Journal of Fluorescence, Vol. 4, No. 4, 1994

Fluorescent Nucleoside Derivatives: Luminescence Study of 4-Dimethylaminopyridinium Chloride Derived from Guanosine

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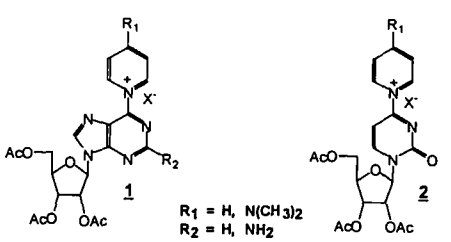
Received October 18, 1993

Photophysical properties of fluorescent *N*-[2-amino-9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)-purin-6-yl]-4-dimethylaminopyridinium chloride (GDMAP) are determined in view of its possible use as a probe in DNA. The fluorescence intensity of GDMAP increases and exhibits double-exponential decay in the presence of common nucleosides. The formation of ground-state complexes with nucleosides is inferred from absorption and emission measurements.

KEY WORDS: Fluorescent nucleoside; probe; fluorescence quenching.

INTRODUCTION

Pyridinium derivatives of purine and pyrimidine nucleosides are a family of ionic compounds of the following general structures:



Scheme I

Both purine, **1**, and pyrmidine, **2**, derivatives in the R_1=H series, have already been shown to possess interesting chemical [1], photochemical [2], and photophysical [3] properties. Among the *N,N*-dimethylaminopyridinium derivatives which have been synthesized recently [4], of particular interest is that derived from guanosine [R_1=N(CH_3)$_2$, R_2=NH_2], abbreviated GDMAP. In contrast to other purine, **1** (R_2=H), or pyrimidine, **2**, derivatives, which are nonfluorescent [4], it exhibits intense fluorescence at room temperature [4,5]. Recent studies have shown that this derivative can be incorporated into synthetic fragments of DNA [5]. This prompted us to study the detailed absorption and luminescence properties of this compound in view of its possible use as a fluorescent probe in nucleic acids. The results of these studies are presented and discussed here.

EXPERIMENTAL

Materials. The solvents (Merck) were of spectroscopic grade and were used as received. *N*-[2-Amino-9-(2',3',5'-tri-*O*-acetyl-β-D-ribofuranosyl)purin-6-yl]-4-dimethylaminopyridinium chloride (GDMAP) was prepared as follows: 6-Chloroguanosine (428 mg, 1 mmol) and 4-dimethylaminopyridine (183 mg, 1.5 mmol) were dissolved in anhydrous THF (15 ml) and heated at about

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1053-0509/94/1200-0283$07.00/0

55°C for 18 h. The crystalline precipitate formed was separated and washed with THF (5 ml) to give 461 mg of GDMAP as stable, yellow crystals; mp 158–161°C. ^{1}H NMR:(DMSO-d_6) 2.03 (s, 6, 2 CH_3CO), 2.12 (s, 3, CH_3CO), 3.32 (s, 6, $N(CH_3)_2$), 4.27–4.45 (m, 3, H4',5',5"), 5.57 (dd, $J_{3'-4'}$ = 4.0 Hz, $J_{3'-2'}$ = 5.8 Hz 1, H3'), 5.31 (dd, $J_{2'-1'}$ = 5.7 Hz, 1, H2'), 6.19 (d, 1, H1'), 7.24 (br s, 2, NH_2), 7.36 (d, $J_{H\alpha-H\beta}$ = 8.1 Hz, 2, H_α), 8.59 (s, 1, H8), 9.37 (d, 2, H_β). ^{13}C NMR: 20.20, 20.38, 20.54 (CH_3CO), 40.82 [$N(CH_3)_2$], 63.07 (C5'), 70.38 (C3'), 72.10 (C2'), 79.94 (C4'), 85.22 (C1'), 108.08 (C3"), 116.31 (C5), 138.34 (C2"), 142.38 (C8), 146.80 (C4"), 156.67 (C4), 157.54 (C2), 159.94 (C6), 169.75, 169.89, 170.54 (3 CO). *Anal.* Calcd for $C_{23}H_{28}ClN_7O_7$: C, 50.2; H, 5.1; N, 17.8. Found: C, 50.1; H, 5.1; N, 17.7.

Steady-State and Time-Resolved Spectroscopy. Absorption and fluorescence spectra were recorded on Perkin–Elmer Lambda 17 and LS50-B spectrophotometers respectively. Fluorescence quantum yields were determined relative to quinine sulfate (ϕ = 0.55 in 1 *N* H_2SO_4) [6]. Ratios of the fluorescence quantum yield of GDMAP in the presence (ϕ_f) and in the absence (ϕ_f^o) of nucleosides, which are summarized in Table II, were measured at a constant concentration of GDMAP (3×10^{-6} *M*). Fluorescence lifetimes were measured using time-correlated single-photon counting [7], using a Model 5000 Fluorescence Lifetime Spectrometer (IBH Consultants) [8]. Samples were excited at 350 nm and emission collected at 480 nm in most of the cases. Reconvolution of fluorescence decay curves was performed using IBH Consultants Version 4 software.

RESULTS AND DISCUSSION

UV Absorption and Fluorescence of GDMAP. The absorption and fluorescence properties of GDMAP in selected solvents are summarized in Table I. The intense absorption extends up to 400 nm (Fig. 1), far beyond the absorption of all common DNA nucleosides. Both the position and the intensity of the first absorption band depend on the polarity of the solvent. The fluorescence emission in water is rather broad ($\nu_{1/2}$ = 5000 cm^{-1}) and well separated from the lowest-energy absorption band. The Stokes shift is relatively large and amounts to ca. 9000 cm^{-1} in water and 5900 cm^{-1} in ethanolic glass at 77 K (Fig. 1). Both UV absorption and fluorescence parameters in water do not change over the wide pH range (1–9). The emission of GDMAP in water is weak but increases significantly in D_2O, alcohols, and, particularly, acetonitrile. The fluorescence lifetime measure-

Table I. Absorption and Fluorescence Parameters of GDMAP in Various Solvents

	Absorption		Fluorescence		
Solvent	λ_{max} (nm)	ε (M^{-1} cm^{-1})	λ_{max} (nm)	ϕ_f	τ_f (ns)
Water	249	6,700			
	312	20,800	498	0.04	0.51
	344	24,000			
D_2O	249	6,700			
	312	20,800	498	0.12	1.46
	344	24,000			
Methanol	249	7,300			
	312	22,600	510	0.13	1.40
	352	17,700			
Ethanol (95%)	251	7,700			
	313	23,800	509	0.13	1.58
	353	18,300			
2-Propanol	250	7,800			
	313.5	23,600	510	0.16	1.83
	354	16,800			
Acetronitrile	250	7,500			
	315	22,300	493	0.43	3.81
	350	21,900			

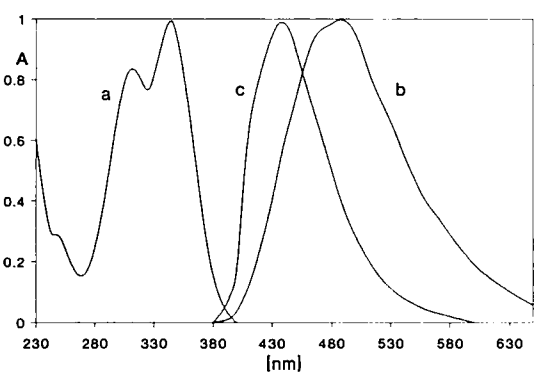

Fig. 1. Room-temperature absorption (a) and fluorescence (b) of GDMAP in H_2O and total luminescence (c) in ethanolic glass (77 K).

ments indicated that the enhancement of ϕ_f is due to the decrease in the rate constant for radiationless decay [$\Sigma k_{nr} = (1-\phi)/\tau$] in these solvents. Steady-state and time-dependent fluorescence measurements of GDMAP in acetonitrile in the presence of various concentrations of water showed that the quenching process at a low water content (<5% vol) is purely dynamic ($k_q = 1\times10^8$ M^{-1} s^{-1}). The high deuterium isotope effect points to the involvement of the excited-state proton transfer reaction in the quenching mechanism. Contrary to previously studied pyridinium salts **1** in R_1=H series, which are efficiently quenched by a number of inorganic ions (Cl^-, Br^-, I^-, SO_4^{-2}) [3], the fluorescence of GDMAP is

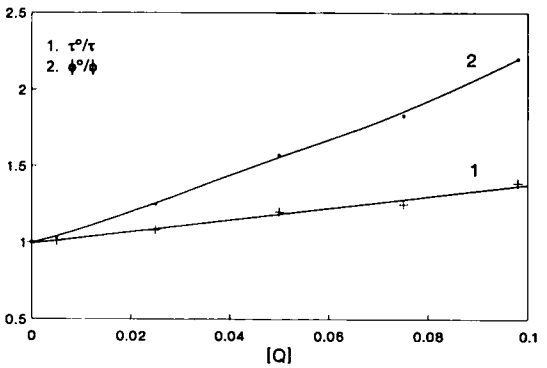

Fig. 2. Stern–Volmer plots for fluorescence quenching of GDMAP by iodide ions.

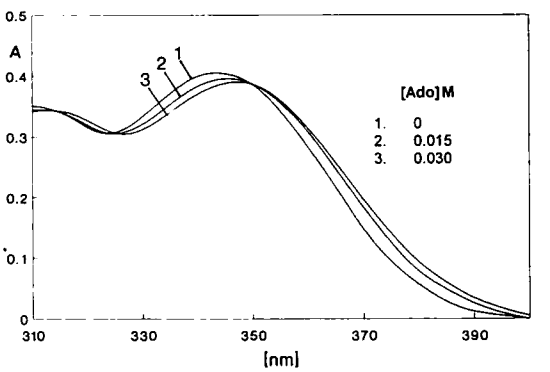

Fig. 3. Changes in the UV spectrum of GDMAP in water upon the addition of adenosine.

quenched exclusively, by I^-. The relevant Stern–Volmer plots obtained from lifetime (τ_o/τ vs $[I^-]$) and steady-state (ϕ_o/ϕ vs $[I^-]$) measurements are shown in Fig. 2. The lifetime data plot is linear over the whole range of I^- concentrations used and gives the Stern–Volmer constant $K_{sv} = k_q\tau = 3.9\ M^{-1}$, from which the dynamic quenching constant $k_q = 7.8 \times 10^9\ M^{-1}\ s^{-1}$ is calculated. The steady state data plot shows a higher initial slope and a distinct upward curvature, indicating a substantial contribution of static quenching, due to the ground-state ion pairing, to the overall quenching process [9].

Absorption and Fluorescence of GDMAP in the Presence of Nucleosides. The addition of common DNA nucleosides (adenosine, guanosine, thymidine, and cytidine) to the aqueous solution of GDMAP results in a small, gradual decrease in the intensity and a small red shift of the long-wavelength absorption band of GDMAP (cf. Fig. 3). The changes in the UV spectra are accompanied by a gradual increase in the intensity and a small blue shift of the fluorescence spectra. Both the extent of the changes in the UV spectra and the magnitude of the fluorescence enhancement depend on the nature and the concentration of nucleoside added (Table II) and are most pronounced in the case of adenosine. These observations clearly show the formation of ground-state complexes between DNA nucleosides and GDMAP.

Table II. Fluorescence Lifetimes and Quantum Yields of GDMAP Measured in the Presence of Various Nucleosides

Nucleoside added			τ_1 (ns) (A_1)	τ_2 (ns) (A_2)	ϕ_f/ϕ_f°
Cytidine,	0.015 *M*	(H_2O)	0.55		1.10
Thymidine,	0.015 *M*	(H_2O)	0.41 (63.4)	0.95 (36.6)	1.11
Uridine,	0.015 *M*	(H_2O)	0.41 (68.1)	0.83 (31.9)	1.06
Adenosine	0.007 *M*	(H_2O)	0.33 (56.5)	1.08 (43.5)	1.21
	0.015 *M*	(H_2O)	0.50 (45.6)	1.18 (54.4)	1.47
	0.020 *M*	(H_2O)	0.42 (39.5)	1.20 (61.5)	1.50
	0.030 *M*	(H_2O)	0.33 (32.5)	1.22 (67.5)	1.56
	0.015 *M*	(DMSO)	1.10		1.02
	0.015 *M*	(CH_3CN)	3.81		1.11

All the GDMAP + nucleoside systems studied exhibit double-exponential decays. The relevant lifetime data are collected in Table II. The two lifetimes remain practically unchanged with increasing nucleoside concentration, while the fractional contribution of the short-lived emission ($\tau_1 \approx 400$ ps) decreases systematically. This emission is therefore ascribed to free, uncomplexed GDMAP which remains in the solution, and the longer one ($\tau_2 > 1$ ns) to the GDMAP–nucleoside complex.

The formation of ground-state complexes is restricted to aqueous solutions exclusively. No changes in the UV and fluorescence spectra and lifetimes could be observed upon the addition of adenosine and other nucleosides to the solutions of GDMAP in organic solvents such as DMSO and acetonitrile. These observation suggests that the stacking interaction between GDMAP and nucleobases plays a major role in the ground-state complexation process.

ACKNOWLEDGMENTS

This work was supported by the Polish Scientific Research Committee, Grant PB 1031/92/02.

REFERENCES

1. R. W. Adamiak, E. Biala, and B. Skalski (1985) *Nucleic Acids Res.* **13**, 2989–3003; (1985) *Angew. Chem. Int. Ed. Engl.* **24**, 1054–1055; G. Wenska, B. Skalski, and Z. Gdaniec (1992) *Can. J. Chem.* **70**, 856–862.

2. B. Skalski, R. P. Steer, and R. E. Verrall (1991). *J. Am. Chem. Soc.* **113**, 1756–1762.
3. B. Skalski, S. Paszyc, R. W. Adamiak, R. P. Steer, and R. E. Verrall (1990) *Can. J. Chem.* **68**, 2164–2170.
4. S. Wnuk, B. Skalski, G. Wenska, and R. W. Adamiak, unpublished.
5. H. Gao, R. Fathi, B. L. Gaffney, B. Goswami, P. Kung, Y. Rhee, R. Jin, and R. A. Jones (1992) *J. Org. Chem.* **57**, 6954–6959.
6. D. F. Eaton (1988) *Pure Appl. Chem.* **60**, 1107.
7. D. V. O'Connor and D. Philips (1984) *Time-Correlated Single Photon Counting*, Academic, London.
8. D. J. S. Birch, G. Hungerford, and R. E. Imhof (1991) *Rev. Sci. Instrum.* **62**(10), 2405–2408.
9. J. R. Lakowicz (1983) *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.