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RESEARCH NOTE

PYRENE FLUORESCENCE LIFETIME AS A PROBE FOR OXYGEN PENETRATION OF MICELLES

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INTRODUCTION

When bound to proteins and membranes, fluorescence probes can provide valuable information about the environment of the binding site (Edelman & McClure, 1968; Brand & Gohlke, 1972). Recently much interest has been shown in aromatic hydrocarbons as fluorescence probes. Because they are non-polar molecules, they should not interact as strongly with charged groups as the amino-napthalene sulfonate probes do. Aromatic hydrocarbons exhibit environment dependent fluorescence lifetime and polarization (e.g. Hautala *et al.*, 1973; Shinitsky *et al.*, 1971; Grätzel & Thomas, 1973). These properties can be used to characterize the probe environment in biological systems. Additionally, the well-known quenching behavior of aromatic hydrocarbons has been exploited to obtain information about the permeability of membrane-like systems (Grätzel & Thomas, 1973; Infelta *et al.*, 1974; Wallace & Thomas, 1973; Pownall & Smith, 1974; Chen *et al.*, 1974) and the accessibility of protein environments (Vaughan & Weber, 1970; Lakowicz & Weber, 1973).

Synthetic micelles have been particularly useful in characterizing the fluorescence and quenching behavior of aromatic hydrocarbons in various environments (e.g. Hautala *et al.*, 1973; Infelta *et al.*, 1974; Pownall & Smith, 1974; Patterson & Vieil, 1974; Chen *et al.*, 1974 Soutar *et al.*, 1974; Cheng *et al.*, 1974). Although oxygen quenching of aromatic hydrocarbon fluorescence has been studied in proteins (Vaughan & Weber, 1970; Lakowicz & Weber, 1973), the question of oxygen permeability in micelles has not been resolved. Previous work in this group suggests that oxygen is soluble in the micelle interior and that oxygen solubility is one of the factors which determines the lifetime of solubilized probes (Hautula *et al.*, 1973). However, Dorrance & Hunter (1972) failed to obtain any increase in fluorescence quantum yield of pyrene upon deoxegenation of aqueous hexadecyltrimethylammonium bromide (HDTBr) solutions containing this hydrocarbon. They suggest that the lack of fluorescence quenching results because a barrier exists to oxygen penetration of micelles containing pyrene molecules. Wallace and Thomas (1973) report rate constants for oxygen quenching of pyrene solubilized in HDTBr and in sodium dodecyl sulfate (SDS) which are slower than the quenching rate constant in water. The effect of oxygen upon the fluorescence lifetime of a probe solubilized in micelles presented in this paper provides further evidence that micelles are penetrated by oxygen. We have studied the pyrene: HDTBr, HDTCl (hexadecyltrimethyl ammonium chloride) and SDS systems in oxygen, air and nitrogen saturated, as well as degassed solutions.

MATERIALS AND METHODS

HDTBr (ethonol–ether, mp 251–252°), HDTCl (acetone), and SDS (ethanol) were purified by recrystallization ($>5\times$, norite $2\times$). Critical micelle concentrations (eosin dye, Corrin & Harkins, 1947) of 0.0008 *M* (HDTBr) and 0.003 *M* (HDTCl) were obtained (literature values 0.0009 *M* and 0.0015 *M*, Mukerjee & Mysels, 1971). Water was redistilled from potassium permanganate. The purified detergents in solution showed no absorption or emission in the spectral regions of interest.

Pyrene (Aldrich, 99%) was recrystallized (ethanol, norite, $3\times$) until colorless crystals were produced. Material obtained by this procedure displayed absorption and emission spectra (cyclohexane) which agree with published spectra (Berlman, 1965). Pyrene fluorescence lifetime in water was found to depend on its purity. The fluorescence lifetime, τ_f, in aqueous solution was ~ 125 ns for pyrene purified in this manner.

The fluorescence lifetimes were determined using single photon counting techniques (Ware, 1971). An air spark lamp (Tao, 1969) gave a pulse of half-width ~ 2 ns. Emissions were analyzed on a system that included a 1/4 m Jarrell-Ash monochrometer, Amperex 56 AVP phototube, Hewlett-Packard 5614L preset counter, and a Northern Econ II multichannel pulse height analyzer (calibrated at 1.55 or 3.12 ns per channel). Fluorescence spectra were recorded on a Perkin-Elmer MPF-2A or MPF-3L spectrophotometer.

Concentrated stock solutions of detergent were added to gas saturated solutions of pyrene ($\sim 10^{-6}$ *M*) in water to obtain the nitrogen and oxygen saturated solutions. Long-necked 1 cm fluorescence cells with Teflon stopcocks were used. Solutions were degassed using three freeze, pump, thaw cycles (mechanical pump, oil diffusion pump) and transferred under vacuum to the cell containing a preweighed amount of detergent. The cell was sealed with a teflon vacuum stopcock (Kontes). After 12 h, the lifetime of a degassed pyrene solution dropped from 328 to 298 ns, showing that the leakage of air back into the cell is slow. The detergent concentration in the degassed samples is

only approximate since the amount of water transferred to the cell is not known exactly.

Conductivity measurements were done on a model 16B2 Industrial Instruments conductivity bridge. Critical micelle concentrations were obtained in the usual manner from plots of reciprocal resistance vs detergent concentration (Mukerjee & Mysels, 1971).

RESULTS AND DISCUSSION

The emission spectra of pyrene ($\sim 10^{-6}$ *M*) in water and in detergent solutions are shown in Fig. 1. Even at low concentrations of pyrene, it might be expected that aggregation occurs in water. However, the emission spectra obtained showed pyrene monomer fluorescence with no evidence of excimer emission ($\lambda \sim 470$ nm) present. On the average, there is less than one pyrene molecule per micelle in detergent solutions. It is, therefore, not surprising that the excimer emission is absent. Pyrene is thought to be solubilized in the micellar phase in detergent solutions because of its hydrophobicity (Pownall & Smith, 1973; Dorrance & Hunter, 1972). Results from pulsed radiolysis experiments (Wallace & Thomas, 1973) and NMR studies (Grätzel *et al.*, 1974) support the solubilization of pyrene in the micellar phase.

In aerated solutions, the lifetime of pyrene in pure water is shorter than in aqueous solutions of HDTCl or SDS (Table 1). We interpret this result to suggest either (a) a lesser solubility of oxygen in the micelle relative to water, (b) a rate constant for oxygen quenching of solubilized pyrene slower than diffusion controlled in bulk solution, or (c) a quenching of pyrene fluorescence by water. The first possibility would be rather surprising. Since oxygen is approximately 20 times more soluble in air-saturated hydrocarbon

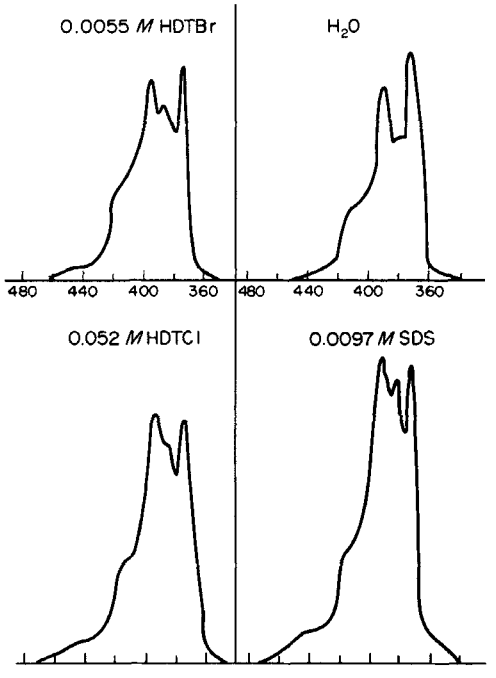

Figure 1. The fluorescence spectra of pyrene in water and detergent solutions; $\lambda_{excit} = 320$ nm.

Table 1. Pyrene fluorescence lifetime (in ns) in environments of various oxygen concentration

	O_2 Saturated	Air	N_2 Saturated	Degassed	Naphthalene* Air
Water	69±4	126±3	226±10	201 206	39
HDTBr(.0055M)	62±6	122±8	165±5	175 158	11
HDTCl(.0065M)	67±8	157±2	281±22	328 268	23
SDS(.0097M)	58±3	158±4	314±10	314 304	60
Cyclohexane*	-	20	202	370	17 degassed 108

*From Hautala *et al.* (1973).

solvents than in water, it is expected that oxygen solubility would be greater in the 'hydrocarbon-like' micelle interior than in water. There is some evidence to support the second possibility. The only reported rates for oxygen quenching of solubilized pyrene (Wallace & Thomas, 1973) *are* slower than the rates observed in water. Fluorescence quenching of several aromatic hydrocarbons by water has been reported (Hautala *et al.*, 1973; Stevens & Strickler, 1973; Vaughan & Weber, 1970) and pyrene may also be quenched in aqueous solutions.

Upon oxygen saturation, the lifetime of pyrene decreases in water and in detergent solutions. These lifetimes are essentially the same (Table 1) and the decrease of pyrene lifetime in micelles is not significantly greater than the decrease in water. These results suggest that oxygen is at least as soluble in micelles as in water. Since we expect that water quenching of pyrene is slow ($k_q(H_2O) \sim 10^5\ mol^{-1}\ s^{-1}$ for naphthalene) in comparison to the rate of oxygen quenching in saturated solutions, only the latter process is considered.

Nitrogen saturation increases the lifetime of pyrene in water, HDTCl, and SDS solutions due to the removal of oxygen. Vacuum degassing the solutions does not siginifcantly alter the lifetime of pyrene from that measured in nitrogen saturated solutions.

Pyrene's lifetime is shorter in air saturated HDTBr than in HDTCl or SDS and more importantly, its lifetime in degassed or nitrogen saturated solutions is shortest in HDTBr. Although the lifetime of pyrene increases with nitrogen saturation or degassing, the increase is small relative to the changes observed in HDTCl, SDS and water. This result is consistent with the Dorrance and Hunter report (1972) that pyrene fluorescence yield in HDTBr was not changed upon deoxygenation. Anomalous behaviour of aromatic hydrocarbon fluorescence in HDTBr has been reported and explained previously (Hautala *et al.*, 1973; Grätzel and Thomas, 1973; Patterson and Vieil, 1973. Bromide ion is known to quench aromatic hydrocarbon fluorescence (Watkins, 1974). The high local concentration of Br^- around the micelle enhances this quenching effect, causing shorter pyrene lifetimes to

be observed in air and N_2 saturated HDTBr solutions. After the removal of oxygen, Br^- quenching dominates the deactivation of excited state pyrene in HDTBr and limits its lifetime.

Fluorescence lifetime studies (Hautala *et al.*, 1973), absorption spectroscopy (Riegelman *et al.*, 1958), and pulsed radiolysis experiments (J. Fendler, unpublished) indicate that naphthalene is solubilized on the micelle-water interface. Pyrene, due to its lower solubility in water, is expected to be solubilized further from the surface, which is supported by pulse radiolysis experiments (Wallace & Thomas, 1973) and NMR studies (Grätzel *et al.*, 1974). A comparison of pyrene and naphthalene fluorescence lifetimes in micelles also suggests a deeper penetration of pyrene into the micelle, since the ratio of pyrene lifetime in micelles relative to water or degassed cyclohexane is larger than for naphthalene lifetime. In addition, the quenching effects in HDTBr are not as great for pyrene compared to naphthalene. The rate constant, k_q, for bromide quenching of naphthalene fluorescence in water is approximately $2 \times 10^8\ mol^{-1}\ s^{-1}$ (Hautala *et al.*, 1973). We have obtained $k_q \sim 1 \times 10^7\ mol^{-1}\ s^{-1}$ for pyrene from quenching of fluorescence intensity (Stern-Volmer analysis) and from lifetime quenching studies (1). Values of the effective Br^- concentration can be obtained from (1) for

$$\tau_0^{-1} = \tau^{-1} + k_q[Br^-] \qquad (1)$$

naphthalene and pyrene, solubilized in HDTBr. We can use the lifetimes in HDTCl (air) for τ_0, since anion quenching is negligible for Cl^- (Watkins, 1974). The τ values are the lifetimes in HDTBr (air). Thus, $k_q[Br^-]$ pyrene $= 1.8 \times 10^{-6}\ s^{-1}$ and $k_q[Br^-]$ naphthalene $= 4.8 \times 10^{-7}\ s^{-1}$. Substituting in the measured values of k_q, we obtain $[Br^-]$ pyrene $= 0.18\ M$ and $[Br^-]$ naphthalene $= 0.24\ M$. The smaller effective Br concentration at the same detergent concentration for pyrene suggests a larger probe to counterion distance. This is consistent with pyrene being solubilized further from micelle surface than naphthalene.

The oxygen effects reported here are known to be micellar since the lifetime of pyrene increases upon micelle formation (SDS and HDTCl). A plot of fluorescence lifetime vs detergent concentration (Fig. 2) exhibits a break characteristic of micelle formation. This method gives critical micelle concentrations (CMC) for HDTCl $\simeq 0.004\ M$ and SDS $\simeq 0.0035\ M$ which compare favorably with values for HDTCl $\simeq 0.005\ M$ and SDS $\simeq 0.0035\ M$ obtained by conductivity measurements (literature values: HDTCl $= 0.001\ M$, SDS $= 0.009\ M$; Mukerjee & Mysels, 1971).

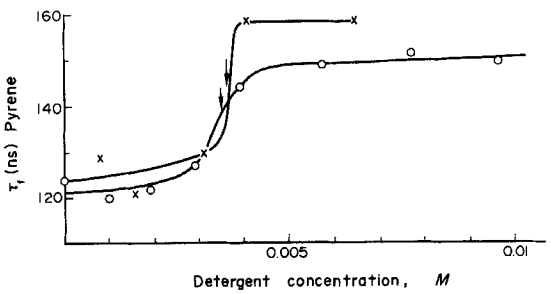

Figure 2. The effect of micelle formation on pyrene fluorescence lifetime. The arrows indicate the approximate CMC's. "×" refers to HDTCl and "O" to SDS.

Conductivity measurements of SDS and HDTCl gave identical values of the CMC in the presence and absence of pyrene implying that pyrene does not significantly perturb the micelle. Saturation by oxygen or nitrogen also does not change the CMC. Upon standing for a week, the lifetime of pyrene in oxygen and nitrogen saturated solutions returned to the values measured in air indicating that no reaction had taken place to cause the observed changes in lifetime. The shape of the fluorescence spectra in the presence of oxygen and nitrogen was unperturbed. In all cases, the lifetime was observed as a single exponential decay confirming the absence of excimer formation. Also, pyrene lifetime in SDS does not vary with the age of the solution, suggesting that the aging effect reported for SDS (Grätzel and Thomas, 1973) does not affect the lifetime measurement.

CONCLUSIONS

The fluorescence lifetime of solubilized pyrene has been used to show that micelles can be oxygenated and deoxygenated. The results suggest that oxygen is at least as soluble in micelles as in water and that oxygen moves across the micelle–water interface. The measured fluorescence lifetime in micelles (especially in HDTBr) is consistent with pyrene being solubilized further from the micelle surface than naphthalene.

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