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1D Radical Motion in Protein Pocket: Proton-Coupled Electron Transfer in Human Serum Albumin

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Proteins and enzymes function by recognizing substrates and, in many biological systems, catalyze chemical reactions at their active regions.[1,2] Considerable interests have been directed to the biological functions of protein dynamics[3,4] and protein−surface interactions.[5−8] The protein surface is complex since many interactions (hydrogen bonding, hydrophobic, hydrophilic, electrostatic, and topological surface interactions) are likely involved in the potential landscape.[9] So far, little is known about how chemical reactions affect the protein−surface interactions and subsequently contribute to ligand motions near or in the binding regions of protein complexes.[9−12]

In this Communication, we employ human serum albumin (HSA) as a model protein to explore protein−surface reactions. Using time-resolved electron paramagnetic resonance (TREPR) spectroscopy, we observe a photoinduced, proton-coupled electron transfer (ET)[13] between 9,10-anthraquinone-2,6-disulfonate ($AQDS^{2-}$) and the amino acid residue of tryptophan in HSA. HSA is the major protein in blood plasma and plays a significant role in transporting several hydrophobic components (fatty acids, hormones, and drugs) by binding them in the protein's pockets.[14] Chemically induced dynamic electron polarization[15] (CIDEP) spectra show that the photoinduced ET takes place from the specific tryptophan residue (W214) to the excited triplet state of $AQDS^{2-}$ bound in a cleft of HSA. Polarized EPR signals clearly demonstrate that the anion radical of the ligand escapes through the pocket toward the bulk water region by undergoing one-dimensional motion to the protein's outer surface.

Figure 1 shows TREPR spectra of HSA (0.2 mM)−$AQDS^{2-}$ (1 mM) in an argon-saturated aqueous solution obtained at (a) 50 ns and (b) 1.5 μs after pulsed laser excitation (355 nm, 5 ns fwhm) of $AQDS^{2-}$ at 294 K. At the initial stage (Figure 1a), a broad emission (E) of microwaves was observed. This emission reveals that the photoexcited triplet state of $AQDS^{2-}$ drives the photoreactions, creating the triplet mechanism[15] (TM) E-CIDEP in free radicals.[16] At 1.5 μs, we obtained emissive CIDEP signals composed of two broad peaks located around $B_0 = 338.5$ and 341 mT. This double-peak feature is different from the signal in Figure 1a. The emission intensity at the lower field is stronger than the higher field emission. This result is explained by the radical pair mechanism[15] (RPM) CIDEP, generating an emission/absorption (E/A)-type signal pattern. The asymmetrical spectral shape in Figure 1a is also explained by the E/A-type RPM. In addition to the broad spectrum in Figure 1b, sharp hyperfine lines are overlapping around 339.1 mT, as enlarged in Figure 1c, and are assigned to the 9,10-anthraquinone-2,6-disulfonate radical anion ($AQDS^{3-\bullet}$).[16] The broad EPR signals in Figure 1a and 1b were assigned to the tryptophan radical cation ($W^{+\bullet}$) and its deprotonated form, neutral radical ($W^{\bullet}$), respectively, by CIDEP simulations (shown by red lines in Figure 1) using reported hyperfine coupling constants (HFCC) of the corresponding tryptophan radicals.[17,18] Since an aromatic anion molecule of warfarin is known to be bound to HSA in subdomain IIA (Figure 1d) by the hydrophobic interaction,[14] $AQDS^{2-}$ is also expected to be bound in subdomain IIA. In the protein unit shown in Figure 1d, there exists only one tryptophan residue (W214). W214 is in close proximity to the ligand, as shown in Figure 1d. The proximity between W214 in HSA (0.1 mM) and $AQDS^{2-}$ (0.1 mM) was confirmed from a broad charge-transfer absorption band by an UV−visible spectrometer since this absorption band was consistent with the absorption spectrum of charge-transfer complex of L-tryptophan−$AQDS^{2-}$ observed in the presence of concentrated L-tryptophan (30 mM) and $AQDS^{2}$ in water (Supporting Information). Therefore, it is concluded, from the TREPR results, that the photoinduced ET takes place from W214 to $^3AQDS^{2-*}$ in domain II, following deprotonation of $W^{+\bullet}$, that is, W214 + $^3AQDS^{2-*} \rightarrow W214^{+\bullet} + AQDS^{3-\bullet}$ and $W214^{+\bullet} \rightarrow W214^{\bullet} + H^{+}$.[19] We have also analyzed time profiles of the TREPR signals at $B_0 = 338.5$ and 341 mT to obtain the deprotonation rate constant and initial TM and RPM spin polarization created in the tryptophan radicals. (See Supporting Information for details.)

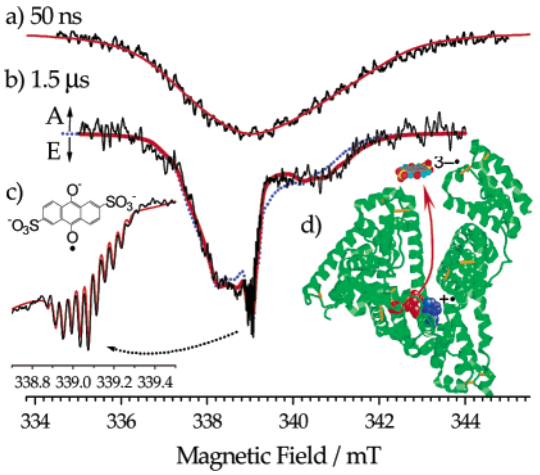

Figure 1. Time-resolved EPR spectra obtained for HSA−$AQDS^{2-}$ system: (a) 50 ns and (b) 1.5 μs after the 355 nm laser irradiation. (c) Enlarged part of 339.1 mT of (b). (d) Ribbon structure of HSA−warfarin complex. This structure was obtained from the Protein Data Bank (ID code 1ha2). The ligand and W214 are shown by red and blue space-filling structures, respectively. CIDEP spectra were fitted, as shown by red lines in (a−c), by considering a one-dimensional diffusion model of eq 1, whereas the conventional 3D diffusion model deviates from the experimental result as denoted by the blue dotted line in (b).

The broad EPR signals of tryptophan radicals indicate that anisotropic hyperfine and Zeeman interactions are not averaged by the protein's rotational motion because of its large molecular size.[20] On the other hand, the sharp hyperfine lines in Figure 1c indicate rapid rotational motion, denoting that the anion radical, $AQDS^{3-\bullet}$, is not bound to the protein but resides in the bulk water region, as shown in Figure 1d. Observation of the RPM contribution supports this result. To generate RPM CIDEP via singlet−triplet ($S-T_0$) mixing in radical pairs (RP), modulation of spin exchange interac-

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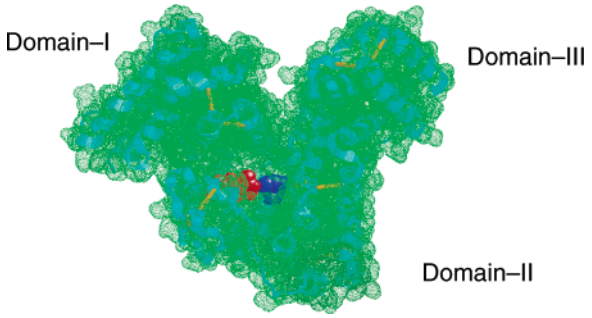

Figure 2. Top view of the protein pocket (space filling model of HSA−warfarin complex of Figure 1). Both the W214 residue (blue) and the ligand (red) at the subdomain IIA are partly visible, indicating that the small radical anion can escape to the bulk water phase through the pocket region after the ET in the HSA−$AQDS^{2-}$ system.

tion ($2J$) is needed[15] and is mediated, in this case, by the translational diffusion of $AQDS^{3-\bullet}$ that ultimately separates the radical ion pairs (RIP) of $W214^{+\bullet}\cdots AQDS^{3-\bullet}$.

According to the RPM theory, CIDEP intensity (P_{RPM}) is dependent on the off-diagonal term (Q) in the spin Hamiltonian under the singlet−triplet ($S-T_0$) representation of the RP. In the conventional RPM model, in which radicals undertake three-dimensional (3D) translation diffusion in fluids, P_{RPM} is expressed[21] as $P_{RPM,3D} = c_0 \cdot \mathrm{sign}(Q) \cdot |Q|^{1/2}$, with $2Q = \beta B_0(g_a - g_b) + \Sigma_m A_{a,m} M_{I,m} - \Sigma_n A_{b,n} M_{I,n}$, where B_0, g, A, and M_I are the magnetic field, the g-values, the HFCCs, and the nuclear spin quantum numbers, respectively, for a and b radicals in the RIP. Just recently, Shushin et al. have modeled the RPM for the case that the radicals undertake one-dimensional (1D) diffusion and found that the P_{RPM} is not proportional to $|Q|^{1/2}$ but only depends on the sign of the Q of the triplet precursor reaction systems[22]

$$P_{RPM,1D} = c_1 \cdot \mathrm{sign}(Q) \qquad (1)$$

Simulations of CIDEP spectra were performed using the 3D and 1D diffusion models, respectively, taking into account the anisotropic hyperfine and **g**-tensors in the tryptophan radicals for the computations of the P_{RPM} and the resonance fields, B_0, both of which are dependent on the orientation of W214 along the B_0 direction (Supporting Information). In $AQDS^{3-\bullet}$, only the isotropic magnetic terms were considered. The emissive contributions from the TM were added to the E/A-type RPM spectra calculated from the P_{RPM} (with the 1D and the 3D models) to reproduce the experimental spectra. The mixture of the TM and the 3D RPM was not capable of reproducing the experimental spectrum (best fit least-squares, blue line in Figure 1b). On the other hand, the experimental results were almost perfectly reproduced, as shown by the red lines in Figure 1, when the 1D diffusion model of eq 1 was utilized.

The combined observations show that the anion radical of $AQDS^{3-\bullet}$ has escaped from the binding region via a restricted 1D motion quite different from the molecular diffusion in the bulk water. Therefore, the RPM CIDEP signals reflect the ligand motion that the RIPs undergo efficient reencounter processes after sufficient-triplet mixings inside the protein's pocket located near W214, as shown in Figure 2 (Supporting Information). This conclusion is consistent with diffusion in a pore size (ca. 2 nm depth of ca. 0.7 nm diameter, as estimated from the X-ray crystal structure[23] of the HSA−warfarin complex[24]) near the W214 region since the effective, center-to-center radical separation is required to be around 1 nm for generation of the RPM.[25] The binding affinity in the domain region has been switched by the photoinduced ET reaction. This result may be explained by switching from the hydrophobic protein binding to the hydrophilic hydration toward the water phase since the carbonyl component in $AQDS^{2-}$ has been changed to the ionic structure via the ET reaction.

In summary, we have observed the ligand motion in a specific protein pocket area (Figure 2) characteristic of 1D diffusion using TREPR spectroscopy. Since $AQDS^{2-}$ is bound to the tryptophan, the present experimental method may be a powerful tool to explore not only characteristics of proteins' local surface structures but also their dynamic biological functions mediated by specific ligand−residue reactions and by subsequent releases of the ligand molecules. This method can therefore be used to investigate drug delivery and protein−substrate catalysis.

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Supporting Information Available: UV−visible spectra of the HSA−$AQDS^{2-}$ and the L-tryptophan−$AQDS^{2-}$ systems. Details of the TREPR experiments, time profile analysis, CIDEP simulations, and inferred 1D motional character in the protein pocket are presented. This material is available free of charge via the Internet at http://pubs.acs.org.

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