Photochemical and Radiolytic Oxidation of a Zinc Porphyrin Bound to Human Serum Albumin

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Abstract: A water-soluble, sulfonated zinc porphyrin, ZnTDCSPP, binds to human serum albumin (HSA) at two distinct sites, which differ according to their accessibility to species dissolved in the bulk aqueous phase. The triplet excited states of dye molecules resident in these two sites react with molecular oxygen, forming $O_2(^1\Delta_g)$, and with 2,6-dimethylbenzo-1,4-quinone, generating net electron-transfer products, on quite different time scales. Singlet molecular oxygen so produced attacks the protein in the vicinity of the bound porphyrin and causes irreversible damage. The ZnTDCSPP π-radical cation, formed by photooxidation in the presence of quinone or by pulse radiolysis, remains bound to HSA but it does not abstract an electron from amino acids in the adjacent protein chain. The triplet state is quenched also by long distance electron transfer under conditions where appropriate electron acceptors are bound to the same protein molecule.

Binding of drugs and other adventitious reagents to serum albumins provides a convenient means for their transportation throughout the body.[1] The protein can accommodate a bewildering array of reagents,[2] including fatty acids, lipids, bilirubin, hematin, metal cations, tryptophan, thyroxine, and many of the tranquilizing drugs. In certain cases, the interactions can be highly specific and binding sites have been identified,[3] although binding can induce conformational changes in the protein structure. It has been shown also that negatively charged porphyrins and metalloporphyrins, and their derivatives, bind strongly to serum albumins.[4] The bound material retains its photoactivity, especially its ability to produce singlet molecular oxygen $O_2(^1\Delta_g)$ upon irradiation with visible light under aerobic conditions.[5,6]

This particular case is of importance because of the growing interest in using porphyrin derivatives as sensitizers for photodynamic therapy.[7] Interaction between such sensitizers and albumin will have a pronounced effect upon the distribution, free concentration, and metabolism of the dye and, in certain cases, could significantly affect the photodynamic activity. There is also the possibility that in vivo irradiation of dye bound to serum albumin could result in destruction of the protein.[8] Previous work has concentrated on determining complexation constants and binding sites for interaction between porphyrins and albumin,[4,9] in resolving the complex fluorescence decay profiles,[5] and in measuring yields for generation of $O_2(^1\Delta_g)$.[5,6] Here, we describe the photochemical and radiolytic properties of a sulfonated zinc porphyrin bound to human serum albumin (HSA) and show that the bound porphyrin can be readily oxidized to the corresponding π-radical cation. In the photochemical studies, both dynamic and static quenching occur according to the nature of the quencher. There is no indication, however, that either the porphyrin triplet excited state or π-radical cation can abstract an electron from amino acids on the adjacent protein.

Experimental Section

Zinc(II) *meso*-tetrakis(2,6-dichloro-3-sulfonatophenyl)porphyrin (ZnTDCSPP) was prepared and purified by a literature method.[10] Elemental analyses, ^{1}H NMR, and reversed-phase TLC studies were consistent with the assigned structure. The compound dissolves readily in water and displays an absorption spectrum[11] typical of this class of zinc porphyrin: λ = 422 nm, ϵ = 69 $\times$ 10^4 M^{-1} cm^{-1}; λ = 556 nm, ϵ = 24 $\times$ 10^3 M^{-1} cm^{-1}; λ = ca. 590 nm, ϵ = 2.1 $\times$ 10^3 M^{-1} cm^{-1}. Human serum albumin (HSA) was obtained from Sigma Chemical (Fraction V, No. A-1887) and defatted according to the method of Chen.[12] Three separate batches of HSA were used without apparent differences. Benzo-1,4-quinone (BQ) (Aldrich Chemical) and 2,6-dimethylbenzo-1,4-quinone (DMBQ) (Aldrich Chemical) were sublimed before use and benzo-1,4-hydroquinone (H_2BQ) (Aldrich Chemical) was twice recrystallized from water. All other reagents were of the highest available commercial purity and were used as received. [It should be noted that ZnTDCSPP possesses an overall formal electronic charge of −4 due to the peripheral sulfonic acid groups. These charges have been neglected throughout the text in order to simplify the representation.]

Absorption spectra were recorded with a Hewlett-Packard 8450A diode array spectrophotometer and fluorescence spectra were recorded with a fully corrected Perkin-Elmer LS5 spectrofluorimeter. Fluorescence lifetimes were measured by the time-correlated, single photon counting technique with a mode-locked, synchronously-pumped, cavity-dumped dye laser as excitation source (λ = 580 nm, response time 270 ps). Laser flash photolysis studies were made with a frequency-doubled, Q-switched Quantel YG481 Nd-YAG laser (λ = 532 nm, pulse duration 10 ns). The laser intensity was attenuated over a factor of 20 with crossed polarizers, and up to 50 individual laser shots were averaged for each measurement. Measurements were restricted to regions where the observed signal showed a linear dependence upon laser energy and the absolute laser intensity was calibrated with respect to zinc(II) *meso*-tetraphenylporphyrin in benzene.[13,14] All solutions were adjusted to possess an absorbance at 532 nm of ca. 0.1 and were purged thoroughly with O_2, N_2, or air according to the experimental needs. Transient absorption spectra were recorded point-by-point, each point being the average of at least 20 separate measurements, and decay kinetics were made by standard computer least-squares iterative procedures.

Yields and decay rates of singlet molecular $O_2(^1\Delta_g)$ were determined by time-resolved luminescence with a Ge photodiode as detector.[15] The solutions were adjusted to possess an absorbance of ca. 0.1 at 532 nm and were purged thoroughly with O_2, N_2, or air. At least 50 separate measurements were averaged for each determination, and the observed decay profiles were analyzed as described before.[6] The detector was calibrated with free-base tetrakis(4-sulfonatophenyl)porphyrin in water as reference material.[16]

Pulse radiolysis experiments were made with a 4 MeV Van der Graaff accelerator delivering 50-ns pulses. The radiation dose was calibrated by thiocyanate dosimetry.[17] Aqueous solutions of ZnTDCSPP (ca. 10^{-4}

(1) Peters Jr., T. *Adv. Protein Chem.* **1985**, *37*, 161.
(2) Means, G. E.; Sollene, N. P.; Mohamed, A. *Adv. Chem. Ser., Modification of Proteins* **1982**, *198*, 325.
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(6) Davila, J.; Harriman, A. *Photochem. Photobiol.* **1989**, *51*, 9.
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(8) It is known[5,6] that $O_2(^1\Delta_g)$ attacks HSA with a bimolecular rate constant of ca. 6 $\times$ 10^8 M^{-1} s^{-1}.
(9) Milanesi, C.; Biolo, R.; Reddi, E.; Jori, G. *Photochem. Photobiol.* **1987**, *46*, 675.
(10) Zipplies, M. F.; Lee, W. A.; Brucie, T. C. *J. Am. Chem. Soc.* **1986**, *108*, 4433.
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(13) Pekkarinen, L.; Linschitz, H. *J. Am. Chem. Soc.* **1960**, *82*, 2407.
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(15) Rodgers, M. A. J.; Snowden, P. T. *J. Am. Chem. Soc.* **1982**, *104*, 5341.
(16) Verlhac, J. B.; Gaudemar, A.; Kraljic, I. *Nouv. J. Chim.* **1984**, *8*, 401.
(17) Harriman, A.; Richoux, M.-C.; Neta, P. *J. Phys. Chem.* **1983**, *87*, 4957.

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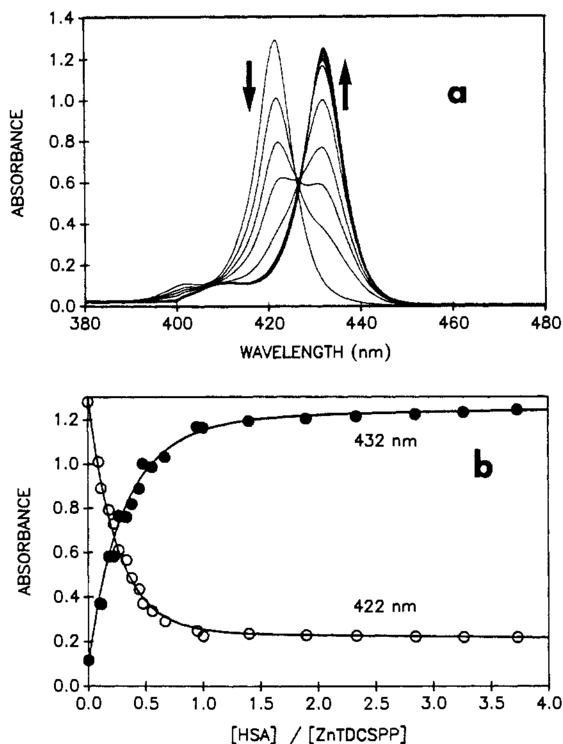


Figure 1. (a) Absorption spectral changes for the Soret region of ZnTDCSPP on addition of successive aliquots of HSA. (b) The same spectral changes expressed in the form of a titration curve: the solid lines have no theoretical significance.

M) containing NaN_3 (10^{-2} M) at pH 7 were purged thoroughly with N_2O. Transient absorption spectra were recorded point-by-point and decay kinetics were analyzed by computer least-squares iterative procedures. All solutions were protected from undue photolysis during the experiments.

The redox potential for one-electron oxidation of ZnTDCSPP (E = 1.02 V vs NHE) was determined by cyclic voltammetry, as described before.[11] When used in conjunction with the measured triplet energy[11] of 1.58 eV, this value suggests that the redox potential for one-electron oxidation of the porphyrin triplet excited state is −0.56 V vs NHE. Remaining redox potentials were taken from the literature:[18] $E°$ values being 0.08, −0.08, and 0.46 V vs NHE for one-electron reduction of BQ and DMBQ and for one-electron oxidation of H_2BQ at pH 7, respectively.

Results and Discussion

Interaction with Human Serum Albumin. At concentrations below 10^{-3} M, ZnTDCSPP exists in aqueous solution in the form of a monomer species as evidenced by NMR and absorption spectral studies. A neutral aqueous solution of ZnTDCSPP (1.85 × 10^{-6} M was titrated with a freshly prepared neutral aqueous solution of HSA and the course of reaction was followed by absorption (Figure 1a) and fluorescence spectroscopy. Identical spectral changes were observed immediately after mixing and after a 24 h incubation period. The most obvious change occurs in the Soret region of the absorption spectrum where the intense band located at 422 nm undergoes a 10 nm red-shift upon addition of HSA. There is a near isosbestic point at 426 nm and the spectral changes are essentially complete upon addition of an equimolar concentration of HSA. There is a corresponding red-shift of 10 nm for the porphyrin visible absorption bands, with a clear isosbestic point at 561 nm.

The spectral changes depicted in Figure 1a can be expressed in the form of a titration curve in which absorbance changes at 422 and 432 nm are plotted as a function of the molar ratio of HSA to ZnTDCSPP (Figure 1b). Uncomplexed dye absorbs predominantly at 422 nm whereas the concentration of complexed dye can be monitored by absorbance changes at 432 nm. The end point for the titration occurs with equimolar concentrations

(18) Wardman, P. *J. Phys. Chem. Ref. Data* In press.

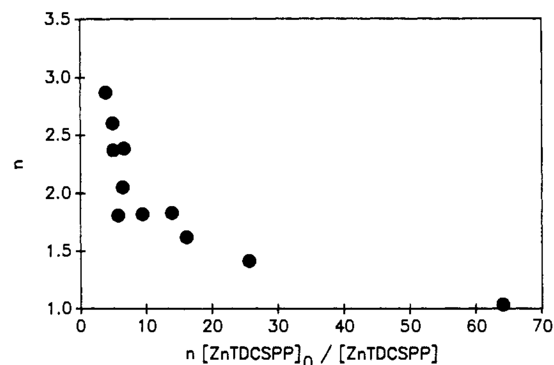


Figure 2. Plot of the absorption spectral data to the Scatchard model:[19] $\cap$ is the relative concentration of complexed ZnTDCSPP per molecule of HSA, $[ZnTDCSPP]_0$ (1.85 × 10^{-6} M) and [ZnTDCSPP] refer to the total and the uncomplexed concentrations of ZnTDCSPP, respectively.

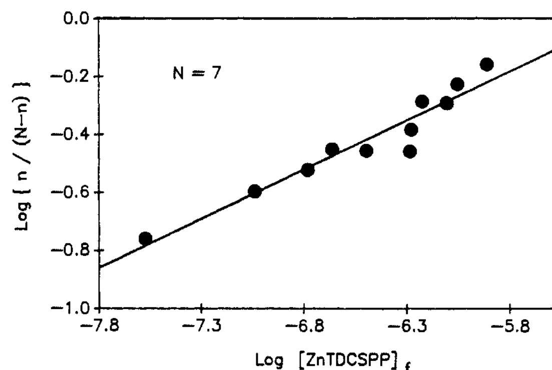


Figure 3. Re-plot of the absorption spectral data in terms of the relative heterogeneity of the system in which $[ZnTDCSPP]_f$ refers to the concentration of free dye (X_f) and with N = 7. The solid curve is a fit to log ($\cap/N - \cap$) versus a log X_f with a = 0.37.

but, from measurements made at 432 nm, there appears to be a further minor spectral change as the molar excess of HSA increases (Figure 1b). The reason for this latter effect is unclear but it should be recalled that ZnTDCSPP is multifunctional and may cross-link protein molecules. Under conditions where only a small fraction of the total concentration of dye is bound to the albumin, the spectral changes are consistent with a model[19] in which the maximum stoichiometry exceeds three molecules of ZnTDCSPP per molecule of protein. As more protein is added to the dye solution, the stoichiometry decreases toward unity without involving well-defined equilibria.

Attempting to analyze the entire titration curves in terms of the Scatchard theory,[19] with its implicit assumption of an unrestricted number of equivalent binding sites on a single protein molecule, was unsuccessful. This theory is based upon there being a single class of binding sites that can accommodate a maximum number N of dye molecules. The theory takes the form of [$\cap = -K_d(\cap/X_f) + N$] where $\cap$ is the relative concentration of bound molecules of dye per molecule of HSA, K_d is the dissociation constant for complexed dye, and X_f is the concentration of free dye. As inferred from Figure 2, this model does not hold for our data since a plot to the above equation is nonlinear. From the plot, N must be greater than 3 and previous studies[4,6] have concluded that up to 7 porphyrin molecules can be accommodated on a single molecule of HSA. Re-plotting the data in the form of [log ($\cap/N - \cap$) = a log $X_f - a$ log K_d] where a is a constant expressing the heterogeneity of the system[19] gives a reasonable fit only for N = 7 (Figure 3), for which a = 0.37. We conclude from these findings that there are at least two non-equivalent binding sites for ZnTDCSPP on each molecule of HSA. The photochemical studies, as described later, require that these sites possess sufficiently similar K_d values for ZnTDCSPP not to complex exclusively to one site when excess HSA is present.

(19) Scatchard, G. *Proc. New York Acad. Sci.* **1949**, *51*, 660.

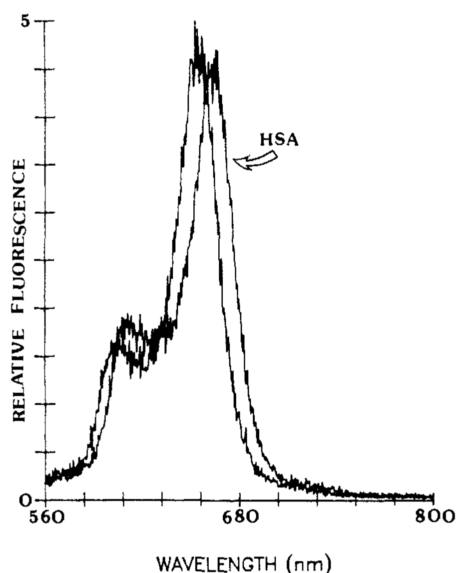


Figure 4. Fluorescence spectra recorded for ZnTDCSPP in dilute aqueous solution and in the presence of a 6-fold molar excess of HSA.

For excitation at 426 nm, titration with HSA causes no apparent changes in porphyrin fluorescence intensity or lifetime but gives an 8 nm red-shift in the peak maxima (Figure 4). During the course of the titration, isoemissive points are maintained at 608, 635, and 667 nm. Again, spectral changes are complete at equimolar concentrations of reagents. For excitation at 278 nm, addition of ZnTDCSPP does not quench fluorescence from the tryptophan residue located on the albumin and an excitation spectrum recorded for the porphyrin emission indicates that excitation energy transfer from tryptophan to ZnTDCSPP does not occur. Consequently, for equimolar solutions the porphyrin is bound to the albumin at a site remote from the tryptophan residue.

For the same titration, the course of reaction was monitored by recording the lifetime of the porphyrin excited singlet and triplet states in neutral, air-equilibrated solution. No changes were observed in the singlet excited state lifetime, as measured by single photon counting methods following laser excitation at 580 nm, which remained at 220 ± 30 ps regardless of the concentration of HSA. The triplet decay profile, as recorded under laser flash photolysis conditions, was found to depend upon the concentration of HSA. Satisfactory analysis of the observed decays required the use of three exponential terms, according to

$$I_t = A_1 \exp(-t/\tau_1) + A_2 \exp(-t/\tau_2) + A_3 \exp(-t/\tau_3) \qquad (1)$$

where A represents the relative amplitude of a component of lifetime τ. The shortest lifetime (τ_1 = 7.4 ± 0.2 μs) corresponded to that derived for ZnTDCSPP in aerated water in the absence of HSA and, accordingly, is attributed to uncomplexed dye. In the presence of HSA, additional triplet lifetimes (τ_2 = 19.4 ± 0.7 μs; τ_3 = 71 ± 3 μs) were observed that are attributed to dye molecules complexed at two distinct sites on the albumin (see later).

The fraction of free ZnTDCSPP compared to the total dye concentration ($A_1/(A_1 + A_2 + A_3)$) was in good quantitative agreement with that derived from the absorption and fluorescence spectral studies. This finding is in accord with the hypothesis that τ_1 can be ascribed to uncomplexed dye whereas both τ_2 and τ_3 can be attributed to bound dye. The ratio A_2/A_3 was 1.9 ± 0.2 at concentrations of HSA up to equimolar, where the contribution of A_1 was negligible, and showed a slight, progressive decrease upon increasing the amount of HSA to a 10-fold molar excess. In N_2-saturated solution, the excited triplet state of the bound dye was found to decay by a monoexponential process (τ = 3.3 ± 0.3 ms). The distinction between the two triplet states, as resolved in air-equilibrated solution, is attributed, therefore, to their relative accessibility to O_2 dissolved in the bulk solution.

The observation that two distinct triplets can be resolved, because of their different rates of reaction with molecular O_2, under conditions where there is a single molecule of ZnTDCSPP per protein molecule can be explained in two ways. First, there could be two distinct binding sites, with similar complexation constants,[19] that differ in their proximity to the bulk aqueous phase. Second, ZnTDCSPP could be complexed at a single binding site which, because of dynamic changes in equilibrium conformations of the protein, experiences two distinct positions relative to the aqueous phase. Since the titration results indicate the presence of at least two non-equivalent binding sites, the former is the more likely explanation for the observed photophysical behavior.

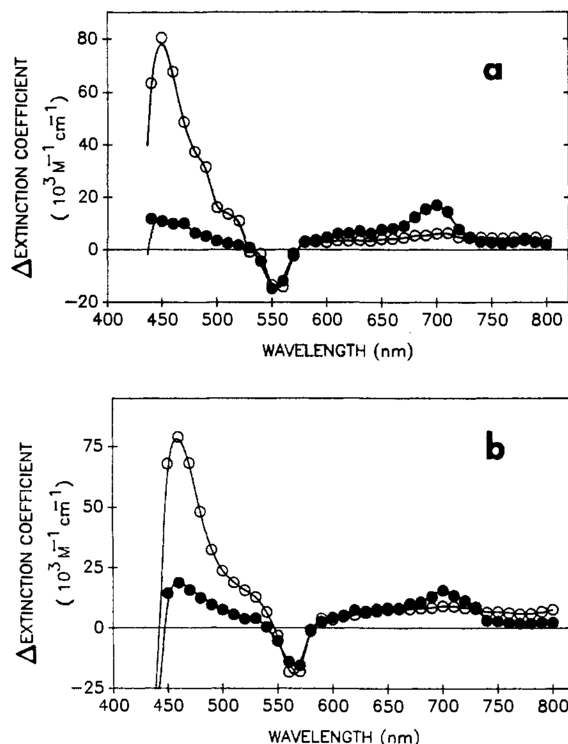


Figure 5. Transient difference spectra recorded (O) for the triplet excited state and (●) for the porphyrin π-radical cation of ZnTDCSPP as observed (a) in water at pH 7 and (b) when complexed to HSA with a 6-fold molar excess of HSA.

From absorption spectral measurements it was found that HSA exhibited a high capacity for BQ, with up to 10 molecules being readily accommodated on a single protein molecule. Association between BQ and amino acid residues on HSA is evidenced[20] by the appearance of a series of intense charge-transfer absorption bands. At least three distinct binding sites could be resolved and there were significant temporal effects whereby particular absorption bands developed over a period of hours. The presence of bound ZnTDCSPP did not prevent binding of BQ onto the same molecule of HSA. In marked contrast, it was found that each molecule of HSA bound a maximum of two molecules of DMBQ. In this latter case, complexation was apparent from the reduced intensity of the DMBQ absorption spectral bands, which were broadened upon binding to HSA, but complexation was not accompanied by the appearance of charge-transfer absorption bands. The different affinities for BQ and DMBQ may arise from steric factors.

Photochemical Studies. Because of the *o*-chlorine substituents, intersystem crossing is extremely effective[21] and the triplet excited state of ZnTDCSPP is formed with a quantum yield of 0.96 ± 0.05. The triplet excited state, which can be monitored by laser flash photolysis, absorbs intensely around 450 nm (Figure 5a) and, at low concentration in deoxygenated aqueous solution, it decays by first-order kinetics with a lifetime of 1.65 ± 0.2 ms. In neutral aqueous solution, the triplet state reacts with molecular oxygen

(20) (a) Moxon, G. H.; Slifkin, M. A. *J. Chem. Soc., Perkin Trans. II* **1972**, 1159. (b) Lorentz, K.; Flatter, B. *Clin. Chem.* **1974**, *20*, 1553.
(21) Solovev, K. N.; Tsvirko, M. P.; Gradyushko, A. T.; Kozhich, D. T. *Opt. Spectrosc.* **1972**, *33*, 881.

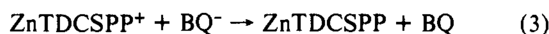
($k = 5.0 \pm 0.4 \times 10^8$ M^{-1} s^{-1}) to form singlet molecular oxygen $O_2(^1\Delta_g)$ with a quantum yield of 0.74 ± 0.07. Binding the dye to HSA does not inhibit triplet state formation, although it does affect the triplet lifetimes, and transient absorption spectra recorded for the bound triplet (Figure 5b) are very similar to those observed in the absence of HSA. Spectra of triplet states formed from ZnTDCSPP molecules resident in the two distinct binding sites on the protein were indistinguishable.[22] Irradiation of bound dye in aerated water generates $O_2(^1\Delta_g)$ with a quantum yield of 0.76 ± 0.09.

Upon prolonged steady-state irradiation ($\lambda > 400$ nm) of ZnTDCSPP in aerated, neutral aqueous solution there is minimal destruction of the porphyrin, as evidenced by absorption spectral measurements during the course of photolysis. From such studies, it was determined that the quantum yield for destruction of ZnTDCSPP was $<10^{-4}$ and, on the basis of this observation, it is concluded that $O_2(^1\Delta_g)$ does not damage the porphyrin nucleus. Similar irradiations of aerated solutions of ZnTDCSPP bound to HSA, under equimolar conditions, resulted in destruction of the protein, as evidenced by loss of absorption spectral bands associated with HSA ($\lambda = 278$ nm) and increased levels of light scattering.[23] Flash photolysis studies, indicated that there were no long-lived transient species present after decay of the porphyrin triplet state that might arise from electron-transfer reactions between the triplet and amino acids on the protein backbone. Since damage to HSA requires the presence of both O_2 and porphyrin and is initiated by visible light it is attributed to attack on the protein by $O_2(^1\Delta_g)$.

In N_2-saturated, neutral aqueous solution containing Na_2SO_4 (0.2 M) as ionic strength mediator, the triplet state of ZnTDCSPP is quenched by both BQ ($k = 4.2 \pm 0.3 \times 10^9$ M^{-1} s^{-1}; $\Delta G° = -0.64$ eV) and DMBQ ($k = 4.0 \pm 0.4 \times 10^9$ M^{-1} s^{-1}; $\Delta G° = -0.48$ eV).[24] The quenching process results in net electron transfer according to

$$\mathrm{ZnTDCSPP^* + BQ \rightarrow ZnTDCSPP^+ + BQ^-} \quad (2)$$

and the zinc porphyrin π-radical cation can be identified easily by its characteristic[25] absorption spectrum (Figure 5a). For both quinones, the π-radical cation decays rapidly ($k = 4.8 \pm 0.9 \times 10^9$ M^{-1} s^{-1}) via diffusion-controlled, reverse electron transfer.

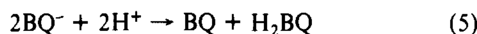


$$\mathrm{ZnTDCSPP^+ + BQ^- \rightarrow ZnTDCSPP + BQ} \quad (3)$$

This latter process is thermodynamically favorable, $\Delta G° = -0.94$ and -1.10 eV respectively for BQ and DMBQ. However, in the presence of excess benzo-1,4-hydroquinone the porphyrin π-radical cation decays via first-order kinetics due to electron abstraction from the added donor.

$$\mathrm{ZnTDCSPP^+ + H_2BQ \rightarrow ZnTDCSPP + BQ^- + 2H^+} \quad (4)$$

The bimolecular rate constant for this process, for which $\Delta G° = -0.56$ eV, was determined to be $8.8 \pm 1.1 \times 10^8$ M^{-1} s^{-1} and, under such conditions, the benzo-1,4-semiquinone π-radical anion decays rapidly via disproportionation.[26]



$$\mathrm{2BQ^- + 2H^+ \rightarrow BQ + H_2BQ} \quad (5)$$

Similar quenching studies were made with ZnTDCSPP bound to HSA, using a 6-fold molar excess of HSA, in N_2-saturated, neutral aqueous solution. It was found that addition of BQ did not affect either the yield or the lifetime of ZnTDCSPP triplet excited state, provided the molar ratio of BQ to HSA was kept below 8:1. Under such conditions, all the BQ is bound to the protein and the absence of quenching suggests that the respective binding sites for BQ and ZnTDCSPP are too far apart for photoinduced electron transfer to occur.[27] Upon increasing the amount of BQ, to give a molar ratio of BQ to HSA of 10:1, the ZnTDCSPP triplet state could no longer be observed by laser flash photolysis and the porphyrin π-radical cation was detected at the end of the laser pulse (Figure 5b).

For molar ratios of BQ to HSA ranging between 8:1 and 10:1 the observed triplet state yield decreased sharply with increasing amount of BQ but the triplet decay profile remained unchanged. Within the accuracy of our measurements, the ratio of the two bound triplets stayed constant. Similarly, the fluorescence yield decreased but τ_s was not affected by the presence of BQ. These experimental findings imply that the additional aliquot of BQ quenches excited state ZnTDCSPP by a static rather than a dynamic process. Presumably, this arises because one or more of the extra BQ molecules bind to the protein at a site near the porphyrin; there is no perturbation of the porphyrin absorption spectrum such that it is unlikely that the BQ molecule responsible for quenching binds directly to the central Zn(II) cation at the center of the porphyrin macrocycle. Fluorescence measurements show that these additional BQ molecules quench less than 20% of the ZnTDCSPP singlet excited state whereas, from the flash photolysis records, the triplet excited state is quenched quantitatively. The inefficient singlet state quenching is attributed to its very short lifetime ($\tau_s = 220 \pm 30$ ps) compared to that of the corresponding triplet state ($\tau_t = 3.3 \pm 0.3$ ms). Since the rate of electron transfer is expected to decrease exponentially with increasing reactant separation distance,[28] effective singlet state quenching requires close contact between reactants whereas triplet quenching can occur over much longer distances.[29]

Decay of ZnTDCSPP π-radical cation, formed from the static quenching process, occurred via second-order kinetics ($k = 1.2 \pm 0.2 \times 10^9$ M^{-1} s^{-1}). This rate constant is significantly lower than found in aqueous solution and the order of reaction implies that one of the redox products is displaced from its binding site into the bulk aqueous phase. One-electron oxidation of ZnTDCSPP has minimal effect upon its structure[30] or overall electronic charge,[31] suggesting that the π-radical cation remains bound to HSA (see later). In contrast, one-electron reduction of BQ converts a neutral molecule into a negatively charged π-radical anion and this change could be sufficient to cause ejection of the anion from the original binding site. As such, regeneration of ground-state species involves interfacial electron transfer between two negatively charged molecules. The rate constant for reverse electron transfer will depend, therefore, upon the ionic strength in the region of the bound π-radical cation and also upon the accessibility of the cation to species in the bulk aqueous phase.

Using a 6-fold molar excess of HSA relative to ZnTDCSPP in N_2-saturated, neutral aqueous solution, the rate constant for deactivation of the porphyrin triplet state was measured as a function of the concentration of added DMBQ. It was found that low concentrations of DMBQ ($<10^{-4}$ M) caused the triplet decay profile to become biphasic. Approximately 65 ± 5% of the total triplet state was quenched by DMBQ, the rate of decay showing a linear correlation with the concentration of added DMBQ, for

(22) The individual triplet spectra were resolved by recording a total triplet absorption difference spectrum in aerated solution and analyzing the decay profile measured at each wavelength as the sum of two first-order rate constants. The rate constants used for the calculation were 5.15×10^4 and 1.41×10^4 s^{-1}. The initial amplitude of each exponential decay component was extrapolated back to the center of the laser pulse, by computer least-squares fitting procedures, and used to construct the individual triplet spectra. Within the accuracy of the technique, the derived spectra were identical.

(23) During photolysis of ZnTDCSPP bound to HSA in aerated solution the position of the Soret band moves progressively, but irreversibly, from 432 to 423 nm. This change, which requires only short irradiation periods, is followed by very inefficient photoreduction of the porphyrin to the corresponding chlorin.

(24) The various $\Delta G°$ values quoted throughout this paper are calculated as the difference between respective redox potentials without correction for electrostatic energy terms.

(25) (a) Neta P. *J. Phys. Chem.* **1981**, *85*, 3678. (b) Harriman, A.; Richoux, M. C.; Neta, P. *J. Phys. Chem.* **1983**, *87*, 4957. (c) Neta, P.; Harriman, A. *J. Chem. Soc., Faraday Trans. 2* **1985**, *81*, 123.

(26) Rao, P. S.; Hayon, E. *J. Phys. Chem.* **1973**, *77*, 2274.

(27) According to ref 3, most small molecules bind to HSA at the two extreme subdomains, which are separated by ca. 8.3 nm. Although the actual binding sites for several molecules, such as aspirin, have been resolved, the location of bound porphyrin molecules remains unknown.

(28) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.

(29) Harriman, A. *Inorg. Chim. Acta* **1984**, *88*, 213.

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(31) The net electronic charge residing on ZnTDCSPP is −4 so that the porphyrin π-radical cation possesses an overall charge of −3.

which the bimolecular quenching rate constant was derived to be $2.8 \pm 0.3 \times 10^7$ M^{-1} s^{-1}. The residual triplet retained a lifetime of 3.3 ± 0.3 ms, regardless of the concentration of added DMBQ, so that the bimolecular quenching rate constant for this triplet is $<10^4$ M^{-1} s^{-1}. Within experimental limits (ca. ±10%), the presence of DMBQ did not affect either the initial yield of triplet or the ratio of quenched to unquenched triplet states.

As with quenching by molecular O_2, these findings imply that there are two types of bound dye which differ by virtue of their accessibility to species dissolved in the aqueous phase. Most (i.e. ca. 65%) of ZnTDCSPP resides in a form that permits dynamic quenching by DMBQ, although the rate is about two orders of magnitude slower than that found for uncomplexed dye, and by O_2. The remaining dye is not readily accessible to DMBQ and reacts only very slowly with molecular O_2. The structure of HSA, which is a predominantly α-helical globin protein, comprises three major domains each of which is made up of two subdomains formed by three or four α-helices linked together by a long section of solvent-exposed (intersub-domain) α-helix.[3] The degree of close-packing of the helices varies throughout the molecule such that many different types of binding site are available. Certainly, the known morphology[3] of HSA is consistent with the type of binding outlined here for ZnTDCSPP.

The dynamic triplet-state quenching of accessible ZnTDCSPP molecules bound to HSA by uncomplexed DMBQ involves net electron transfer, in analogy to reaction 2. The porphyrin π-radical cation can be observed by transient absorption spectroscopy at the end of the quenching process. This species decays via second-order kinetics with a rate constant of $1.3 \pm 0.2 \times 10^9$ M^{-1} s^{-1}. Again, this finding is consistent with interfacial electron transfer between free DMBQ π-radical anion and protein-bound ZnTDCSPP π-radical cation.

Radiolytic Studies. Further information about the reactivity of the porphyrin π-radical cation, isolated from the benzoquinone π-radical anion, was sought from pulse radiolysis experiments. In N_2O-saturated, neutral aqueous solution containing NaN_3 (10^{-2} M), the azidyl radical oxidizes ZnTDCSPP to the porphyrin π-radical cation ($\Delta G° = -0.30$ eV)[32] with a bimolecular rate constant of $2.2 \pm 0.2 \times 10^9$ M^{-1} s^{-1}.

$$\mathrm{ZnTDCSPP} + N_3^{\bullet} \rightarrow \mathrm{ZnTDCSPP}^{+} + N_3^{-} \quad (6)$$

The π-radical cation, which is most conveniently monitored at 700 nm (Figure 5a), is formed with a radiation yield[33] of $G = 6.0$ and decays slowly via second-order kinetics with a first half-life of 5 ± 2 ms. On the basis of earlier work with related zinc porphyrins,[34] this process is assigned to disproportionation, for which the bimolecular rate constant was derived to be $2 \pm 1 \times 10^7$ M^{-1} s^{-1}.

$$2\mathrm{ZnTDCSPP}^{+} \rightarrow \mathrm{ZnTDCSPP} + \mathrm{ZnTDCSPP}^{2+} \quad (7)$$

The resultant porphyrin π-dication decays via first-order kinetics ($k = 10 \pm 3$ s^{-1}) due to nucleophilic attack by water or hydroxide ions.

Similar pulse radiolysis studies were made with ZnTDCSPP bound to HSA, using a 3-fold molar excess of protein. In N_2O-saturated, neutral aqueous solution containing NaN_3 (10^{-2} M), the ZnTDCSPP π-radical cation was formed with $G = 2.2$. This relatively low yield implies that azidyl radicals are lost to competitive dimerization[35] and/or oxidation of amino acid residues in the protein chain.[36] The absorption spectrum assigned to the ZnTDCSPP π-radical cation did not change, in profile or intensity, over 1 s. This finding implies that the π-radical cation remains complexed to HSA and that disproportionation is inhibited by the large diffusion coefficient of the protein.[37] The high stability of the ZnTDCSPP π-radical cation implies that it does not abstract an electron from HSA, at least on these time scales.

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