

Compared Behavior of Hydrophobic Fluorescent NBD Probes in Micelles and in Cyclodextrins

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Four hydrophobic NBD derivatives, differing by the length of the fatty chain, were introduced in micelles of Triton X100 as well as in α - and β -cyclodextrins. The spectroscopic data indicated that the probes were more efficiently protected from water in micelles than in cyclodextrins. The insertion of the probes in both media was discussed.

KEY WORDS: NBD; micelle; cyclodextrin; fluorescence.

For a number of years, supramolecular organized assemblies have been of interest to the pharmaceutical chemist, either as drug carrier or more recently as targeting system. Both non-ionic micelles and cyclodextrins (CD) have been widely investigated as a means of producing a clear stable solution of poorly water-soluble drugs, suitable for intravenous administration [1,2].

In the present work, probes based on the nitro-benzoxadiazolyl (NBD) group were used as models of hydrophobic compounds, to gain new insights into the solubilization processes. Probes of this family display the distinct advantage of being very sensitive to their environment, and their solvatochromic properties are now well known [3]. This is the reason why they have been extensively used for the study of biological membranes [4] and, more recently, of the micellar medium [5]. The NBD moiety was directly grafted on four alkyl amines, the chain length of which varied from 3 to 18 carbon atoms (Scheme 1). In a previous work, these probes were

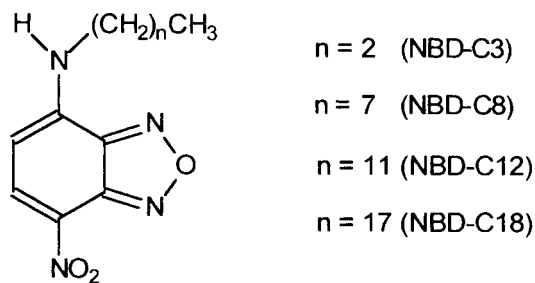
incorporated into anionic, cationic, and neutral micelles, with the aim to study the influence of the alkyl chain length upon the fixation of the fluorophore into the micelle [6]. The spectroscopic properties of these dyes were investigated here in the presence of native α and β cyclodextrins. They were compared with the spectroscopic characteristics previously obtained with Triton X100, a neutral surfactant [6].

In the NBD probes, the nitrogen atom undergoes a strong electron-withdrawing effect from the nitro-benzoxadiazolyl group. Consequently, these dyes were not protonated in aqueous solution at a pH close to neutral and were very hydrophobic. To have them solubilized in water, as well as in micellar and CD solutions, it was necessary that the probes were first dissolved in ethanol. Then an aliquot of the ethanolic solution was added to the aqueous solution. The concentration in ethanol was 1.3% v/v for the surfactant solution and 3% v/v for water and CD solutions. Using this method, the solubility limit of the probes was 3×10^{-4} M for NBD-C3. It was found to be below 1×10^{-6} M for the other members of the series. In the presence of TX100 (10^{-2} M), the four probes were soluble at a concentration of 2×10^{-5} M.

Below the solubility threshold, in all the media investigated here, the absorption spectrum of each com-

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Scheme 1. Chemical structure of NBD-labeled alkylamine.

pound showed an intense charge transfer band at high wavelength. The absorption maxima were very close to the excitation maxima reported in Table I. For higher probe concentrations, the solutions showed a red glint, and a shoulder was observed at high wavelength, indicative of the presence of dye microcrystals [6]. It is interesting to note that a discrepancy was observed in pure water regarding the position of the absorption maximum of NBD-C12. This maximum was situated at a lower wavelength than that of the other members of the series. This discrepancy disappeared in the presence of micelles and CD. For NBD-C3, NBD-C8, and NBD-C12, in the presence of TX100 (10^{-2} M), the absorption spectrum was shifted towards the blue, by comparison with the spectrum in pure water. The shift reached 10 nm for NBD-C8 and

Table I. Fluorescence characteristics of NBD probes in different media. Excitation (λ_{ex}) and emission (λ_{em}) maximum wavelength, and fluorescence quantum yield (error 15%). The excitation spectra were recorded at the emission maximum, the emission spectra were obtained by exciting at the absorption maximum. *a*: From ref [6].

		λ_{ex}	λ_{em}	$\Phi \times 10^2$
NBD-C3	H ₂ O	482	559	1.7
	TX100	478	545	3.6
	α -CD	480	556	2.2
	β -CD	481	549	2.5
NBD-C8	H ₂ O	482	559	1.4
	TX100 ^a	472	539	6.4
	α -CD	479	552	2.3
	β -CD	480	549	2.5
NBD-C12	H ₂ O	466	560	—
	TX100 ^a	472	536	7.2
	α -CD	478	552	2.9
	β -CD	478	549	2.5
NBD-C18	H ₂ O	482	560	—
	TX100 ^a	472	537	6.5
	α -CD	472	548	0.8
	β -CD	475	548	0.8

NBD-C18. In β -CD (10^{-2} M) solution, the absorption spectrum was close to that obtained in water. The presence of α -CD also had a weak effect upon the absorption spectrum.

For fluorescence measurements, the dye concentration was 1.5×10^{-6} M for NBD-C3 to NBD-C12, and 1×10^{-6} M for NBD-C18. The four probes showed similar emission properties in pure water (Table I). In the presence of TX100, a strong blue shift of up to 24 nm was observed for the long chain probes. The shift was weaker for NBD-C3. The presence of β -CD led to a moderate blue shift (10–12 nm), whatever the probe. Addition of α -CD led to similar or weaker shifts. The quantum yield in water could only be measured with accuracy for NBD-C3 and NBD-C8. An increase in the quantum yield was observed in the presence of micelles and CD, by comparison with water. For instance, the quantum yield of NBD-C8 was increased by 4.5 in TX100 and by 1.8 in the presence of β -CD. For NBD-C3, the quantum yield in TX100 was about half that measured for long-chain probes. This suggests either that the location of this probe in the micelle is different, or that a proportion of the probe remains in the aqueous phase. In contrast, a similar quantum yield was encountered for NBD-C3 to NBD-C12 in the presence of CD, this value only collapsing for NBD-C18. The variations in the fluorescence properties indicate that the dye environment was less polar and protic in the presence of TX100 or CD than in pure water. The dye protection against the water environment seems to be more effective in micelles. For TX100, it is likely that the dyes were located in the interfacial region, presumably between the surfactant polar heads [6]. For CD, the exact nature of the interaction with the dyes must still be precised.

The strength of the interaction with CD was analyzed. The increase of the fluorescence intensity was monitored while the β -CD concentration was allowed to vary from 0 to 10^{-2} M (Fig. 1). The data were processed, assuming a 1:1 interaction. A satisfactory fit was obtained. The association constant was found to be $55 \pm 10 \text{ M}^{-1}$ and $1550 \pm 100 \text{ M}^{-1}$ at 23°C for NBD-C3 and NBD-C8 with β -CD, respectively. The constant found for NBD-C3 is weak compared with the mean binding constants reported for 1:1 inclusion complexes of CD [7]. However, the constant found for NBD-C8 corresponds to a fairly stable complex.

The possibility for dyes to be incorporated in the CD cavity was examined. A molecular modeling was performed by taking into account a solvent cage. To build a model of β -CD, the structure of a β -CD hydrate clathrate, determined by X-rays analysis, was taken from the literature as previously described [8]. It appeared that the

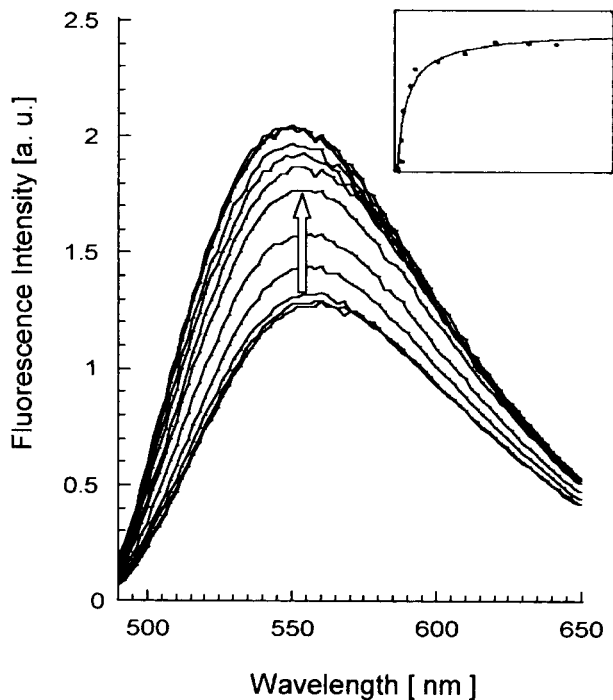


Fig. 1. Fluorescence spectrum of NBD-C8 (1.5×10^{-6} M) in deionized water (3% ethanol) in the presence and absence of β -CD, at 19°C. From bottom to top: $[\beta\text{-CD}] = 0$ to 1×10^{-2} M. At this dye concentration, the fluorescence intensity did not vary during many hours. Insert: data fit assuming a 1:1 stoichiometry.

NBD aromatic cycle was centered at the middle of the CD cavity, with the nitro group protruding at the exterior. No hydrogen bond was evidenced between NBD and CD, and no water molecule remained inside the cavity. It is also known that alkyl chains can be well incorporated in

α -CD [2]. The incorporation of NBD probes within the β -CD cavity could agree well with the effects observed in fluorescence spectroscopy. However, no direct proof of inclusion could be obtained. Different methods were used, and failed to provide a solid complex of the dyes with β -CD. Consequently, no X-rays analysis could be undertaken.

Some information about the heterogeneity of interaction sites was looked for. The dependence of the emission spectrum upon the excitation wavelength was analyzed. With TX100, a shift of the emission maximum by 7–10 nm was observed for the four probes, when the excitation wavelength was allowed to vary between the maximum of the absorption band and its red-edge [6]. This effect has been mainly attributed to a slow exchange between different solubilization sites at the micelle surface (e.g., more exposed to water or buried deeper between the head groups). In the presence of β -CD, a 6 nm-shift was observed in the same conditions, whatever the probe used. Therefore, the magnitude of the red-edge effect was slightly higher in micellar medium than in β -CD.

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