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SHORT COMMUNICATION

Combined Quenching Mechanism of Anthracene Fluorescence by Cetylpyridinium Chloride in Sodium Dodecyl Sulfate Micelles

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Abstract The Stern-Volmer quenching constant (K_{SV}) for quenching of anthracene fluorescence in sodium dodecyl sulfate (SDS) micelles by pyridinium chloride has been reported previously to be 520 M^{-1} based on steady state fluorescence measurements. However, such measurements cannot distinguish static versus dynamic contributions to the overall quenching. In the work reported here, the quenching dynamics of anthracene in SDS micelles by cetylpyridinium chloride (CPC), an analogue of pyridinium chloride, were investigated using both steady state and time resolved fluorescence quenching. Concurrent measurement of the decrease in fluorescence intensity and lifetime of anthracene provide a quantitative evaluation of collision induced (i.e. dynamic) versus complex formation (i.e. static) quenching of the anthracene fluorophore. The results reveal that a combined quenching mechanism is operative with approximately equal constants of 249±6 M^{-1} and 225±12 M^{-1} for dynamic and static quenching, respectively.

Keywords SDS · Anthracene · Alkylpyridinium chloride · Time-resolved fluorescence quenching · Surfactant micelles

Introduction

Understanding micellar microenvironment properties are important for surfactant applications in industry. The aggregation number, size, shape, microviscosity, and micropolarity of a micelle are characteristic features of its microenvironment and can vary substantially with conditions. These and other important properties associated with micelle microenvironment have been shown to be effectively probed using fluorescence quenching measurements, starting in 1978 when Turro and Yekta published a method utilizing fluorescence quenching to determine aggregation number for SDS micelles [1]. Numerous studies have since shown that fluorescence quenching is a powerful tool in the investigation of micelles and their microenvironment for elucidation of aggregation number, microviscosity, and additive effects [2–4].

Polycyclic aromatic hydrocarbons (PAHs) are known to strongly partition into micellar cores, and as a result, are often used as micellar probes in fluorescence quenching studies [5–7]. Alkylpyridinium halides are known to quench PAHs by photoinduced-electron transfer [8] in which the electron donor can be either the excited fluorophore or the quencher [9]. Dynamic quenching occurs when the quencher diffusively encounters a fluorophore that is in the excited state. In static quenching, a nonfluorescent complex is formed between the ground state fluorophore and the quencher.

Dynamic and static quenching both exhibit a linear dependence on quencher concentration. Often, both types of quenching can occur simultaneously within a single sample [6, 7, 10]. In such cases, the so-called Stern-Volmer (S-V) plot (I_0/I vs. [Q]) [11] shows upward curvature due to the second-order dependence on [Q] [12, 13]. The data in these cases can be fit to a quadratic equation that results from multiplying the S-V linear responses for dynamic and static quenching:

$$\frac{I_0}{I} = (1 + K_D[Q])(1 + K_S[Q]) = 1 + (K_D + K_S)[Q] + K_D K_S [Q]^2 \quad (1)$$

This situation is often encountered in micellar systems due to the different locations available for fluorophore residence after partitioning and quencher association [6, 7, 12, 13]. For example, a fluorescent probe can be located in or near the Stern layer or deep in the core of the micelle. Alternately, the quencher might be associated with the micelle exterior by electrostatic interaction, incorporated into the interior of the

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micelle, or partitioned between these two domains. All of these possibilities lead to different fluorescence quenching behavior as discussed by Kalyanasundaram [14]. The absolute values of the static and dynamic quenching constants provide insight into the locations of the probe and quencher and the local environments in which each resides.

In a micellar system, dynamic quenching is predominant when both quencher and fluorophore are free to diffuse in a micelle within which both species are co-solubilized, or when the quencher diffuses from bulk solution to the exterior of the micelle. Conversely, static quenching is observed when the quenching event is rapid relative to the fluorescent lifetime, as when quencher and fluorophore are co-confined in a small volume, such as within a specific domain of a micelle [15]. Depending on the dominant mechanism, information about the microenvironment of the micelle can be extracted such as reactant mobility, relative free volume, and chemical effects of the micelle environment on the quenching reaction.

It is significant that static quenching is not time-dependent; fluorescence is observed only from fluorophores that are not near a quencher throughout the experiment. As a result, the average lifetime of the fluorophore is unperturbed, and the ratio of fluorescence lifetime in the presence of quencher (τ) is identical to that in the absence of quencher (τ_0):

$$\frac{\tau_0}{\tau} = 1 \tag{2}$$

In contrast, for dynamic quenching, the average fluorescence lifetime decreases at the same rate as the intensity [16]:

$$\frac{\tau_0}{\tau} = \frac{I_0}{I} \tag{3}$$

Thus, the most straightforward way to confirm the contributions of one or both mechanisms to fluorescence quenching is to acquire fluorescence lifetime data in addition to steady-state fluorescence data.

The goal of the current work is to elucidate the contributions of static and dynamic quenching of anthracene fluorescence by a pyridinium quencher in SDS micelles. This work extends the earlier studies of Pownall and Smith wherein the quenching behavior of anthracene in ethanol and detergent micelles was reported [5]. In ethanol, both pyridinium chloride (PC) and cetylpyridinium chloride (CPC) quench anthracene in a diffusion-controlled dynamic process resulting in comparable K_{SV} values of 42±1 and 45±0.5 M^{-1}, respectively [5]. However, the quenching of anthracene fluorescence by PC in SDS micelles is characterized by a greatly increased K_{SV} value of 520±20 M^{-1}. This increase was attributed to electrostatic attraction between the negatively charged SDS micelles and the positively charged pyridinium quenchers [5]. (K_{SV} is used throughout this work to correspond to a quenching process of unspecified mechanism; K_D and K_S are used for dynamic and static quenching, respectively.) Since only steady-state fluorescence intensity data were acquired in this previous work, Pownall and Smith could not differentiate between static and dynamic contributions to the overall quenching of anthracene fluorescence in SDS micelles [5]. Thus, to expand our understanding of fluorescence quenching in anthracene-alkylpyridinium chloride systems in SDS, steady state fluorescence quenching (SSFQ) and time resolved fluorescence quenching (TRFQ) measurements are performed on anthracene in SDS micelles with CPC as a quencher with explicit determination of values for static (K_S) and dynamic (K_D) quenching constants.

Material and Methods

Materials

Cetylpyridinium chloride (CPC), sodium dodecylsulfate (SDS, ~99 %), and anthracene (sublimed grade ≥99 %) were purchased from Sigma-Aldrich. SDS and CPC were purified by recrystallization from ethanol; anthracene was used as received. Absolute ethanol (Decon Laboratories Inc., 200 proof) was used for quenching studies in homogenous solutions and to introduce anthracene to the aqueous micelle solutions. Water was purified with a Milli-Q UV Plus System (Millipore Corp.) with a final resistivity and total organic content of 18 MΩ/cm and <6 ppb, respectively.

Purified CPC and SDS stock solutions were prepared each day in pH 8, 10 mM phosphate buffer. After dissolution, the aqueous solution pH was verified to be 8.0±0.1, and if necessary, adjusted with dilute NaOH or HCl. The stock solutions were then filtered sequentially through Acrodisc® 25 mm syringe filters with 0.45 μm, 0.2 μm, and 0.1 μm Supor® membranes (Pall Corporation). Solutions were prepared by adding an appropriate amount of ~1 mM anthracene in ethanol to a Teflon vial. The ethanol was allowed to evaporate before the SDS stock solution was added. The final solution contained [SDS micelle]:[anthracene] ~100 assuming an aggregation number of ~60 for SDS micelles [17]. After the solution was prepared, the headspace was purged with Ar, the vial was tightly capped, and the mixture gently stirred for 30 min to allow anthracene to partition into the SDS micelles. Partitioning was initially monitored by observing an increase in the anthracene fluorescence signal; the signal was observed to maximize after stirring in the surfactant solution for ~20 min indicating that complete partitioning had occurred.

Fluorescence Quenching

All fluorescence measurements were made using a Photon Technologies Inc. (PTI) Quanta Master 40 spectrofluorometer

system equipped to perform both steady-state (SSFQ) and time-resolved fluorescence quenching measurements (TRFQ). Temperature was kept constant at 20 °C with a TLC 50™ Quantum Northwest thermoelectric temperature control system, and the sample chamber purged with a steady stream of Ar. Aliquots of the quencher were added to the micelle/fluorophore solution and allowed to stir for 5 min. Intensity and lifetime measurements were performed sequentially before the next aliquot of quencher solution was added. A Xe arc lamp provided a λ_{ex} of 355 nm for SSFQ measurements, and a 370 nm MHz PTI pulsed diode laser was used for TRFQ studies. Fluorescence intensities at a λ_{em} of 402 nm were used in both experiments, corresponding to the $\nu_{1\rightarrow0}$ transition for anthracene. Corrections for dilution of SDS and anthracene were deemed unnecessary as the total volume of quencher added was only ~5 % of the total solution volume. Inner filter effects were evaluated using absorbance measurements and found to be negligible for the concentrations used. The instrument response function (IRF) for TRFQ was measured using a dilute solution of 20-nm silica particles (Nanostructured & Amorphous Materials, Inc.) at a concentration of ~10 mg/L under the same conditions as sample measurements with $\lambda_{em}=\lambda_{ex}$.

Fluorescence decay curves were fit to a sum of exponentials using a nonlinear weighted least squares procedure to determine the fluorescence lifetimes in the absence and presence of quencher [9]. The equation was expanded to the minimum number of components required to obtain a residual (χ^2) below 1.5 before the fit was deemed acceptable.

Results and Discussion

The quenching of anthracene fluorescence by CPC in ethanol was measured using both SSFQ and TRFQ to verify the results of Pownall and Smith; the corresponding Stern-Volmer plot is shown in Fig. 1. For quencher concentrations below the critical micelle concentration (CMC) of CPC in ethanol (~11 mM) [18], quenching is purely diffusion controlled, since the SSFQ and TRFQ data are identical. The slope of these plots gives a value for K_D of 50.1±5.0 in good agreement with the K_{SV} value reported by Pownall and Smith of 45±0.5 M^{-1} [5].

Figures 2a and b show the full steady-state fluorescence spectra obtained using 355 nm excitation and the time-resolved intensity at 402 nm with 370 nm excitation, respectively, for anthracene in SDS micelles with increasing CPC concentration. These results are used to construct the Stern-Volmer plots shown in Fig. 3. From the upward curvature in the SSFQ data (black line) with linear TRFQ data (red line) and a non-zero slope that is not equivalent to that of the SSFQ data, it is apparent that a combined quenching mechanism is operative. From the slope of the TRFQ data, a value for K_D of 249±6 M^{-1}

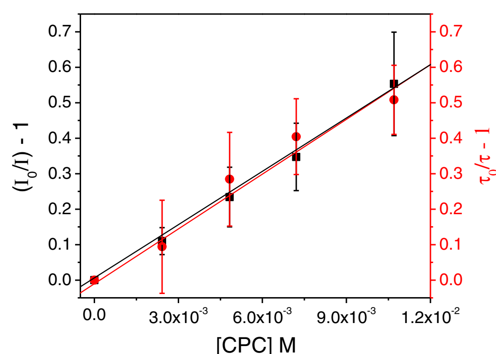

Fig. 1 Stern-Volmer plots for 10 μM anthracene in EtOH with CPC as quencher; CPC concentration <11 mM to prevent formation of CPC micelles. Red circles are TCPSC data fit to $y=50.1\times+0.006$ (solid red line), $R^2=0.986$; black squares represent steady-state data fit to $y=51.5\times-0.01$ (*solid black line*), $R^2=0.999$

is calculated. By inserting this value and the fit slope of the SSFQ data into equation (1), a value for the static quenching constant (K_s) of 225±12 M^{-1} is calculated. The sum of K_D and K_S is 474±13 M^{-1} in good agreement with the value for K_{SV} of 520±20 M^{-1} reported by Pownall and Smith [5].

The slight difference between the K_{SV} determined here as the sum of K_D and K_S compared to that reported by Pownall and Smith may be attributable to the chemical nature of the two quenchers used. These researchers used PC as the

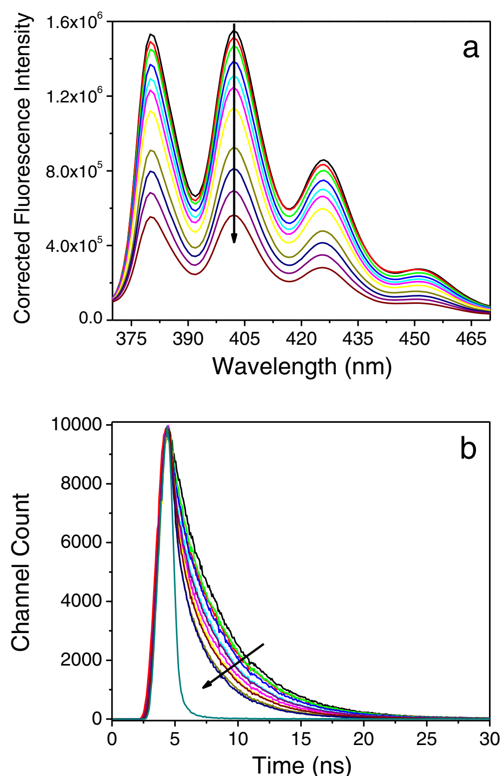

Fig. 2 **a** Steady-state fluorescence spectra with λ_{ex} 355 nm, and (**b**) TCSPC fluorescence response at λ_{em} 402 nm with λ_{ex} 370 nm from 1 μM anthracene in 50 mM SDS solution with increasing concentrations of CPC from 0 to 2.22 mM in direction of arrow. Instrument response function (IRF) shown in dark cyan for TCSPC data

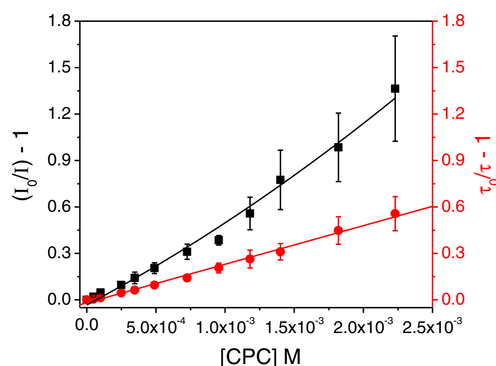

Fig. 3 Stern-Volmer plots for 5 μM anthracene in 50 mM aqueous SDS with CPC quencher in 10 mM, pH 8 phosphate buffer. Red circles are TCSPC data fit to a linear equation (red line); black squares represent steady-state data fit to Eq. (1) (*black line*)

quencher whereas CPC, which contains a hexadecyl alkyl chain, was used in this work. PC, which is highly soluble in aqueous media, is thought to reside on the surface of SDS micelles [5]. In contrast, Lunardi et al. studied the quenching of alkylacridone fluorophores (n_C=1, 4, and 10) in SDS micelles with dodecyl- and propylpyridinium quenchers and suggested that alkylpyridinium probes reside in the Stern layer of the micelle with the alkyl chains extended toward the hydrophobic micellar core [19]. Indeed, alkylpyridinium quenchers in SDS micelles are considered immobile for chain lengths longer than decylpyridinum [20]. Thus, in contrast to PC, CPC is hypothesized to be immobilized within the SDS micelle with the pyridinium headgroup in the Stern layer. This slight difference in chemistry is proposed to rationalize the small difference in total K_{SV} values between the two studies.

Finally, anthracene, although hydrophobic, is sufficiently polarizable that it resides closer to the Stern layer than deep in the core [21–23]. This position may allow the CPC quencher and the anthracene to either be within the sphere of action or to create a quenched complex. The sphere of action is estimated to be no more than the first solvation sphere of the reactant molecules (~1 nm) [9, 24], and given that the SDS micelle is ~3.6 nm in diameter [25, 26], the probability that anthracene and CPC are located within the sphere of action at the time of excitation without forming a complex is not high. Thus, the static contribution to anthracene quenching by CPC is proposed to occur by complex formation between the pyridinium headgroup and the anthracene. This complex formation would be expected to be general for any alkylpyridinium molecule.

Conclusions

The goal of this work was to expand knowledge of the quenching behavior of anthracene fluorescence in SDS micelles by alkylpyridinium molecules. Both SSFQ and TRFQ were used to verify a similar degree of absolute quenching to that observed by Pownall and Smith [5]. In homogeneous ethanol solutions, the quenching is purely dynamic with a K_D value of ~50 M^{-1}. The overall quenching constant for anthracene in SDS micelles with any pyridinium quencher is ~500 M^{-1}; here, this quenching was shown to be due to approximately equal contributions from static and dynamic quenching. This quenching is much more efficient in SDS micelles relative to that in homogeneous solution, most likely due to a higher local quencher and fluorophore concentration. The addition of a static quenching component in the micellar environment suggests that the SDS stabilizes complex formation between anthracene and the pyridinium headgroups.

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