 eV).[14] However, the spin state of the electron-transfer
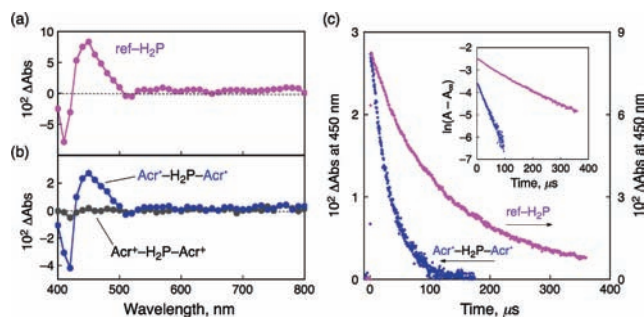
Figure 4. (a) Transient absorption spectrum of ref-H_2P (1.0 μM). (b) Transient absorption spectra of $Acr^+-H_2P-Acr^+$ (1.0 μM) and $Acr^\bullet-H_2P-Acr^\bullet$ produced by the addition of $KO_2^{\bullet-}-18$-crown-6 (5.0 μM) in deaerated toluene at 298 K taken 2.0 μs after laser excitation at $\lambda_{ex} = 430$ nm. (c) Decay time profiles at $\lambda = 450$ nm for ^{3}ref-H_2P^* and $Acr^\bullet$-$^3H_2P^*$-$Acr^\bullet$.

product may be different. The spin state of $Acr^\bullet-H_2P-Acr^\bullet$ is two independent doublets (see above), whereas the spin state of the electron-transfer product ($Acr^+-H_2P^{\bullet-}-Acr^\bullet$) is either a singlet or a triplet because the $H_2P^{\bullet-}$ moiety can interact with the $Acr^\bullet$ moiety. Thus, electron transfer from the $Acr^\bullet$ moiety to the $^1H_2P^*$ moiety in $Acr^\bullet-H_2P-Acr^\bullet$ to produce $Acr^+-H_2P^{\bullet-}-Acr^\bullet$ (or $Acr^\bullet-H_2P^{\bullet-}-Acr^+$) is spin-forbidden. The endergonic electron transfer from the $Acr^\bullet$ moiety to the $^1H_2P^*$ moiety is also spin-forbidden. This may be the reason why no quenching of $^1H_2P^*$ was observed in $Acr^\bullet-H_2P-Acr^\bullet$.

The electron-transfer quenching of the fluorescence of $^1H_2P^*$ by the Acr^+ moiety was also confirmed by nanosecond laser flash photolysis measurements. No transient absorption due to the triplet excited state of the H_2P moiety was observed in $Acr^+-H_2P-Acr^+$ because of electron transfer from $^1H_2P^*$ to Acr^+, which is faster than the intersystem crossing from $^1H_2P^*$ to $^3H_2P^*$ (black line in Figure 4). In the case of $Acr^\bullet-H_2P-Acr^\bullet$ produced by the two-electron reduction of $Acr^+-H_2P-Acr^+$ with 2 equiv of $KO_2^{\bullet-}-18$-crown-6, however, the transient absorption band ($\lambda_{max} = 450$ nm) was clearly observed, as in the case of ref-H_2P (blue line in Figure 4b vs the reference spectrum in Figure 4a). The triplet lifetime of $Acr^\bullet-^3H_2P^*-Acr^\bullet$ is shorter than that of ref-H_2P (Figure 4c), probably because of electron transfer from the $Acr^\bullet$ moiety to the $^3H_2P^*$ moiety followed by fast back electron transfer.

The energy diagrams for the photodynamics of $Acr^+-H_2P-Acr^+$ and $Acr^\bullet-H_2P-Acr^\bullet$ are summarized in Scheme 2. The singlet excited state ($^1H_2P^*$) produced upon photoexcitation of $Acr^+-H_2P-Acr^+$ is quenched by spin-allowed electron transfer from the $^1H_2P^*$ moiety to the Acr^+ moiety followed by fast back electron transfer. from the $Acr^\bullet$ moiety to the $H_2P^{\bullet+}$ moiety, and therefore, it exhibits little fluorescence. Once the two Acr^+ moieties are reduced by $O_2^{\bullet-}$ to produce $Acr^\bullet-H_2P-Acr^\bullet$, the $^1H_2P^*$ moiety is not quenched in toluene by either endergonic electron transfer from the $^1H_2P^*$ moiety to the $Acr^\bullet$ moiety or spin-forbidden electron transfer from the $Acr^\bullet$ moiety to the $^1H_2P^*$ moiety, so it exhibits much stronger fluorescence than $Acr^+-H_2P-Acr^+$.

In a polar solvent such as MeCN, the formation of $Acr^\bullet-H_2P-Acr^\bullet$ 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 $Acr^\bullet-H_2P-Acr^\bullet$ ($Acr^\bullet-^1H_2P^*-Acr^\bullet$) was observed in MeCN because of fast electron transfer from the $Acr^\bullet$ moiety to the $^1H_2P^*$ moiety.[15]

Scheme 2
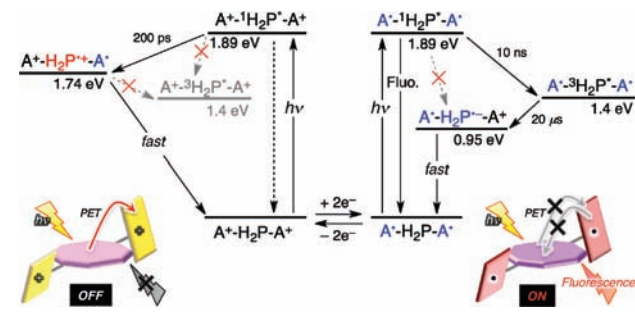
In conclusion, we have developed an efficient fluorescence sensor for detection of $O_2^{\bullet-}$ using an acridinium ion-linked porphyrin triad ($Acr^+-H_2P-Acr^+$) that is responsive to electron-transfer reduction of the Acr^+ moiety. The present study provides a new strategy for fluorescence sensors that are responsive to one-electron reductants such as $O_2^{\bullet-}$.

■ ASSOCIATED CONTENT

ⓢ **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 ($\varepsilon = 2.38$ 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 $^1H_2P^*$ moiety may occur rapidly prior to the spin—spin interaction between the Acr$^\bullet$ and $H_2P^{\bullet-}$ moieties because of the larger driving force for electron transfer in MeCN.