DOI: 10.1002/asia.200800114

A Femtosecond Study of the Interaction of Human Serum Albumin with a Surfactant (SDS)

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Dedicated to Professor Ryoji Noyori on the occasion of his 70th birthday

Abstract: The interaction of a protein, human serum albumin (HSA) with a surfactant (sodium dodecyl sulfate, SDS) was studied by femtosecond upconversion. HSA was labeled covalently with a probe (CPM, 7-dimethylamino-3-(4-maleimidophenyl)-4-methylcoumarin). Binding of SDS to HSA is found to accelerate the solvation dynamics ~1.3-fold. The solvation dynamics in HSA displays two time components: 30 ps (20%) and 800 ps (80%). When ~10 SDS molecules bind to HSA the components are 15 ps (40%) and 800 ps (60%). It is argued that SDS may increase the solvent exposure of the probe (CPM); it may also displace the buried water molecules in the immediate vicinity of CPM.

Keywords: femtosecond solvation dynamics · fluorescent probes · proteins · surfactants

Introduction

Human serum albumin (HSA) is synthesized in the liver. It binds to a fairly diverse range of drugs, un-esterified fatty acids, and various organic compounds; it therefore acts as an almost universal carrier for many important molecules. HSA consists of 585 amino acids with an average molecular weight (M_r) of 65 kDa.[1] HSA is asymmetric in structure, with six sub-domains assembled to a nearly heart-shaped structure. Its shape resembles a rough equilateral triangle with an edge length (l) of ~80 Å and a depth (d) of ~30 Å.[1] There is a long-standing interest in the binding of long-chain fatty acid and surfactant molecules to HSA.[2–5] Binding of a surfactant to a protein plays an important role in molecular recognition at cell surfaces and surfactant-induced denaturation of a protein during gel electrophoresis.[2–7] HSA has been reported to have five principle binding sites for a long- or medium-chain fatty acid, three of which bind quite strongly.[2] Curry et al. carried out a detailed crystallographic study on the binding of a long-chain fatty acid (myristic acid) to HSA.[3] They reported three domains (I, II, and III) as the binding sites. Gelamo et al. studied binding of sodium dodecyl sulfate (SDS) to HSA using CD and molecular modeling. According to them, even at a very low SDS concentration (~100 μM) about 10 molecules of SDS remain bound to each protein molecule.[4] HSA contains 35 cysteine moieties with 17 disulfide bridges (Cys–Cys), and only one free thiol (Cys34).[1] The lone free cysteine (Cys34) is located in domain I. Therefore, if the free cysteine SH group is labeled by a solvation probe it will reside close to a surfactant binding site and report the effect of SDS binding to HSA.

Herein we report our study of the effect of SDS binding to the hydration layer of HSA using femtosecond solvation dynamics. For this purpose, we covalently attached a fluorescent probe at the free thiol group (Cys34) of the protein. The covalent attachment ensures that the probe is not relocated when the surfactant binds to the protein. Buzady et al. previously studied solvation dynamics in an acrylodan-labeled HSA using a transient absorption technique.[6] They detected a 3-ps component of solvent relaxation. However, they did not look for a possible slower component. Earlier we studied solvation dynamics in the HSA/SDS system using a noncovalent probe and a picosecond setup.[7]

The dynamics of water in the hydration layer of proteins has received a great deal of attention recently. In bulk water, solvation dynamics is very fast, with a major sub-picosecond (<0.1 ps) component.[8] However, many proteins and organized assemblies display a substantially slow component.[9–14] Herein we show that the local environment in

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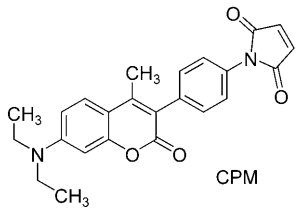
the hydrophobic pocket around the fluorescent probe 7-dimethylamino-3-(4-maleimidophenyl)-4-methylcoumarin (CPM) is modified as a result of the binding of the surfactant molecule. We used a very low concentration of SDS



(150 μM), much lower than its critical micellar concentration (CMC) of 8000 μM. At 150 μM SDS, the protein essentially retains its three-dimensional structure.[5a]

Results

Steady-state spectra

Figure 1a shows the emission spectrum of the solvation probe (CPM) covalently attached to HSA in 0.1 M phosphate buffer. The CPM–HSA conjugate exhibits an absorption maximum at ~390 nm and an emission maximum at 460 nm. With the addition of SDS, both the absorption and emission maxima of HSA-bound CPM undergoes a red shift (Figure 1). With HSA/SDS at a molar ratio of 1:10, with nearly 10 anionic SDS molecules bound to the positively charged protein, the emission maximum is red shifted by ~3 nm (Figure 1b, inset). The red shift suggests that in the presence of SDS, the microenvironment of CPM with HSA becomes more polar than that with HSA alone.

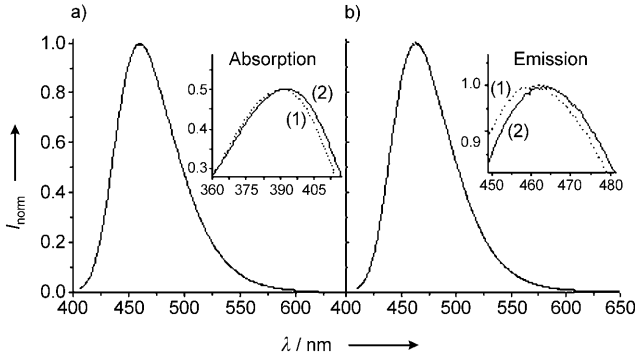


Figure 1. Emission spectra of CPM in the CPM–HSA complex (15 μM) in the a) absence and b) presence of SDS (150 μM) at an excitation wavelength of 405 nm. The insets show the absorption and emission peaks for (1) HSA (-----) and (2) HSA/SDS (——).

Anisotropy decay

In water, a coumarin dye (coumarin 480) shows a fast anisotropy decay with a time constant of ~70 ps.[15] Anisotropy decay of CPM was recorded in methanol because CPM is almost insoluble in water. Figure 2a shows the anisotropy decay of CPM in methanol as well as that of CPM bound to HSA. From Figure 2a it is evident that the anisotropy decay

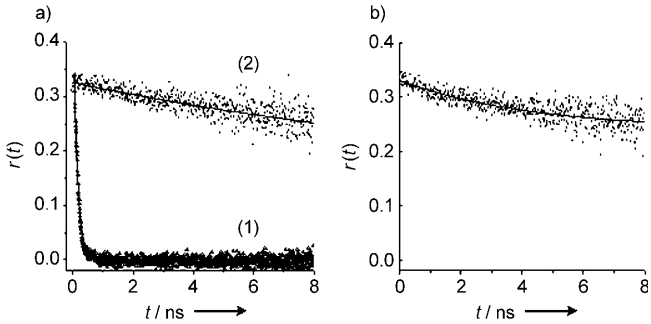


Figure 2. Fluorescence anisotropy decay (λ_{ex} = 405 nm, λ_{em} = 460 nm): a) (1) CPM in methanol and (2) CPM bound to HSA; b) CPM bound to HSA (15 μM) in 150 μM SDS. Points denote experimental data, and the line represents the best fit to a bi-exponential decay.

of CPM bound to HSA is much slower than that of CPM in methanol. The high initial anisotropy value also suggests that the picosecond setup captures almost the entire rotational dynamics for CPM bound to HSA (and HSA/SDS, Figure 2a and 2b).

In methanol, CPM exhibits a fast anisotropy decay with a time constant of ~100 ps (Figure 2a). The anisotropy decay of CPM bound to HSA displays a major component >10 ns. The slow component (>10 ns) of anisotropy decay may be attributed to the large volume and long tumbling time of HSA. HSA is a nearly heart-shaped molecule that can be approximated to an equilateral triangle with dimensions of 80 Å for each of the three sides, and a thickness of 30 Å.[1] For a probe covalently attached to HSA, the time constant (τ_P) of the overall rotation or tumbling is given by:

$$\tau_P = \frac{\eta V}{k_B T} \quad (1)$$

for which η is the solution viscosity and V is the volume of HSA. This corresponds to a rotational relaxation time of ~20 ns. A similar slow rotational relaxation time (>20 ns) was reported earlier for HSA in the case of a noncovalent probe (anilinonaphthalene sulfonate, ANS),[17a] a Ru^{II} complex,[17b] and for erythrosin bound to BSA.[17c]

The detection of such a long component of anisotropy decay (20 ns) requires a dye with a fluorescence lifetime on a similar order (~20 ns).[17a] Unfortunately, the lifetime of the covalent CPM probe is too short (~3.5 ns). Because of the very low fluorescence intensity of CPM at long times (>10 ns) the signal-to-noise ratio is not very good for CPM bound to HSA or HSA/SDS (Figure 2). Thus the long component of anisotropy (~20 ns) could not be accurately determined for CPM bound to HSA (or HSA/SDS). To get a rough approximation, both the anisotropy decays (with

HSA and HSA/SDS) were fitted to a bi-exponential decay with a relatively fast component (1.35 ns for HSA and 0.7 ns for HSA/SDS) and a long component of ~20 ns.

In summary, CPM bound to HSA exhibits a very slow (>10 ns) anisotropy decay, which is much slower than that of CPM in methanol (~100 ps or 0.1 ns). The very slow anisotropy decay suggests that the signal arises from CPM bound to HSA and contribution of free CPM (if any) in bulk water is negligible.

Time-resolved studies: picosecond and femtosecond decays

The fluorescence transients of CPM in CPM–HSA complex both in the presence and absence of SDS were found to be wavelength dependent (Figure 3 and 4). For both systems, a rise precedes the decay at the red end. No such rise is observed at the blue end, and only a decay is detected. Such wavelength dependence of emission decays, and in particular the rise, is clear proof of solvation dynamics. It should be mentioned that we did not detect any rise component in our picosecond setup (Figure 3). This is reasonable because the rather large CPM probe is bound to an exposed site (Cys34).

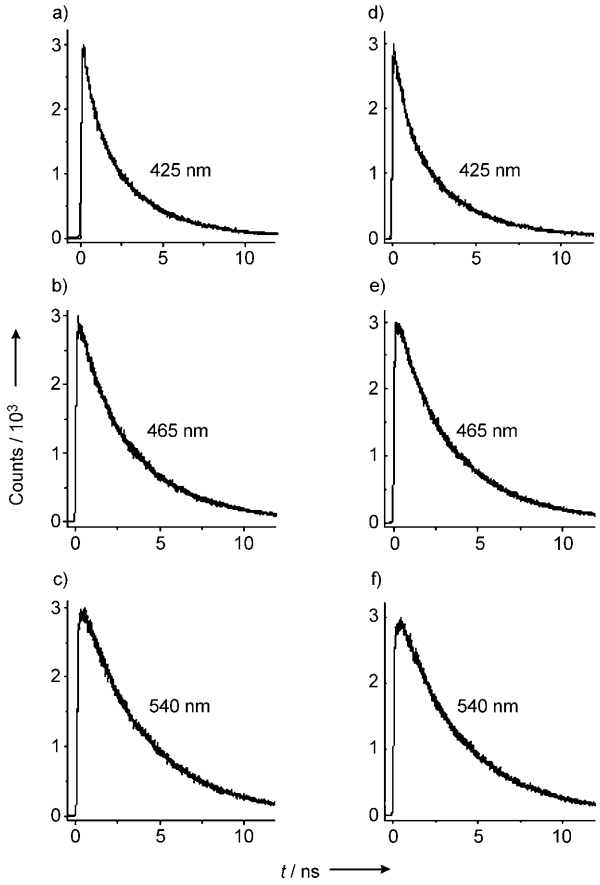


Figure 3. Picosecond decay of CPM ($\lambda_{ex}=405$ nm) in CPM–HSA complex (50 μM) in the a–c) absence and d–f) presence of SDS (150 mM). Values of λ_{em} are indicated.

In the case of CPM bound to HSA, the femtosecond fluorescence transient at 425 nm (blue end) exhibits three decay components: 2, 22, and 1920 ps (Figure 4). In the absence of SDS, a rise component (2.2 ps) precedes long decay components (300 and 3600 ps) at the red end (emission wavelength of 540 nm; Figure 4). Upon addition of SDS to the CPM–HSA aggregate, the corresponding decay components at the blue end are 3.3, 30, and 1900 ps, and at the red end (540 nm) there is a single rise component (1.2 ps) with two long decay components (57 and 3800 ps).

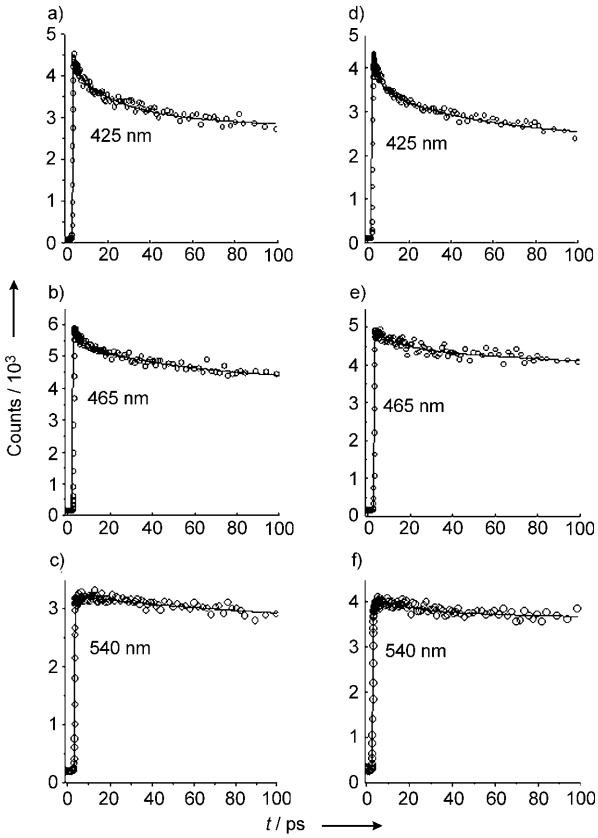


Figure 4. Femtosecond transients of CPM ($\lambda_{ex}=405$ nm) in CPM–HSA complex in the a–c) absence and d–f) presence of SDS (150 mM). Values of λ_{em} are indicated.

Figure 5 shows the time-resolved emission spectra (TRES) of C480 in CPM–HSA in the absence and presence of SDS. The total dynamic Stokes shifts ($DSS=\nu(0)-\nu(\infty)$) in CPM–HSA and CPM–HSA/SDS complexes were found to be 400 and 500 cm^{-1}, respectively (Table 1). Figure 6 shows the decays of the solvent response function, $C(t)$, for the CPM–HSA complex in the absence and presence of SDS, and Table 1 summarizes the decay parameters of $C(t)$.

Discussion

The main findings of this work are as follows: Solvation dynamics for HSA and HSA/SDS are significantly slower than

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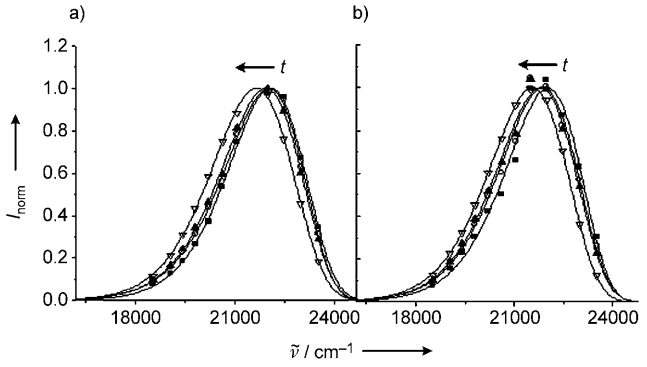

Figure 5. Time-resolved emission spectra (TRES) of CPM ($\lambda_{ex}=405$ nm) in CPM–HSA complex in the a) absence and b) presence of SDS (150 mM) at 0 (■), 24 (○), 500 (▲), and 3500 ps (▽).

Table 1. Parameters obtained from the decay of $C(t)$ for CPM in various systems at 295 K

System	$\Delta\nu_{obs}$ [cm^{-1}][a]	a_1	τ_1 [ps][a]	a_2	τ_2 [ps][a]	$\langle\tau\rangle$ [ps][b]
CPM–HSA	400	0.20	30	0.80	800	650
CPM–HSA + SDS[c]	500	0.40	15	0.60	800	500

[a] SE ± 10%. [b] $\langle\tau\rangle = a_1\tau_1 + a_1\tau_2$. [c] 15 μM CPM–HSA conjugate; 150 μM SDS.

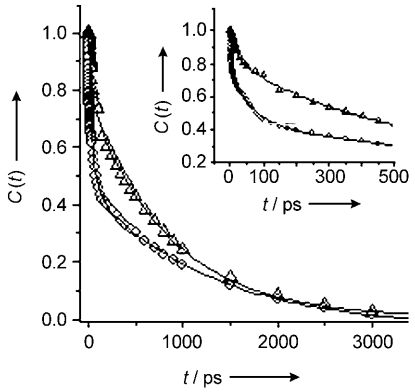

Figure 6. Complete decay of solvent response function $C(t)$ of CPM in CPM–HSA complex for $\lambda_{ex}=405$ nm in the absence (△) and presence (○) of 150 mM SDS. The points denote the actual values of $C(t)$, and the solid lines denote the best fit. Initial portions of the decays are shown in the inset.

that in bulk water (<1 ps). The average solvation time for HSA/SDS is 500 ps, which is ~30% faster than that for HSA (~650 ps, Table 1). The anisotropy decay of CPM bound to HSA exhibits a very long component (>20000 ps) and becomes faster with the addition of SDS. The time scales of solvation and anisotropy decays are very different and hence, they originate from different kinds of motion. The anisotropy decay arises from the motion of the CPM probe. The solvation dynamics may be attributed to the motion of the polar entities (water molecule, polar residues, and head groups of SDS). The major source of the ultraslow components of solvation (30 and 800 ps in the case of HSA) is the strong hydrogen bond as well as other electrostatic interactions in the protein.[11–14] As noted in many recent simulations, such interactions lead to the formation of a hydrogen bond network around a protein, stabilized by quasi-stable hydrogen bonds ("pinning sites").[11–14] The hydrogen bond network and bound-to-free interconversion gives rise to anomalously slow dynamics of the structured and quasi-bound water molecules ("biological water") around a protein.[11–14]

Interestingly, the binding of SDS at a site near the probe (CPM) leads to a red shift of the emission spectra and acceleration of the anisotropy decay and solvation dynamics. Because the probe is covalently bound to the protein, relocation of the probe on binding of SDS can be ruled out. The red shift of the emission maximum may arise from the increase in local polarity of the microenvironment because of the presence of the ionic head group of SDS and the counterions. The ~30% acceleration of solvation dynamics may be explained as follows: First, SDS seems to displace the tightly bound slow water from the immediate vicinity of the probe and as a result, contribution of the slow water molecules decreases. Second, SDS may change the local conformation of the side chain, making the CPM probe more exposed to bulk water.

Conclusions

This work demonstrates the presence of slow water molecules inside HSA near the Cys34 residue, to which a solvation probe is covalently bound. Binding of SDS near this site causes an acceleration of the anisotropy decay and solvation dynamics. This is attributed to displacement of the slow and buried water molecules by SDS and the increased exposure of the probe.

Experimental Section

Laser-grade CPM (7-dimethylamino-3-(4-maleimidophenyl)-4-methylcoumarin, Exciton) and sodium dodecyl sulfate (SDS, Aldrich) were used as received. Human serum albumin (HSA) was purchased from Sigma and used without further purification. The steady-state absorption and emission spectra were recorded with a Shimadzu UV-2401 spectrophotometer and a Spex FluoroMax-3 spectrofluorimeter, respectively.

The CPM–HSA complex was prepared according to the method reported by Wang et al., with minor modifications.[18a] A stock solution containing HSA (75 μM) was prepared in 0.1 M phosphate buffer (pH 7.0). A sufficient quantity of CPM (in the minimum amount of *N,N*-dimethylformamide) was added to 10 mL of this solution to give a molar ratio of HSA to CPM of 1:1. The mixture was stirred gently and maintained at room temperature for 15 h. It was then dialyzed for 4 days at 4 °C against 500 mL phosphate buffer (0.1 M) with a change of dialysis buffer after every 12 h. The labeled protein was then passed through a G-50 Sephadex column to dislodge any unreacted label. The concentration of labeled protein was measured by the method of Lowry et al.[18b] The labeling efficiency was determined to be 85%. During our experiment, however, the concentration of labeled protein was kept fixed ~15 μM. We used SDS at a concentration of 150 μM, which is 10-fold greater than that of the protein (HSA) and ~50-fold less than the CMC of SDS (8000 μM).

In our femtosecond up-conversion setup (FOG 100, CDP) the sample was excited at 405 nm. Briefly, the sample was excited using the second harmonic of a mode-locked Ti sapphire laser at a repetition rate of 80 MHz (Tsunami, Spectra Physics), pumped by 5-W Millennia (Spectra Physics). The fundamental beam was frequency-doubled in a nonlinear crystal (1 mm BBO, $\theta = 25°$, $\phi = 90°$). The fluorescence emitted from the sample was up-converted in a nonlinear crystal (0.5 mm BBO, $\theta = 38°$, $\phi = 90°$) using a gate pulse of the fundamental beam. The up-converted light was dispersed in a monochromator and detected with photon-counting electronics. A cross-correlation function obtained by using the Raman scattering from ethanol displayed a full width at half-maximum (FWHM) of 350 fs. The femtosecond fluorescence decays were fitted using a Gaussian shape for the excitation pulse.

Knowledge of the long decay components is required for fitting the femtosecond data. The components were detected using a picosecond setup in which the samples were excited at 405 nm using a picosecond diode laser (IBH Nanoled-07) in an IBH Fluorocube apparatus. The emission was collected at a magic-angle polarization using a Hamamatsu microchannel plate (MCP) photomultiplier (5000U-09). The time-correlated single-photon counting (TCSPC) setup consisted of an Ortec 9327 CFD and a Tennelec TC 863 TAC. The data were collected with a PCA3 card (Oxford) as a multichannel analyzer. The typical FWHM of the system response using a liquid scatterer is ~90 ps. The fluorescence decays were deconvoluted using IBH DAS6 software.

To fit the femtosecond transient, we first determined the long picosecond components by deconvolution of the picosecond decays. The long picosecond components were then kept fixed to fit the femtosecond data. The time-resolved emission spectra (TRES) were constructed by using the parameters of best fit to the fluorescence decays and the steady-state emission spectrum following the procedure described by Maroncelli and Fleming.[16] The solvation dynamics are described by the decay of the solvent correlation function $C(t)$, defined as:

$$C(t) = \frac{\nu(t) - \nu(\infty)}{\nu(0) - \nu(\infty)} \tag{2}$$

in which $\nu(0)$, $\nu(t)$, and $\nu(\infty)$ are the peak frequencies at time 0, t, and ∞, respectively.

To study fluorescence anisotropy decay, the analyzer was rotated at regular intervals to get perpendicular ($I_\perp$) and parallel ($I_\parallel$) components. The anisotropy function $r(t)$ was then calculated using the formula:

$$r(t) = \frac{I_{//}(t) - GI_\perp(t)}{I_{//}(t) + 2GI_\perp(t)} \tag{3}$$

The G value of the picosecond setup was determined by using a probe with very rapid rotational relaxation, such as coumarin 480 in methanol. The G value was found to be 1.5.

Acknowledgements

Thanks are due to the Department of Science and Technology, India (Project Number: IR/I1/CF-01/2002 and J. C. Bose Fellowship) and the Council for Scientific and Industrial Research (CSIR) for generous research support. U.M., S.G., A.A., and S.D. thank CSIR for awarding fellowships. K.B. thanks Professors B. Bagchi and B. Bhattacharyya for many illuminating discussions.

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Received: March 19, 2008
Revised: May 4, 2008
Published online: July 30, 2008

