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Fluorescent molecular probes VI
The spectral properties and potential biological applications of water-soluble Dapoxyl™ sulfonic acid

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Abstract

Dapoxyl sulfonic acid was characterized as a new water-soluble fluorescent solvatochromic dye. This molecule contains a 'push–pull' electron transfer system from the 5-phenyl moiety to the 2-phenyl ring that generates the environment-sensitive fluorescence. The strong environment-sensitive fluorescence of Dapoxyl sulfonic acid can be explored to study a variety of biological events and processes, in addition to its long emission wavelength, large extinction coefficient, high fluorescence quantum yield and large Stokes shift and excellent photostability. ©2000 Elsevier Science S.A. All rights reserved.

Keywords: Fluorescence; Dapoxyl dyes; Oxazole; Solvent effect; Solvatochromic; Twisted intramolecular charge transfer

Abbreviations: 1,8-ANS, 8-anilinonaphthalene-1-sulfonic acid; 2,6-ANS, 6-anilinonaphthalene-2-sulfonic acid; Dapoxyl, 5-(4″-dimethylaminophenyl)-2-(4′-sulfophenyl)oxazole; DMSO, dimethylsulfoxide; DPO, 2,5-diphenyloxazole; TICT, twisted intramolecular charge transfer

1. Introduction

In the past decades fluorescence techniques have become firmly established and widely employed tools of analytical sciences, and are now routinely used in the detection, quantitation, identification and characterization of inorganic and organic compounds and of biological structures and processes [1,2]. Their inherently high sensitivity and selectivity have been the major driving forces for this development. This trend has also been greatly accelerated by the recent advances in fluorescent reagents and instrumentation [1,3,4].

Fluorescent molecules whose spectra or quantum yields are sensitive to their environments, are valuable in the study of heterogeneous media, organized media and biological media, and many fluorescent solvatochromic dyes have been developed for these applications [5–8]. Dansyl chloride has been used to make a variety of bioconjugates, whose environment-sensitive fluorescence has been successfully used to follow a variety of biological processes [9]. 8-Anilinonaphthalene-1-sulfonic acid (1,8-ANS) has been used to determine the relative hydrophobicities of the binding sites in a number of proteins and to detect the protein conformational changes induced by ligand binding [10]. Similarly, lanthanide ions were used as probes to determine the presence of water and its mobility at the cation binding sites of proteins [11]. Probes such as pyrene [6] and hypocrellins [12] were used extensively to study the local polarity of micelles, silica gel, zeolite and other organized media. These studies were directed to evaluate the microenvironment surrounding the fluorescent solvatochromic probes and such probes are of practical value in the development of fluorescent sensors.

Among these fluorescent solvatochromic dyes, we have demonstrated that Dapoxyl™ dyes are superior environment-sensitive fluorescent probes that possess long emission wavelengths, large extinction coefficients, high fluorescence quantum yields, large Stokes shift and great solvent sensitivity [13]. In this paper, we report the fluorescence properties of water-soluble Dapoxyl sulfonic acid, sodium salt (5-(4″-dimethylaminophenyl)-2-(4′-sulfophenyl)oxazole, sodium salt). Such a fluorescent compound is obviously promising probe for certain biological applications.

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2. Materials and methods

Absorption, excitation and fluorescence spectra were recorded on an Aminco SPE-5000 and an Aminco SPF-500C, respectively. NMR spectra were obtained on a Bruker YLIV370.040. β-cyclodextrin was obtained from Sigma Chemical (St. Louis, MO), and used as received. All the solvents of spectral grade and 2,5-diphenyloxazole were purchased from Aldrich Chemical (Milwaukee, WI), and were used without further purification. Dapoxyl sulfonic acid, sodium salt was prepared from Dapoxyl suflonyl fluoride as previously reported, and the reagent is currently commercially available from Molecular Probes, Inc. (Eugene, Oregon). Dioleoyl phosphatidylethanolamine (DOPE) liposome was prepared according to the procedure of Szoka [14].

The fluorescence quantum yields were determined using quinine sulfate in 5 M H_2SO_4 as the reference standard (Φ_F=0.55) [15]. The concentrations of the samples were adjusted to obtain an absorbance of 0.25 (1 cm cell) at the peak wavelength. The concentration of the reference was also adjusted to have an absorbance of 0.25 at the same excitation wavelength of the dye tested. Under these conditions, the fluorescence quantum yield of the tested compound (Φ_F^X) in the indicated solvent was calculated from the following formula, considering that the peak area (A_R) of the emission spectrum of the reference and that of the tested dye (A_X) can be readily determined:

$$\Phi_F^X = \frac{A_x \Phi_F^R}{A_R}$$

where Φ_F^R and Φ_F^X are the fluorescence quantum yields of the reference and the testing dye, respectively. The measurements were done in triplicate and the estimated errors were no more than 1%.

3. Results and discussion

3.1. Excitation and fluorescence spectra

The excitation and fluorescence spectra of the Dapoxyl sulfonic acid were shown in Fig. 1. The excitation and fluorescence spectra of the Dapoxyl dye are considerably red shifted and broadened relative to that of 2,5-diphenyl oxazole (the parent compound). Additionally, the Dapoxyl sulfonic acid demonstrated much stronger solvent-dependent fluorescence than 2,5-diphenyloxazole. These spectral properties imply that the observed longer excitation and fluorescence bands of the Dapoxyl sulfonic acid might result from intramolecular charge transfer involving the dimethylamino moiety and the sulfonyl group (Scheme 1). This is consistent with our previous observations [13].

The excitation wavelength and intensity of the Dapoxyl sulfonic acid are fairly insensitive to the solvent polarity or

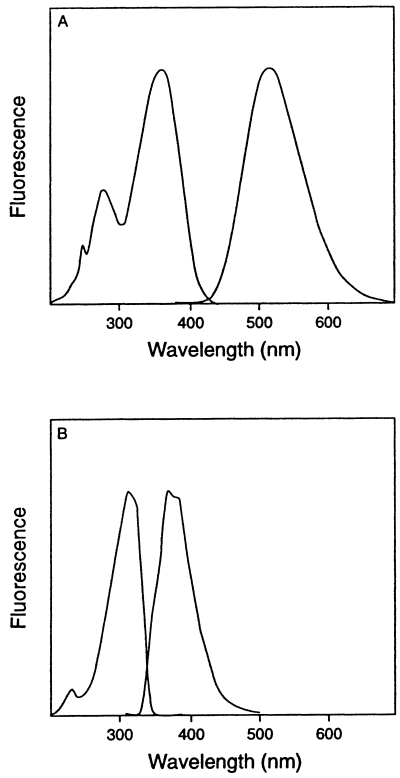
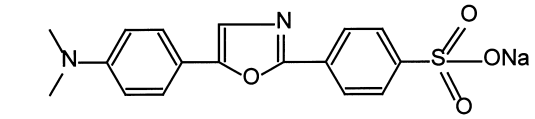
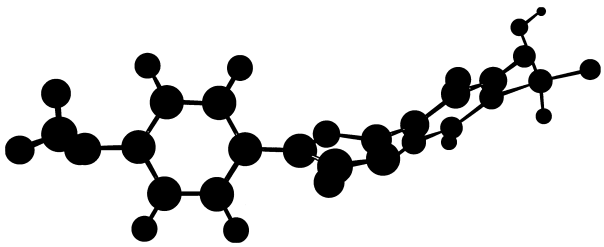


Fig. 1. The excitation and fluorescence spectra of Dapoxyl sulfonic acid, sodium salt and DPO in methanol (1 μM).

the hydrogen-bonding ability of the solvent (data not shown). This insensitivity indicates that the interaction of the participating ground and excited states with the solvent is such that the energy gap between the ground state and the corresponding Franck–Condon excited states responsible for the observed electronic transition is independent of the solvent polarity and hydrogen-bonding ability.

Unlike the excitation spectra, both the fluorescence wavelength and quantum yield of the Dapoxyl sulfonic acid change dramatically with the solvents of different polarity. For example, the fluorescence of Dapoxyl sulfonic acid





Scheme 1. The chemical structure and MOPAC-optimized configuration of Dapoxyl sulfonic acid, sodium salt.

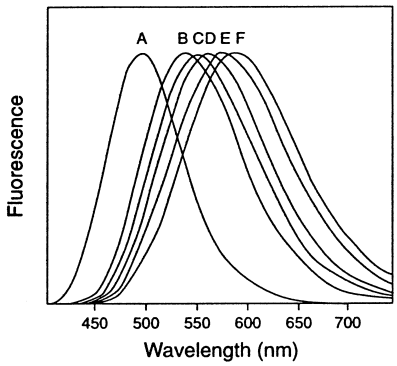


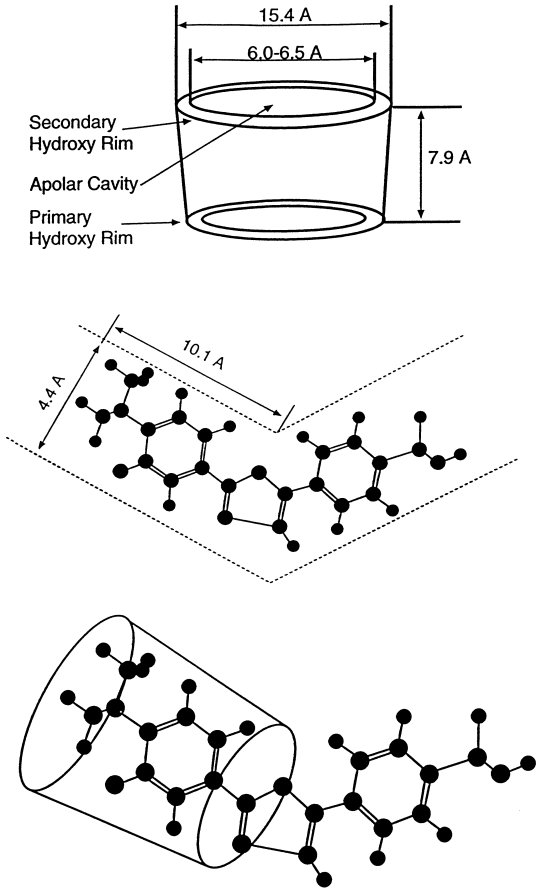
Fig. 2. The fluorescence spectra of Dapoxyl sulfonic acid, sodium salt in acetonitrile-water (1 μM). A=acetonitrile; B=4:1 acetonitrile/water; C=3:2 acetonitrile/water; D=2:3 acetonitrile/water; E=1:4 acetonitrile/water; F=1:9 acetonitrile/water.

shows a 144 nm red shift when the solvent is changed from acetonitrile to 9:1 water-acetonitrile (see Fig. 2). The red-shifted and broadened spectra of Dapoxyl sulfonic acid with increase in solvent polarity indicates the intramolecular charge transfer of the molecule at excited states [16], which are also very likely to be twisted in geometry, given the flexible structure of the Dapoxyl sulfonic acid. The twisted structure of the excited states of the Dapoxyl sulfonic acid was supported by MOPAC and MM2 derived force field calculations as shown in Scheme 1 [17]. Thus, the broad featureless emission might be assigned to twisted intramolecular charge transfer (TICT). The marked solvent sensitivity of the Dapoxyl sulfonic acid is derived from the large dipole moment developed in the excited state as a consequence of facile charge delocalization between the dimethylamino moiety and the sulfonyl group.

3.2. Cyclodextrin systems

Cyclodextrins (CDs) are water-soluble cyclic oligosaccharides which, with their torus shape and relatively apolar interior, can selectively incorporate molecules on the basis of size and polarity characteristics. The commonly available cyclodextrins are α-CD (cyclohexaamylose), β-CD (cycloheptaamylose), and γ-CD (cyclooctaamylose), characterized by increasing cavity diameters. It is well known that the less polar cavity of CDs effectively binds many dyes, and thus changes their excitation and fluorescence spectra [7,18]. These CDs have been used as effective models to study various interactions in biological systems.

The molecular modeling of Dapoxyl sulfonic acid indicated that the compound is a 'V' shaped molecule consisting of two 4.4×10.1 Å wings with an angle of 118° (Scheme 1), which matches well the β-CD cavity opening of 6.5 Å diameter (Scheme 2). The absorption spectrum of Dapoxyl sulfonic acid in the presence of β-CD (1 mM) showed very little change with respect to that in water (data not shown). Only a slight red shift (see Table 1) and little change in the molar excitation coefficients were observed in the aqueous



Scheme 2. The interaction of Dapoxyl acid, sodium salt with β-cyclodextrin at ground state. A: The dimensions of β-cyclodextrin; B: The MM2-optimized configuration of Dapoxyl sulfonic acid, sodium salt at ground state; C: the incorporation of Dapoxyl sulfonic acid, sodium salt into β-cyclodextrin.

CD solutions compared with CD-free solution. This mimics the excitation change of the Dapoxyl sulfonic acid from a more polar solvent to a less polar solvent in organic systems (*vide surpra*). However, such little change in the excitation spectra of the dye makes excitation spectroscopy difficult to use as an effective way to study the CD-Dapoxyl dye complexation.

Fluorescence is a property of the excited state, and, in principle, it should not be used to study ground-state complexation. However, given the extremely short singlet lifetime involved, it is safe to assume that fluorescence

Table 1
Spectral properties of Dapoxyl sulfonic acid, sodium salt in aqueous β-cyclodextrin and aqueous ethanol solutions

Composition	λ_{max} (nm)	λ_F (nm)	ϕ_F	Stokes shift (nm)
100% water	348	584	0.04	236
Water+3 mM β-CD	353	560	0.31	205
100% ethanol	357	500	0.72	143
80% ethanol–20% water	356	524	0.70	168
50% ethanol–50% water	356	547	0.48	191
20% ethanol–80% water	354	562	0.30	208

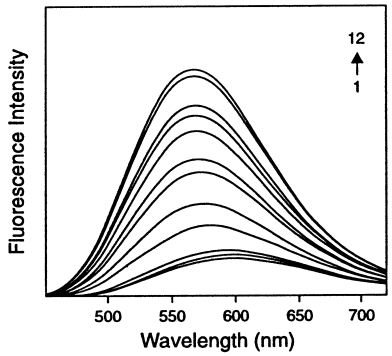


Fig. 3. The fluorescence spectra of Dapoxyl sulfonic acid, sodium salt (2 μM) in the presence of different concentrations of β-cyclodextrin. 1: [CD]=0 μM; 2: [CD]=1 μM; 3: [CD]=10 μM; 4: [CD]=50 μM; 5: [CD]=100 μM; 6: [CD]=150 μM; 7: [CD]=200 μM; 8: [CD]=300 μM; 9: [CD]=400 μM; 10: [CD]=500 μM; 11: [CD]=700 μM; 12: [CD]=1000 μM.

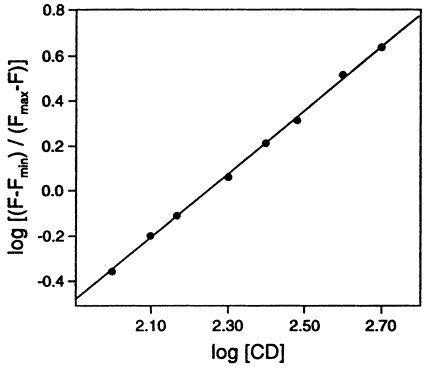


Fig. 4. The plot of $\log[(F-F_{\min})/(F_{\max}-F)]$ against [CD], the concentration of β-cyclodextrin. The concentration of Dapoxyl sulfonic acid, sodium salt is 0.2 μM, and those of β-cyclodextrin are changed from 100 to 1000 μM. The fluorescence spectra was obtained using 365 nm excitation, the isobestic point.

deactivation is much faster than complexation. This allows us to use this property to investigate ground-state complexation of the Dapoxyl sulfonic acid with CDs. As shown in Fig. 3, a significant fluorescence enhancement with a corresponding blue shift is observed upon complexation of Dapoxyl sulfonic acid with β-CD. A quantitative treatment of the fluorescence changes is complicated by the fact that both complexed and uncomplexed dyes emit. The association constant of Dapoxyl sulfonic acid with β-CD can be obtained from the following equation assuming the 1:1 stoichiometry of Dapoxyl sulfonic acid-β-CD complex and considering that the isobestic point wavelength of the excitation spectra of Dapoxyl sulfonic acid (in the presence of different concentrations of β-CD) is the excitation wavelength [19]:

$$\frac{(F - F_{\min})}{(F_{\max} - F)} = K_a[\mathrm{CD}]$$

where [CD] represents the concentration of β-CD; $F_{\max}$ and $F_{\min}$ are the fluorescence intensities of Dapoxyl sulfonic acid in the absence of β-CD and in the presence of saturated β-CD, respectively; F is the fluorescence intensity of Dapoxyl sulfonic acid in the presence of a certain β-CD concentration, K_a is the association constant of Dapoxyl sulfonic acid with β-CD. As shown in Fig. 4, the plot of $\log[(F-F_{\min})/(F_{\max}-F)]$ against log[CD] is quite linear (correlation coefficient=1.014), indicating that the complexation of Dapoxyl sulfonic acid with β-CD might have 1:1 stoichiometry. The association constant of Dapoxyl sulfonic acid with β-CD is determined to be 5488 M^{-1}, which is greater than those of β-CD with fluoresceins [20] and rhodamines [21], the classic fluorescent dyes, indicating that β-CD has higher affinity for Dapoxyl sulfonic acid.

It has been reported by numerous workers that the polarity of β-CD cavity is similar to that of ethanol. Recently Hansen and co-workers [22] measured the rate of excited-state proton transfer of the complexes of 1-aminopyrene and β-naphthol with a modified β-CD. These authors concluded that the basicity in the proximity of the CD hydroxyl rim is similar to that of a 1:4 water/ethanol mixture. Thus, the fluorescence spectra of Dapoxyl sulfonic acid in the aqueous CD solutions were compared with those in aqueous ethanol. As seen in Table 2, the fluorescence wavelength, quantum yield and Stokes shift of the compound in saturated β-CD solutions were quite close to those in 4:1 water-ethanol, rather than 100% ethanol or 1:4 water/ethanol. This result can be explained by assuming the partial incorporation of Dapoxyl sulfonic acid into β-CD due to the size of Dapoxyl sulfonic acid as shown in Scheme 2. This hypothesis was supported by a calculation using an intermolecular interaction model as described in the manual of Alchemy™ 2000 [17].

Table 2
Determination of critical concentrations of micellar solutions using Dapoxyl sulfonic acid

Micellar systems	Critical concentrations (mM)	
	This work	Literature[a]
Sodium dodecyl sulfate	8.2	8.0
Cetyl trimethylammonium bromide	1.0	0.92
β-D-octylglucupyranosides	21	25

[a] From [23].

3.3. Micellar and liposome systems

Micelles are conglomerates of surface-active species in solution. A number of fluorescent compounds have been used to probe the micellar microenvironment [6]. Among these probes, pyrene is the most widely used one because of the well-established variation of its emission spectrum with the polarity of the immediate surroundings. However, the poor water solubility and small environment-dependent fluorescence change cause some practical problems of using pyrene, in particular, for aqueous biological systems. For example, the intensity ratio of the first (372 nm) and third (383 nm) peaks in the fluorescence spectrum of pyrene is

defined as the environment-sensitivity parameter, and this value changes only from 1.89 (in dimethylsulfoxide) to 0.56 (in methylcyclohexane). Additionally, the short wavelength of pyrene often overlaps with the fluorescence of biological constituents such as tyrosine, tryptophan, NADH and NADPH, etc.

The strong environment-dependent fluorescence of the Dapoxyl sulfonic acid was used to study micellar systems. As shown in Table 2, the critical concentrations of three typical micelles were readily obtained by either fluorescence wavelength or intensity changes of the compound. These results are consistent with the previously reported data [23]. The new probe is more easily used due to its larger spectral response.

Liposomes are closed spherical phospholipid vesicles. In the past decades, liposomes have gained popularity as model membranes and drug delivery systems [24–27]. Some fluorescent compounds have been used to investigate a variety of liposomes. Our preliminary data (not shown) indicated that the strong environment-sensitive fluorescence of the Dapoxyl sulfonic acid, sodium salt, might be readily explored to probe the microenvironment of liposomes and the interactions between drugs and liposomes. We believe that the Dapoxyl sulfonic acid is a convenient probe to study a variety of micelles, liposome and membranes.

4. Conclusions

As discussed above, Dapoxyl sulfonic acid is an attractive fluorescent probe because of its long emission wavelength, large extinction coefficient, high fluorescence quantum yield, large Stokes shift, great environment sensitivity and excellent photostability. The fluorescence changes of the dye are well correlated with the polarity of solvent, solvent composition or the surrounding environment, e.g., polarity parameter $E_T(30)$. This fluorescence-environment dependence can be used to develop new fluorescent molecular probes to study a variety of biological events and processes. For example, We have prepared Lysotracker™ Blue/White, a Dapoxyl sulfonic acid derivative, to selectively label lysosomes of live cells. The probe is primarily localized in lysosome membranes and emits blue fluorescence, whereas the remaining population of the dye is localized in cytoplasm fluoresces white. This phenomenon was predicated from the environment-sensitive fluorescence of the Dapoxyl dye (see Fig. 2). The similar phenomena were also observed with ER Tracker™ Blue/White that is another Dapoxyl sulfonic acid derivative. The compound has been used to selectively stain the endoplasmic reticulum of live cells. The Dapoxyl sulfonic acid derivatives have proven to be particularly attractive probes in ratio imaging because the environment-sensitivity of the Dapoxyl sulfonic acid derivatives is manifested as an alteration of both fluorescence wavelength and intensity that is readily detectable in living cells with fluorescence ratio imaging.

Furthermore, the development of Dapoxyl dye-based fluorescent molecular probes should be facilitated by the ready availability of a versatile set of reactive Dapoxyl sulfonic acid derivatives.

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