ELSEVIER

Biophysical Chemistry 57 (1996) 291–295

Biophysical Chemistry

Two-photon induced fluorescence of linear alkanes; a possible intrinsic lipid probe

Ignacy Gryczynski, Anna Razynska, Joseph R. Lakowicz *

Center for Fluorescence Spectroscopy, Department of Biological Chemistry, University of Maryland, School of Medicine, 108 North Greene Street, Baltimore, MD 21201, USA

Accepted 19 April 1995

Abstract

We measured the fluorescence emission spectra and intensity decays of the linear alkane tetradecane when excited at 300 nm by two-photon excitation. The unquenched lifetime of tetradecane in neat solution is near 4.4 ns. The emission of tetradecane centered at 210 nm is collisionally quenched by oxygen, n-propanol and water. These results suggest that aliphatic groups in non-polar environments can display good fluorescence, and that the aliphatic side chains of detergents and lipids may serve as an intrinsic fluorescent probe of micelles and bilayers.

Keywords: Lipid probe; Two-photon excitation; Vacuum ultraviolet

1. Introduction

The fluorescence from aromatic molecules is often used to study the physical properties of biological macromolecules [1–3]. However, unsaturated cyclic and linear alkanes also display significant fluorescence when excited in the vacuum ultraviolet (VUV) region of the spectrum near 140–160 nm [4–7]. The need for VUV excitation has resulted in the limited study of alkane fluorescence because the light sources are weak, it is necessary to exclude oxygen, and quartz optics are not sufficiently transparent. However, we have recently shown that the cyclic alkanes cyclohexane and methylcyclohexane can be excited by two-photon excitation (TPE) near 300 nm, and the emission over 200 nm is readily transmitted by standard quartz optics [8]. The use of TPE circumvents the need for VUV conditions, and suggests the use of the intrinsic fluorescence of saturated hydrocarbons as an intrinsic probe.

In the present report we extend these studies to a linear alkane tetradecane. Observation of emission from a linear alkane with TPE would support the notion of using the emission from detergent and lipid side chains as an intrinsic fluorophore. Our experiments demonstrated that the emission of tetradecane is collisionally quenched by water, alcohol and oxygen, which in turn suggests that the emission of aliphatic groups can be a sensitive indicator of exposure of the methylene chains to solvent.

2. Materials and methods

Tetradecane was obtained from Kodak (99% pure), and purified by repeated washing with sulfuric

* Corresponding author.

SSDI 0301-4622(95)00066-6

acid, water, dried and passaged over activated silica gel and alumina until the absorption onset between 190 and 210 nm remained unchanged. Following this treatment the optical density at 200 nm was less than 0.4.

Two-photon excitation was accomplished using the frequency-doubled output of a rhodamine 6G (R6G) dye laser at 300 nm, with the light focused to an approximate 10-μm diameter spot as described previously [8]. The emission was observed using a diaphragm to spatially select the focal region, and a stack of two interference filters (210 nm, Oriel) or a monochromator to isolate the emission.

The intensity decays were measured using the frequency-domain (FD) method [9] with a 3.795 MHz cavity-dumped and frequency-doubled pulse train and GHz detector [10]. Steady-state intensities, emission spectra, and FD data were obtained on the same instrument.

The frequency-domain intensity decay data were analyzed using the multi-exponential model

$$I(t) = \sum_i \alpha_i e^{-t/\tau_i}, \quad (1)$$

where α_i are the pre-exponential factors and τ_i the decay times, as described previously for FD data [11,12]. The fractional intensity of the ith component can be calculated using

$$f_i = \alpha_i \tau_i / \sum_j \alpha_j \tau_j \quad (2)$$

3. Results

The emission spectrum of tetradecane is shown in Fig. 1, for excitation at 300 nm. The emission maximum is at 210 nm in this uncorrected spectrum. The intensity depends on the square at the incident light intensity as expected for TPE (insert).

The emission of tetradecane is decreased in the presence of n-propanol. The decrease in intensity follows Stern–Volmer kinetics, as seen by the linear intensity Stern–Volmer plot (Fig. 2, ●). Quenching of tetradecane by n-propanol is most likely collisional in that the Stern–Volmer quenching constant of 37 M^{-1}, and the unquenched lifetime of 4.4 ns (below), results in a bimolecular quenching constant

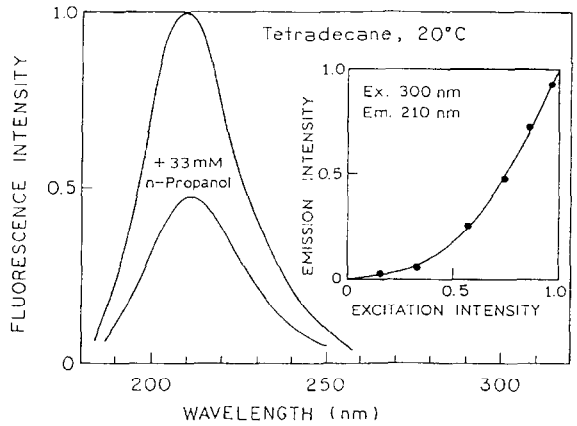

Fig. 1. Emission spectra of tetradecane with two-photon excitation at 300 nm. Spectra are shown for neat tetradecane (—) and in the presence of 33 mM n-propanol. The insert shows the quadratic dependence on the emission at 210 nm, with 300 nm excitation.

of $8.4 \cdot 10^9$ M^{-1} s^{-1}. This value is near that expected for 100% efficient collisional quenching in a solvent of the viscosity of tetradecane (2.18 cP). Tetradecane and n-propanol do not appear to form observable exciplexes since the emission spectrum is unchanged in the presence of n-propanol, but future more detailed studies could alter the initial conclusion. Quenching by inner filter effects or fluorescence resonance energy transfer seems unlikely since n-propanol does not absorb significantly at the excitation (300 nm) or emission (210 nm) wavelengths.

The nature of n-propanol quenching can be determined by measurement of the fluorescence lifetime.

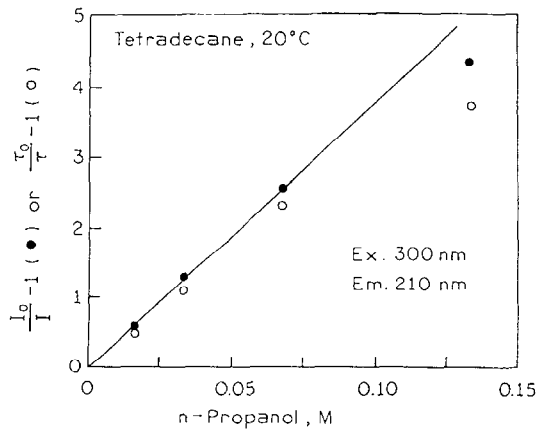

Fig. 2. Stern–Volmer intensity (●) and lifetime (○) plot for n-propanol quenching of tetradecane.

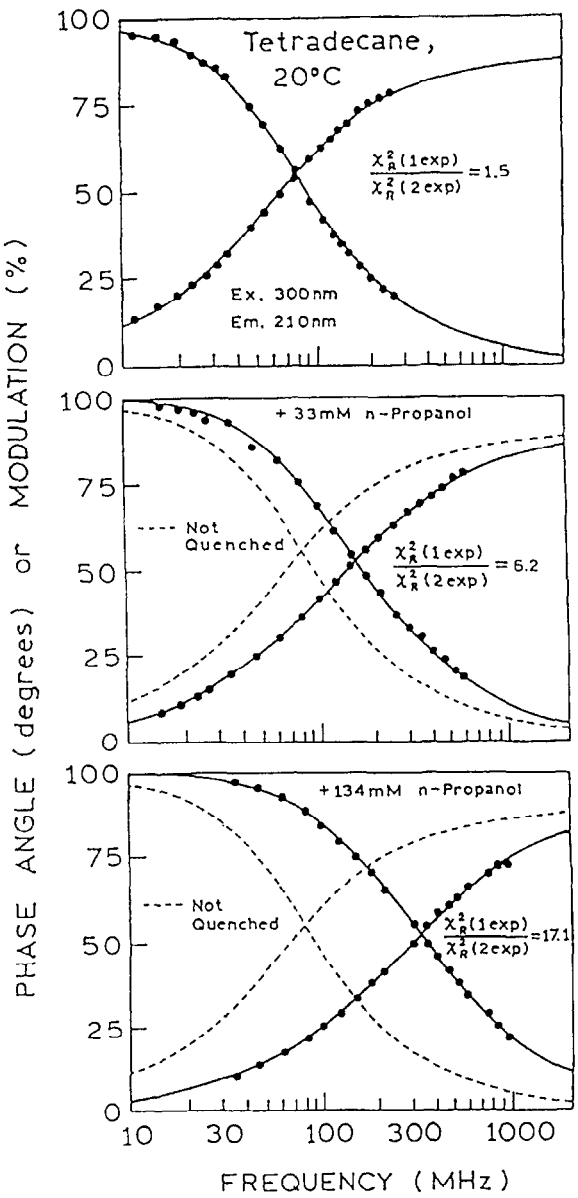

Fig. 3. Frequency-domain intensity decay of tetradecane for a neat solution (top) and with 33 mM (middle) and 134 mM (bottom) n-propanol.

Frequency-domain intensity decays of tetradecane are shown in Fig. 3. In the absence of n-propanol the intensity decay is dominantly a single exponential of 4.36 ns (Table 1), which accounts for 97% of the total intensity. With present instrumentation a 4.4 ns decay time can be regarded as comfortably long to allow detailed studies of factors affecting the emission of tetradecane. At present we cannot explain the small component with a long 36 ns decay time. A similar small but long-lived component was also observed for cyclohexane and methylcyclohexane

Table 1
Intensity decays of tetradecane with two-photon excitation, in the presence of propanol, water or dissolved oxygen

Compound	n	α_i	f_i	τ_i (ns)	χ^2_R
O_2 free	1	1	1	4.36	1.4
	2	0.996	0.971	4.29	0.6
		0.004	0.029	36.10	
Air	1	1	1	3.01	2.3
	2	0.995	0.971	2.95	
		0.005	0.029	18.86	1.5
Air, H_2O saturated	1	1	1	2.85	0.9
Air, 17 mMn-propanol	1	1	1	2.31	1.2
	2	0.945	0.900	2.20	
		0.055	0.100	4.21	0.6
33 mM n-propanol	1	1	1	1.44	8.1
	2	0.626	0.416	0.95	
		0.374	0.584	2.22	1.3
67 mM n-propanol	1	1	1	0.92	10.2
	2	0.597	0.364	0.55	
		0.403	0.636	1.44	0.9
134 mM n-propanol	1	1	1	0.70	31.9
	2	0.814	0.607	0.52	
		0.186	0.393	1.43	1.9

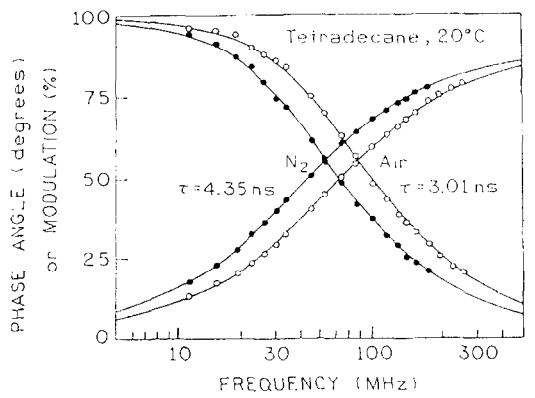

Fig. 4. Frequency-domain intensity decay of tetradecane in the absence (●) and presence (○) of dissolved oxygen from the equilibration with air.

[8], suggesting an optical origin of this component. The component could originate from the cuvette or optical filters, which could be excited by the 210 nm emission of tetradecane. Since this component is excited in proportion to the intensity of the tetradecane emission, it is difficult to eliminate. Although we cannot explain the origin of this component, its presence does not affect the conclusions of this paper.

The presence of n-propanol in tetradecane results in a shift in the frequency response to higher frequencies (Fig. 3, middle and bottom panels), which indicates a decrease in the decay time of tetradecane. The mean decay time of tetradecane decreases from 4.4 to 0.70 ns as the concentration of n-propanol is increased to 134 mM (Table 1). The decrease in lifetime is comparable to the decrease in intensity (Fig. 2), demonstrating that n-propanol quenching of tetradecane is dynamic. Another indication of the dynamic nature of n-propanol quenching is the modest multi- or non-exponential decay in the presence of n-propanol. This can be seen by the elevated χ_R^2 values for the single exponential fits (Table 1). Such effects are known to occur due to transient effects in diffusional quenching [13].

Tetradecane fluorescence is readily quenched by dissolved oxygen (Fig. 4). Equilibration with air, rather than nitrogen, decreases its decay time from 4.4 to 3.0 ns. Using the known solubility of oxygen in liquid alkanes like dodecane (about 0.009 M/atmosphere of O_2 [14]), the bimolecular quenching constant is $4.8 \cdot 10^{10}$ M^{-1} s^{-1}, which is slightly above the diffusion controlled limit. In previous studies [16] we observed oxygen bimolecular quenching constants near $2.2 \cdot 10^{10}$ M^{-1} s^{-1} in dodecane. The present values of $4.8 \cdot 10^{10}$ M^{-1} s^{-1} could be the result of absorption of oxygen at 200 nm, which could in effect increase the interaction distance for quenching. Irrespective of these details, the sensitivity of alkane fluorescence to dissolved oxygen suggests that alkane fluorescence can provide an intrinsic probe of oxygen transport in membranes. Oxygen quenching of alkanes will be the subject of a separate study.

Tetradecane also appears to be collisionally quenched by water, as seen by a decrease in the decay time from 3.0 to 2.85 ns when equilibrated with water (Table 1). The extent of quenching by water is limited by its low solubility in alkanes [15]. Using the known water solubility of near 0.01% W (0.005 M), we estimate the biomolecular quenching constant to be $3.5 \cdot 10^9$ M^{-1} s^{-1}.

4. Conclusion

The fluorescence of linear alkanes can be observed without VUV excitation when excited by a two-photon process. The steady-state intensity and intensity decay of the linear alkane tetradecane are sensitive to both oxygen and hydroxyl groups. Improved laser sources are needed to determine whether alkane fluorescence can be observed from aqueous dispersion of amphipathic molecules.

Acknowledgements

This work was supported by an NIH grant to the Center for Fluorescent Spectroscopy (NCRR RR-08119). Joseph R. Lakowicz expresses appreciation to the University of Maryland School of Medicine and the Medical Biotechnology Center for their support.

References

[1] T.G. Dewey, Biophysical and Biochemical Aspects of Fluorescence Spectroscopy, Plenum Press, New York, 1991.
[2] O.S. Wolfbeis, Fluorescence Spectroscopy, New Methods and Applications, Springer-Verlag, New York, 1993.
[3] S.G. Schulman, Molecular Luminescence Spectroscopy, Methods and Applications: Part 3, John Wiley & Sons, New York, 1993.
[4] F. Hirayama and S. Lipsky, J. Chem. Phys., 51 (1969) 3616.
[5] F. Hirayama, W. Rothman and S. Lipsky, Chem. Phys. Lett., 5 (1970) 296.
[6] W. Rothman, F. Hirayama and S. Lipsky, J. Chem. Phys., 58 (1973) 1300.
[7] A.H. Halpern and W.R. Ware, J. Phys. Chem., 74 (1970) 2413.
[8] J.R. Lakowicz and I. Gryczynski, Biospectroscopy, 1 (1995) 3–8.
[9] J.R. Lakowicz and I. Gryczynski, in J.R. Lakowicz (Editor), Topics in Fluorescence Spectroscopy, Vol. 1, Plenum Press, New York, 1991, 293 pp.
[10] G. Laczko, J.R. Lakowicz, I. Gryczynski, Z. Gryczynski and H. Malak, Rev. Sci. Instrum., 61 (1990) 2331.

[11] E. Gratton, J.R. Lakowicz, B. Maliwal, H. Cherek, G. Laczko and M. Limkeman, Biophys. J., 46 (1984) 479.
[12] J.R. Lakowicz, H. Cherek, B. Maliwal, G. Laczko and E. Gratton, J. Biol. Chem., 259 (1984) 10967.
[13] M.R. Eftink, Fluorescence Quenching: Theory and Applications, in J.R. Lakowicz (Editor), Topics in Fluorescence Spectroscopy, Plenum Press, New York, 1991, pp. 53–126.
[14] Landolt-Bornstein, Physikalische und Chemische Konstanten, 2. Teil, Bandteil B, 1962, p. 267.
[15] J.A. Riddick, W.B. Bunger and T.K. Sakano, in Techniques of Chemistry, Vol. II, Organic Solvents, John Wiley and Sons, NY, 1986.
[16] J.R. Lakowicz and G. Weber, Biochemistry, 12 (1973) 4161–4170.