Molecular Umbrellas

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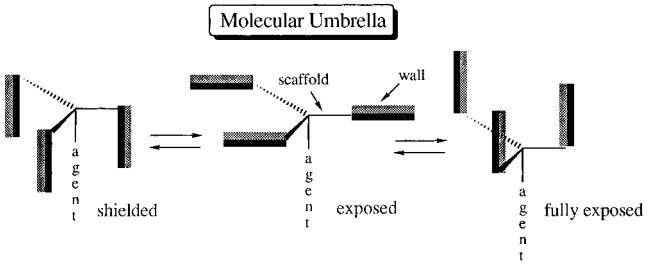
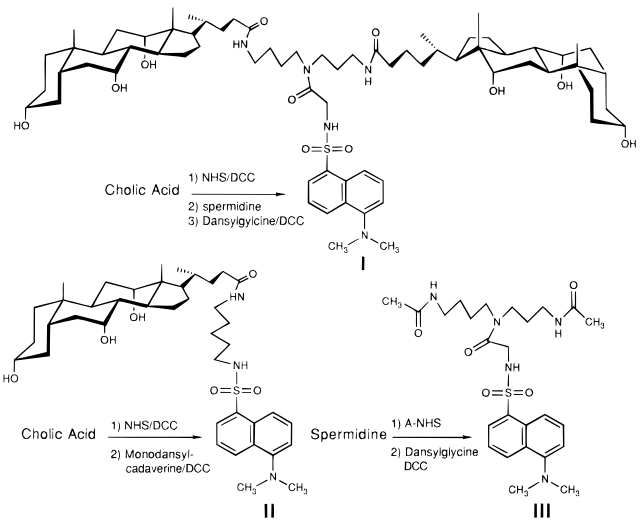
In this paper we introduce a new concept in surfactant chemistry that is based on molecules that mimic the structure and function of umbrellas, i.e., molecules that can cover an attached agent and shield it from an incompatible environment. We term such structures "molecular umbrellas" and report herein the first representative example. Potential applications of this new class of compounds in the areas of drug design and drug delivery are briefly discussed.



Our construction of molecular umbrellas hinges on the use of amphiphilic molecules that maintain a hydrophobic as well as a hydrophilic face.[1−3] In essence, two or more such amphiphiles (umbrella walls) are coupled to a suitable scaffold either before or after a desired agent is attached to a central location. Under appropriate environmental conditions, the amphiphilicity of each wall combines with the hydrophobicity or hydrophilicity of the agent to produce a "shielded" conformation. For those agents that are hydrophobic, "immersion" in water favors a shielded conformation such that intramolecular hydrophobic interactions are maximized and the external face of each wall is hydrated. When immersed in a hydrocarbon solvent, the umbrella favors a fully exposed conformation where solvation and intramolecular dipole−dipole and hydrogen-bonding interactions can be optimized. For those umbrellas that bear a hydrophilic agent, these same forces are expected to produce shielded and fully exposed conformations in hydrocarbon and aqueous environments, respectively, i.e., the opposite conformational preferences.

In this study, we have prepared an umbrella molecule using cholic acid as "wall material", spermidine as the scaffold, and an environmentally-sensitive fluorescent probe, 5-dimethyl-amino-1-naphthalenesulfonyl (dansyl), as the agent. Due to the relative hydrophobicity of the dansyl moiety, it was anticipated that such umbrellas would favor exposed or fully exposed conformations in solvents of low polarity and a shielded conformation in water. Cholic acid was specifically chosen because it possesses the requisite amphilicity and because it can be readily conjugated through its carboxylic acid group.[2,4] In addition, umbrella frameworks derived from cholic acid and spermidine were expected to be potentially biocompatible, since both compounds occur naturally in mammalian cells.

Scheme 1



Activation of cholic acid by conversion to its *N*-hydroxysuccinimide ester, followed by condensation with the primary amino groups of spermidine and subsequent coupling with dansylglycine afforded the double-walled umbrella, **I** (Scheme 1). An analogous single-walled structure (**II**) was also prepared using monodansylcadaverine as starting material. Compound **III**, which was chosen as a control, was synthesized by acetylation of spermidine with acetic acid *N*-hydroxysuccinimide ester (A-NHS), followed by condensation with dansylglycine.[5]

The fluoresence emission spectra of 2 μM solutions of **I** in varying dimethoxyethane/water mixtures are shown in Figure 1. Incremental replacement of DME with water resulted in a continuous decrease in fluorescence intensity and a shift in λ_{max} to longer wavelengths, until a composition of 10/90 (DME/water, v/v) was reached. Further increases in water content resulted in a *reversal* in the spectral changes; i.e., the fluorescence intensity increased and λ_{max} shifted toward shorter wavelengths.[6] In pure DME, water, and hexane, the fluorescence intensities that were observed were very similar; the corresponding λ_{max} values were 501, 496, and 495 nm, respectively. In sharp contrast, incremental increases in water content for DME/water solutions of **II** produced a continuous shift in λ_{max} to longer wavelenghs and a continuous decrease in fluorescence intensity *over the entire range of solvent mixtures used* (in 100% water, the observed fluorescence was barely detectable); exactly analogous behavior was observed for **III**.

The observed changes in the fluorescence emission spectrum of **I** with increasing water content, on going from pure DME

(1) (a) Stein, T. M.; Gellman, S. H. *J. Am. Chem. Soc*. **1992**, *114*, 3943. (b) McQuade, D. T.; Barrett, D. G.; Desper, J. M.; Hayashi, R. K.; Gellman, S. H. *J. Am. Chem. Soc.* **1995**, *117*, 4862.

(2) (a) Cheng, Y.; Ho, D. M.; Gottlieb, C. R.; Kahne, D.; Bruck, M. A. *J. Am. Chem. Soc*. **1992**, *114*, 7319. (b) Venkatesan, P.; Cheng, Y.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 6955.

(3) Burrows, C. J.; Saute, R. A. *J. Inclusion Phenom*. **1987**, *5*, 117.

(4) Hjelmeland, L. M.; Nebert, D. W.; Osborne, J. C. *Anal. Biochem*. **1983**, *130*, 72.

(5) Dansylgycine, monodansylcadaverine, and A-NHS were commercially available (Sigma) and used as obtained. Compounds **I**, **II**, and **III** gave satisfactory ^{1}H NMR (360 MHz) and HRMS analyses.

(6) The cmc of **I**, which was determined in pure water by UV methods (Shoji, N.; Ueno, M.; Meguro, K. *J. Am. Oil Chem*. **1978**, *55*, 297) was 2.5 μM. In addition, the fluorescence intensity of **I** in pure water was directly proportional to its concentration within the range of 0.5 and 5.0 μM; a discontinuity at the 5.0 μM concentration, however, was noted. This discontinuity is presumed to reflect the onset of aggregation. The fact that the "turning point" for **I** (i.e., the DME/water ratio at which a minimum fluorescence intensity is observed) occurs at exactly the same DME/water ratio (10/90 v/v) when 1.0 and 0.5 μM solutions of umbrella are employed also argues against the possibility that this turning point reflects a critical micelle concentration.

(7) MacGregor, R. B.; Weber, G. *Nature* **1986**, *319*, 70.

(8) In preliminary studies, we have found that a molecular umbrella can enhance the partitioning of a polar compound from water into an organic phase. Thus, spermidine, bearing a cholic acid group at each end, significantly enhances the partitioning of picric acid from water into chloroform. Although the structure of this umbrella/picric acid complex remains to be established, a likely possibility is one in which the umbrella adopts a shielded conformation that covers over picric acid and holds it in place through acid/base interaction with the secondary amine group of spermidine: Vigmond, S.; Janout, V.; Regen, S. L., unpublished results.

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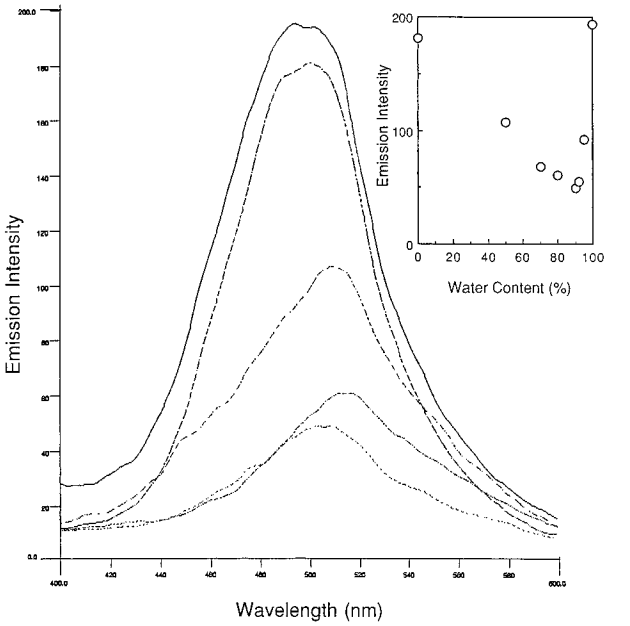

Figure 1. Representative fluorescence emission spectra of 2 μM solutions of **I** in DME/water mixtures measured at 23 °C. Highest to lowest intensity spectra were observed using the following ratios of DME/water (v/v): 0/100 (pure water), 100/0 (pure DME), 50/50, 20/80, and 10/90. The excitation wavelength and spectral bandwidth used in all cases were 330 and 5 nm, respectively. Inset: Plot of relative emission intensity at λ_{max} for **I** as a function of water content (v/v) for a series of DME/water mixtures measured at 23 °C. Similar data were obtained using 0.5 and 1.0 μM concentrations of the molecular umbrella.

to 10/90 DME/water (v/v), clearly reflect an increase in polarity of the microenvironment surrounding the dansyl moiety.[7] Such changes indicate the presence of exposed and/or fully exposed conformations. The dramatic reversal in these spectral changes that accompanies further increases in water content shows that **I** functions like an umbrella by shielding the dansyl moiety in highly polar media. In this regard, the λ_{max} for **I** in water, which lies between those observed in DME and in hexane, reflects a microenvironment that is very hydrophobic. The fact that a single-walled analog (**II**) cannot effectively shield the dansyl moiety indicates that a minimum of two walls is necessary in order to have a functional umbrella. This finding also provides compelling evidence that the shielded conformation of **I** has *both* walls covering over the dansyl group; with **II**, one side of the fluorophore must remain completely exposed to the solvent at all times.

Molecular umbrellas of the type reported herein offer intriguing possibilities for creating new classes of drugs and drug carriers. One can envision, for example, the sequestration of cytotoxic, nonpolar cross-linking agents within an umbrella (agents that would otherwise decompose in water or react with water-soluble nucleophiles) and their release into target membranes by "flipping" from a shielded to a fully exposed state. Alternatively, molecular umbrellas may serve as novel vehicles for transporting polar drugs across biological membranes. Specifically, a logical sequence of events would be (i) diffusion of a drug/umbrella conjugate to a biomembrane surface in a fully exposed state, (ii) insertion into the outer monolayer leaflet by flipping into a shielded state, (iii) diffusion to the inner monolayer leaflet, and (iv) entry into the cytoplasm via the sequential reversal of steps ii and i.

Efforts aimed at exploiting molecular umbrellas with a view toward drug design and drug delivery are now under intensive investigation in our laboratories.[8]

Acknowledgment. We are grateful the National Institutes of Health (PHS Grant GM51814) for support of this research.

Supporting Information Available: One figure containing the data used to determine the cmc of **I** (1 page). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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