

SYNTHESIS AND FLUORESCENCE PROPERTIES OF A
PROBE FOR MEMBRANE ANIONIC SITES⁺

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SUMMARY. A convenient synthesis of the fluorescent probe, dansyl cadaverine is reported. The probe binds to erythrocyte membranes and brain lipids with changes in fluorescence spectra but does not interact with bovine serum albumin, trypsin and chymotrypsin. Evidence for its affinity for anionic binding sites is presented. Two classes of binding sites are observed with erythrocyte membranes using tryptophan energy transfer experiments.

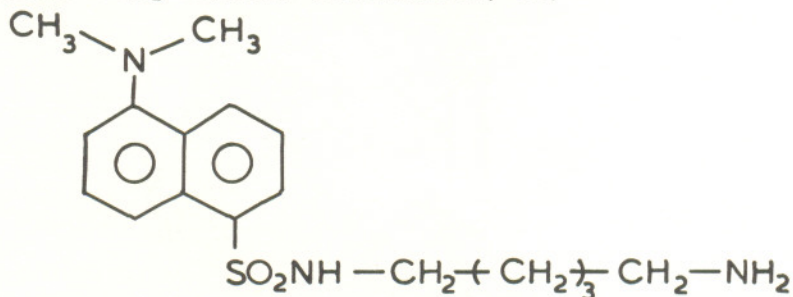
INTRODUCTION.

Fluorescent probe methods have found wide application in studies of biological systems (1,2). The anionic probe 1-anilino-8-naphthalene sulfonate (ANS)* has found widespread use in studies of protein and membrane structure. Recently, we have critically evaluated the use of anionic probes in studies of cation binding to membranes (3). The facility with which ANS binds to both proteins and lipids renders interpretation of fluorescence data ambiguous. It is therefore necessary to introduce new probes, that have a greater degree of binding specificity. In this communication we report a

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* Abbreviations: ANS, 1-anilino-8-naphthalene sulfonate; DC, dansyl cadaverine; BSA, bovine serum albumin.

convenient synthesis of dansyl cadaverine (N-(5-aminopentyl)-5-dimethylamino-1-naphthalene sulfonamide, DC)



and present evidence for its affinity for anionic lipid sites in biomembranes.

MATERIALS AND METHODS.

a) Synthesis of dansyl cadaverine: Dansyl chloride was prepared by the procedure of Mendel (4). A solution of 1 gm dansyl chloride in 10 ml dry ether was added over a period of one hour to a solution of 1.3 ml cadaverine in 10 ml dry ether. The reaction was carried out below 5°C in a nitrogen atmosphere with continuous stirring. The solid that separates out during the reaction was filtered and dissolved in methylene chloride and the solution washed with NaHCO₃, water and finally dried over sodium sulfate. Evaporation yielded a greenish white solid that was recrystallised from benzene-ethyl acetate. Yield 50-60%, M.P = 134-136°C, Mass spectrum M⁺ = 335. NMR, IR and UV spectra confirm the structure. The product was homogeneous by TLC on silica gel, using petroleum ether, chloroform, methanol 2:2:1.

b) Membrane, lipid and protein samples: Erythrocyte membranes were prepared by the procedure of Dodge et al. from sheep blood (5). Brain lipids were extracted from monkey brains by the method of Folch et al. (6). Mixed gangliosides from monkey brain were isolated by the procedure due to Gammack (7). Lecithin, cephalin, bovine serum albumin (BSA), trypsin and α-chymotrypsin were obtained from Sigma Chemical Co.

c) Fluorescence measurements: Spectra were recorded using a manual Perkin Elmer MP203 spectrofluorimeter and are uncorrected. The excitation wavelength was 340 nm. For energy transfer measurements the excitation was 290 nm. Matched 1cm cuvettes were used. The fluorescent probe concentration was 20 μg/ml. Protein concentrations were fixed at 200 μg/ml. Erythrocyte membrane protein concentration was determined by the procedure of Lowry et al. (8). Lipid dispersions were prepared by shaking dry lipid in buffer, to yield a concentration of 200 μg/ml. All solutions were prepared in 10 mM Tris-HCl buffer at pH 7.4.

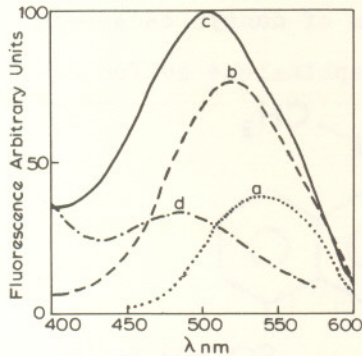


Fig 1. Uncorrected fluorescence spectra of DC. Excitation 340 nm. a) DC in Tris buffer. b) DC + 200 $\mu\text{g}/\text{ml}$ brain lipids. c) DC + erythrocyte membranes, 200 $\mu\text{g}/\text{ml}$ protein. d) DC + erythrocyte membranes. Excitation 290 nm.

RESULTS AND DISCUSSION.

Dansyl cadaverine, prepared by a long sequence of reactions, has been used in studies of fibrin crosslinking (9). The synthesis reported here is far simpler and results in highly pure preparations required for fluorescent probe studies. Pincus *et al.* have shown by fluorescence microscopy that DC binds to plasma membranes of intact cells (10). Free DC, in Tris buffer at pH 7.4, has a single emission peak at 540 nm on excitation at 340 nm. (Fig 1a). Fig 1b and c show the effects of addition of erythrocyte membranes and brain lipids on the emission spectrum of DC. There is a marked increase in the intensity of emission and a pronounced blue shift of the fluorescence maximum. While a small increase in intensity was observed in the presence of lecithin, cephalin did not alter the fluorescence spectrum. The proteins BSA, trypsin and α -chymotrypsin were also ineffective in changing the emission properties of DC. The addition of ganglioside dispersions resulted in a small but reproducible

Table I

Fluorescence parameters of DC in the presence of proteins and lipids

Protein/lipid	max emission	Relative intensity
Control (Tris HCl, pH7.4)	540 nm	1
Erythrocyte membranes	505 nm	2.5
Brain lipids	515 nm	2
Gangliosides	535 nm	1.2
Lecithin	540 nm	1.3
Cephalin	540 nm	1
Trypsin	540 nm	1
α -chymotrypsin	540 nm	1
BSA	540 nm	1.1

Table II

Fluorescence parameters of DC in different solvents

Solvent	max emission	Relative intensity
Tris HCl pH 7.4	540 nm	1
Methanol	505 nm	13.25
n-Butanol	500 nm	44.75
Ether	470 nm	189

enhancement in DC emission. These results are summarised in Table I. The effect of solvent polarity on the fluorescence spectrum of DC is shown in Table II. Pronounced blue shifts and intensity enhancements are observed with decreasing solvent polarity. This parallels the behaviour of other naphthalene derived fluorescent probes (1).

Our results indicate that DC binds strongly to whole brain lipids and erythrocyte membranes, but interacts weakly with lecithin and does not bind to many proteins. It is likely therefore that DC binds strongly to anionic lipids. This may be contrasted with the behaviour of ANS, which binds strongly to lecithin (11), BSA (12) and to a variety of membranes (1). The small fluorescence enhancement of DC in the presence of gangliosides may involve interaction with the carboxylate groups of neuraminic acid. At high cation concentrations (300 mM NaCl) the fluorescence of DC does not show any enhancement in the presence of erythrocyte membranes. Preliminary studies indicate that increasing Na^+ or Ca^{++} concentration results in a decrease of DC fluorescence, in the presence of membranes, due to displacement of DC molecules from membrane binding sites. These results further support our contention that DC interacts specifically with negatively charged sites. The use of DC in studies of ion binding to membranes should provide data, complementary to that obtained using ANS as a probe (3). While non-polar hydrocarbons like N-phenyl naphthylamine (1) and pyrene (13) are specific probes of the hydrocarbon regions of lipids, DC appears to be the first amphipathic probe with an affinity for anionic lipids.

A striking feature of the spectrum of DC bound to erythrocytes is that while excitation at 340 nm leads to emission at 505 nm, excitation at 290 nm results in fluorescence at 485 nm (Fig 1d). DC molecules that are favourably positioned for excitation energy transfer from tryptophan residues on membrane proteins (1), emit at lower wavelengths, suggesting a more non-polar environment. The

intensity of emission is only 30% of that obtained by direct excitation at 340 nm implying that only a small fraction of DC molecules are bound at this site. The observation of a binding site which is spatially proximate to proteins for a probe that has an affinity for anionic lipids has interesting possibilities. It is conceivable that probes like DC may be used to gain spectroscopic evidence for the existence of integral proteins in biological membranes (14). We are further investigating the synthesis and fluorescence properties of homologous probes, in which the distance between the polar amino group and the naphthalene ring is systematically varied.

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