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Fluorescent Molecular Rotors with Specific Hydrophilic Functions: Glucosamine and Inositol Derivatives

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New fluorescent molecular rotors having hydrophilic functional groups (such as a sugar or an inositol group) were synthesized. The aim was to obtain impermeant and uncharged probes, with a defined orientation within a model membrane bilayer or in a cell membrane. Their fluorescence properties, which are dependent on solvent polarity and viscosity, were successfully applied to characterize organized media: for example, the CMC of surfactants and the transition temperature of DPPC liposomes were evaluated.

KEY WORDS: Amphiphilic probes; fluorescent molecular rotors; micelles; liposomes.

INTRODUCTION

Fluorescent molecular rotors are flexible fluorescent molecules which have been proven to be sensitive to both the local polarity and the local viscosity (rigidity) of the surroundings, in various solvents as well as inside organized assemblies or in polymers (solutions and solid matrices) [1–6].

Indeed, we have used these fluorescent probes widely to study polymer properties: their abilities were applied to detect the vitrification effect in bulk polymerization of MMA[4] into PMMA [7] or to assess hydrophobic microdomains in amphiphilic polymers [8,9].

Presently, our objective is to attach hydrophilic functional groups (such as a sugar or an inositol group) to fluorescent molecular rotors with the aim of obtaining impermeant and uncharged probes, with a defined orientation within a model membrane bilayer first or in a cell membrane for further investigations (fluidity, lipid–protein interactions, structural changes, location of proteins, etc.).

In a preceding study, we started development of such amphiphilic probes involving glucidic linkage onto pyrene derivatives [10].

The work is now pursued with molecular rotors because the conjugation, in addition to the aromatic ring, allows red-shift absorption of the probe, which is preferable for studies in cellular biology to overcome the intrinsic fluorescence of cell components (flavines, NADH, etc.).

In the present paper, we describe the new fluorescent molecular rotors' synthesis and photophysical properties. The fluorescence dependence on solvent polarity and viscosity was successfully applied to characterize organized media, for determination of the CMC of surfactants and temperature transition of DPPC liposomes.

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[4] *Abbreviations used*: CH_2Cl_2, methylene chloride; CMC, critical micellar concentration; CTAB, cetyltrimethylammonium bromide; DCCI, 1,3-dicyclohexylcarbodiimide; DMAP, 4-(*N,N*-dimethylamino)pyridine; DMF, *N,N*-dimethylformamide; DPPC, DL-α-dipalmitoylphosphatidylcholine; HOBT, 1-hydroxybenzotriazole hydrate; MMA, methylmethacrylate; NADH, nicotinamide adenine dinucleotide, reduced; PMMA, poly(methylmethacrylate); SDS, sodium dodecyl sulfate; SUV, small unilamellar vesicles; T_M, main phase transition temperature.

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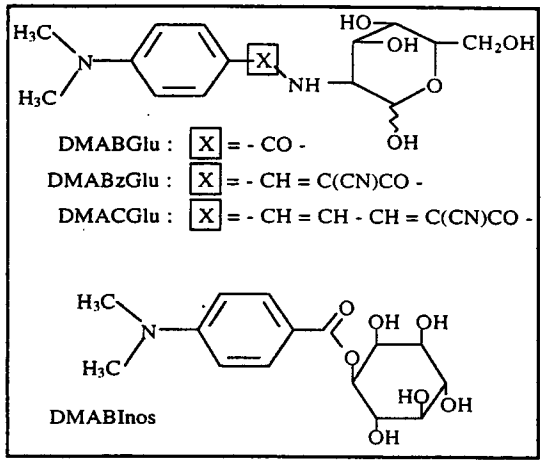

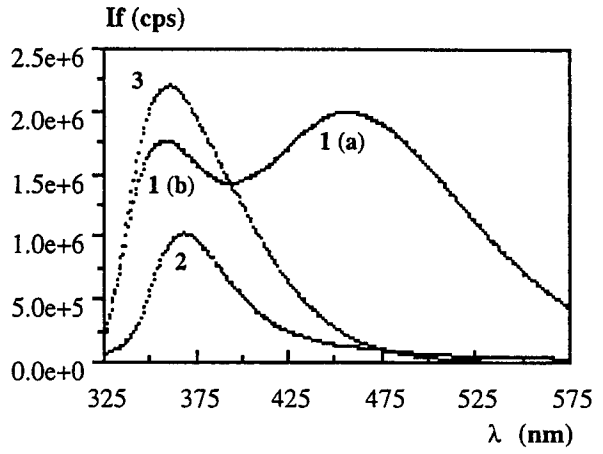

Fig. 1. Fluorescence spectra for the rotor DMABGlu in ethyl acetate 1 ($\Delta\lambda_{1/2}$ = 74 nm, band b; $\Delta\lambda_{1/2}$ = 144 nm, band a), cyclohexane 2 ($\Delta\lambda_{1/2}$ = 76 nm), and glycerol 3 ($\Delta\lambda_{1/2}$ = 92 nm) at 25°C (λ_{ex} = 300 nm).

EXPERIMENTAL

General Procedures

The parent molecular rotors were synthesized by a Knoevenagel condensation [11] involving an equimolar mixture of aldehyde [4-(*N*,*N*-dimethylamino) benzaldehyde or cinnamaldehyde] (Aldrich) and an active methylene compound (ethyl cyanoacetate) with 1.2 mol equiv of magnesium oxide in methanol at room temperature during 1 day, with respective yields of 96 and 78%.

Saponification of the ethyl ester rotors conducted with 8 mol eq of KOH in MeOH at room temperature for 10 h afforded the corresponding acids at 80 and 60% yields, respectively.

4-(*N*,*N*-dimethylamino)benzoic acid was obtained from Aldrich.

The glucosamine derivatives were obtained by amidation of each acid with 2-amino-2-deoxy D-glucose (whose hydroxyl groups were protected with acetyl functions to facilitate the reaction) with coupling reagents DCCI–HOBT in DMF–CH_2Cl_2 (hydroxyl groups were recovered by deacetylation with a catalytic amount of sodium methoxide) as described in Ref. 10 (yields: DMABGlu, 38%; DMABzGlu, 45%; DMACGlu, 40%).

DMABInos was prepared by esterification of 4-(*N*,*N*-dimethylamino)benzoic acid with a suitable diisopropylidene-protected inositol derivative [12,13] with coupling reagents DCCI–DMAP in CH_2Cl_2 (hydroxyl groups were recovered by acidic acetal removing, yield, 22%).

Small unilamellar vesicles (SUV) of DPPC (Aldrich) were prepared as follows: the phospholipid and the probe were dissolved in chloroform, and the solvent was removed using a rotary evaporator leaving a thin film which was suspended in Tris/HCl (0.1 *M* at pH 7.2) and sonicated for 15 min at 45°C (Vibra Cell Ultrasonic Instruments; 20 kHz).

Physicochemical Measurements

Absorption spectra were measured with a Perkin Elmer (Lambda 2) UV/visible spectrophotometer.

Fluorescence emission spectra were recorded on a SPEX Fluorolog-2 spectrofluorometer, equipped with a thermostated cell compartment (rotor concentration was in the range of $5\cdot10^{-6}$ to 10^{-5} *M*).

RESULTS AND DISCUSSION

Fluorescence in Organic Solvents

The absorption and fluorescence spectra of these new probes are analogous to the spectra of their parent compounds. The fluorescence wavelengths depend on the solvent polarity, whereas fluorescence intensities reflect a viscosity effect.

For DMABGlu (see Fig. 1) fluorescence spectra are characteristic of benzene derivatives, i.e., in polar solvents, such as EtOAc, a two-band fluorescence emission (dual fluorescence) is observed. It might correspond to two kinds of excited states: a higher-energy non-CT excited state (b state) with a rather planar conformation and a twisted intramolecular charge transfer state (TICT; a state) stabilized by solvation coming from the b state with a torsional motion of the dimethylamino group around the amino–phenyl bond. In a nonpolar solvent

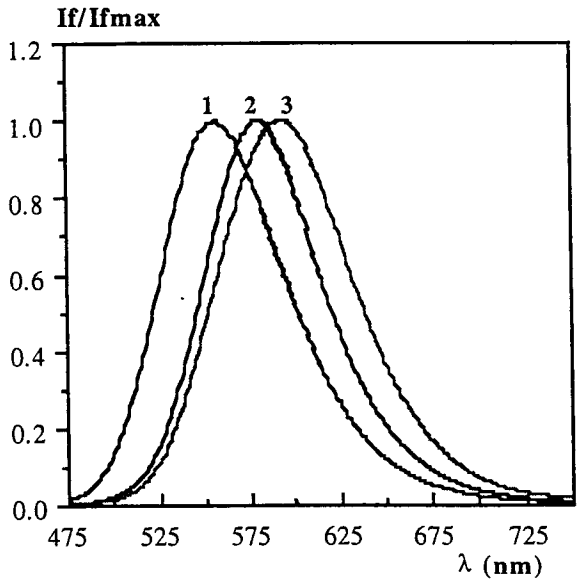

Fig. 2. Fluorescence spectra (normalized intensities) for the rotor DMACGlu in ethyl acetate 1 ($\Delta\lambda_{1/2}$ = 80 nm), methanol 2 ($\Delta\lambda_{1/2}$ = 75 nm), and glycerol 3 ($\Delta\lambda_{1/2}$ = 102 nm) at 25°C (λ_{ex} = 450 nm).

Table I. Absorption and Fluorescence Emission Data on Glucosamine Rotors in Solvents at 25°C[a]

Rotor	Solvent	Toluene	EtOAc	EtOH	MeOH	Gly-cerol	Water
	$E_T(30)$	33.9	38.1	51.9	55.4	57.0	63.1
	η	≈0.55	0.441	≈1.1	0.547	954	0.894
DMABGlu	$\lambda_{a\,max}$	289	294	301	303	307	306
	$\lambda_{f\,max}$	359	359	362	362	363	374
			457	497	508		
	Φ_f	0.067	0.310	0.053	0.020	0.270	0.004
DMABzGlu	$\lambda_{a\,max}$	412	406	410	411	421	422
	$\lambda_{f\,max}$	458	466	471	475	487	492
	Φf	0.002	0.0025	0.002	0.0018	0.076	0.0012
DMACGlu	$\lambda_{a\,max}$	450	441	450	451	471	457
	$\lambda_{f\,max}$	540	556	575	581	589	592
	Φ_f	0.005	0.018	0.033	0.033	0.344	0.015

[a] $E_T(30)$ (kcal·mol^{-1}), polarity parameter, values defined by Dimroth and Reichardt [14]; η (cP), viscosity; $\lambda_{a\,max}$ (nm), absorption wavelength at its maximum; $\lambda_{f\,max}$ (nm), emission wavelength at its maximum; Φ_f, fluorescence quantum yield.

(cyclohexane) and in a high-viscous solvent (glycerol), only the short wavelength band is observed.

For DMABInos, a behavior similar to that of DMABGlu was observed.

For DMABzGlu and DMACGlu, only one single band was observed, the signal of which was red shifted with increasing solvent polarity (see Fig. 2 for DMACGlu).

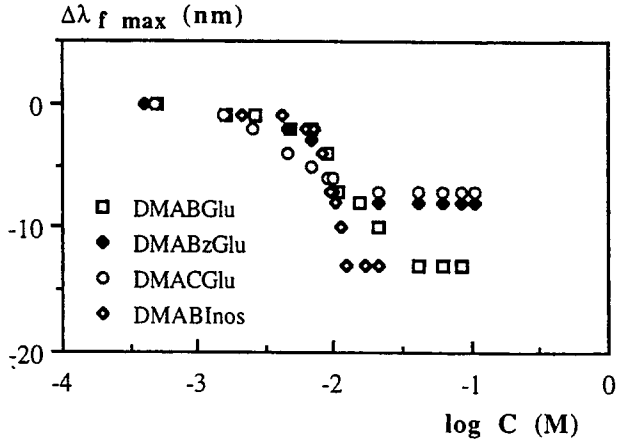

Fig. 3. Wavelength shift variations at the maximal fluorescence emission with SDS concentration for the different rotors, at 25°C.

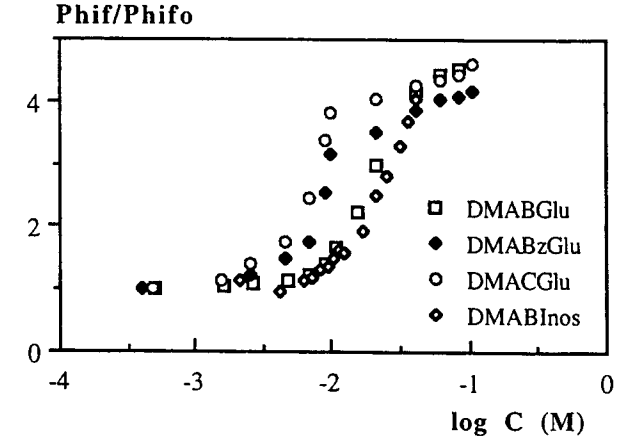

Fig. 4. Fluorescence quantum yield ratio Φ_f/Φ_{fo} with SDS concentration for the different rotors, at 25°C.

Photophysical data on glucosamine rotors in homogeneous solvents are gathered in Table I.

Fluorescence in Micelles: Determination of the CMC of Surfactants

The use of the new fluorescent molecular rotors, either with the polarity aspect based on the fluorescence emission wavelength value (see Fig. 3) or with the cohesion behavior according to the fluorescence emission efficiency (see Fig. 4), has allowed us to determine the CMC for surfactants of different classes: SDS (anionic), CTAB (cationic), and Triton X-100 (nonionic).

Results for SDS are shown in Figs. 3 and 4.

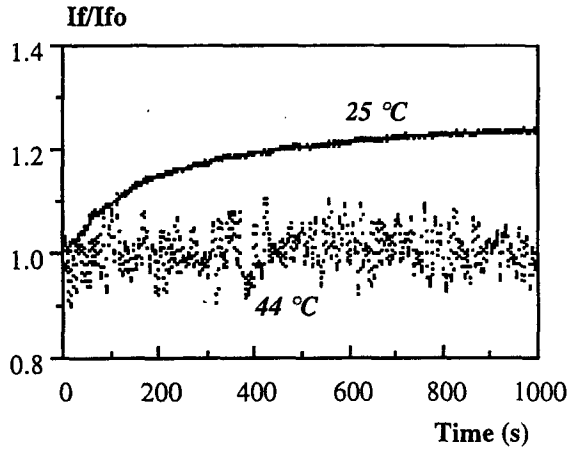

Fig. 5. Incorporation of DMABzGlu ($1.6 \cdot 10^{-5}$ *M*) into SUV of DPPC (10^{-3} *M*) at 25 and 44°C (λ_{ex} = 420 nm, λ_f = 475 nm; measurements made with the SPEX in the kinetic mode).

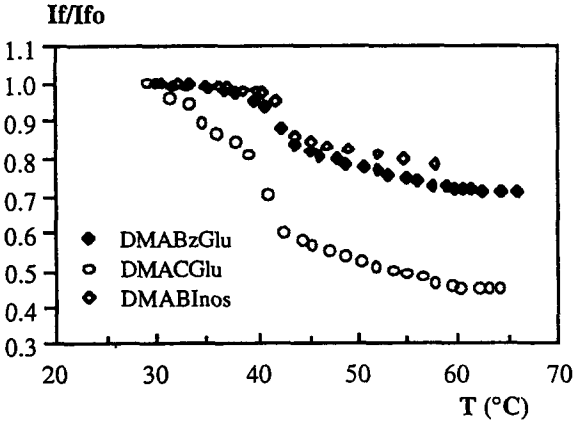

Fig. 6. Relative variation of fluorescence intensity versus temperature for different rotors included in SUV of DPPC. Ifo corresponds to the fluorescence intensity at 30°C. DMABzGlu: λ_{ex} = 420 nm; λ_f = 475 nm. DMACGlu: λ_{ex} = 460 nm; λ_f = 580 nm. DMABInos: λ_{ex} = 310 nm; λ_f = 359 nm.

Rotors as Polarity Probes

The blue shift of $\lambda_{f\,max}$, attributed to the polarity decrease in the probe's environment when the micelles are formed, provides the CMC value for the surfactant. In the case of SDS (see Fig. 3), the CMC values ($3.5 \cdot 10^{-3}$ to $7 \cdot 10^{-3}$ *M*) correlate well with the literature data (i.e., $8 \cdot 10^{-3}$ *M* [15]).

Rotors as Viscosity Probes

Molecular fluorescent rotor emission is generally viscosity dependent. An increase in the viscosity induces a diminution of the free volume of the medium, with a subsequent slowing-down of the motions of the rotor, if any. This leads to a diminution of the nonradiative relaxation processes, with a consequent increase in the fluorescence quantum yield Φ_f. This phenomenon is due to the lower mobility of the molecular rotor when the environmental medium is undergoing organization, which corresponds to a more viscous microenvironment for the probe.

The formation of micelles, which is accompanied by an increase in the local order, leads to an increase in the fluorescence quantum yield.

For SDS micelles (see Fig. 4), the fluorescence quantum yield ratio Φ_f/Φ_{fo} (where Φ_{fo} represents the fluorescence quantum yield in water) is roughly fourfold increased when the micelles are formed.

The CMC values calculated from Fig. 4 are in the range of $4 \cdot 10^{-3}$ to $6.3 \cdot 10^{-3}$ *M* and are in good agreement with the previous values.

Fluorescence in Liposomes: Detection of T_M

Incorporation of hydrophilic rotors in phospholipid vesicles was followed at two temperatures. As an example, Fig. 5 corresponds to the incorporation of DMABzGlu.

The increase in fluorescence intensity is representative of the incorporation of the rotor into SUV of DPPC: uptake of the probe is complete and rapid at a temperature above T_M (44°C), whereas the incorporation rate is lowered under T_M (25°C).

The fluorescence efficiency of the different hydrophilic rotors was used to characterize SUV of DPPC exhibiting a gel-to-liquid phase transition temperature near 41°C (see Fig. 6).

CONCLUSION

Glucidic and inositol derivatives of molecular rotors which are amphiphilic but electroneutral, thanks to the modifications of their photophysical features to polarity and viscosity variations of the surroundings, experience surfactant aggregation to form micelles and gel–liquid changes during the phase transition of SUV of DPPC.

As they insert almost-instantaneously into artificial membranes, they should find wide applications in the field of membrane research. Investigations in this field are in progress.

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