Emission Properties Of Potential-Responsive Probe Di-4-ANEPPS

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Di-4-ANEPPS is a potential responsive probe. It is used to follow changes in membrane potential on perfused animal hearts. Di-4-ANEPPS and other probes from ANEPPS group respond to increases in membrane potential with a decrease in fluorescence excited at approximately 440 nm and an increase in fluorescence excited at 530 nm. This fluorescence shift and intensity change is used in fluorescence emission ratio measurements. Potential responsive probes are sensitive to solvent properties in these kind of measurements. We try to find out how much di-4-ANEPPS dependent on solvent polarity is.

1 Introduction

Di-4-ANEPPS is a potential responsive probe. It is used to follow changes in membrane potential on perfused animal hearts, variety of tissue and cardiac cells and cell systems.

ANEP dyes have very similar spectral properties. ANEP maximum molar extinction coefficients for absorption in methanol are about 36,000 $cm^{-1}M^{-1}$ at about 498 nm (for the zwitterionic ANEPPS dyes) or 517 nm (for the cationic ANEPEQ and ANEPPQ dyes). Absorption and fluorescence spectra of the ANEP dyes are highly dependent on their environment. The dyes are essentially nonfluorescent in water and become quite strongly fluorescent upon binding to membranes.

The absorption and fluorescence emission maxima of di-8-ANEPPS bound to model phospholipid membranes are about 465 nm and 635 nm, respectively. Fluorescence is insensitive to pH and photostability is generally high.

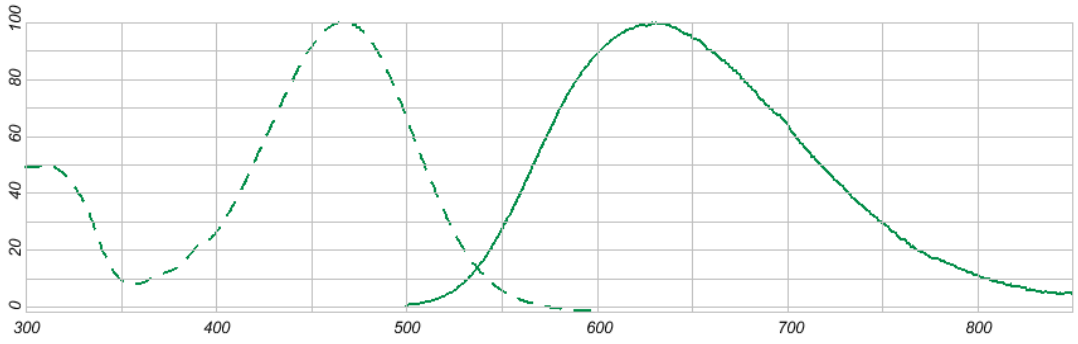

Fig. 1. Emission spectra of ANEPPS in a model of phospholipid membrane.

Di-4-ANEPPS and other probes from ANEPPS group respond to increases in membrane potential with a decrease in fluorescence excited at approximately 440 nm and an increase in fluorescence excited at 530 nm. This fluorescence shift and intensity change is used in fluorescence emission ratio measurements. Di-4-ANEPPS and di-8-ANEPPS intensity change is up to 10% of fluorescence intensity per 100 mV changes.

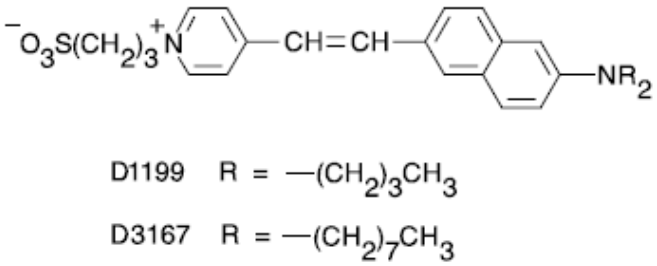

Fig. 2. ANEP dyes structure. D1199 and D3167 radicals are di-4-ANEPPS and di-8-ANEPPS.

The dipole moment of an aromatics molecule in the excited state $\boldsymbol{\mu_e}$ differs from that in ground state $\boldsymbol{\mu_g}$. Most of polarity probes undergo intramolecular charge transfer upon excitation so that $\boldsymbol{\mu_e} > \boldsymbol{\mu_g}$. Following excitation, the solvent cage undergoes a relaxation leading to a relaxed state of minimum free energy. The increasing of the polarity of the solvent, the lower energy of the relaxed state, and the larger red-shift of the emission spectrum. It is important to note that the rate of solvent relaxation depends on the solvent viscosity.

2 Methods

We used a variety of solvents to check some main spectral properties of di-4-ANEPPS described above. Especially we tried to find out how much di-4-ANEPPS is dependent on solvent polarity.

Solvents used in this study were in spectrophotometric grade (Sigma): methanol, ethanol, 1-propanol, 1-octanol, *tert*-butanol, chloroform, dimethylsulfoxide (DMSO), and acetone. The used water was triple-distilled. Model surfactants were anionic sodium dodecyl sulfate (SDS), cationic cetytrimethylammonium bromide (CTAB) and phospholipid dipalmitoylphosphatidylcholine (DPPC) as cell membrane model (all obtained from Sigma).

Stock solutions of probe ANEPPS (Invitrogen, Inc.) were prepared in acetone in final concentration 10^{-4} mol L^{-1}. Probe stock solution was introduced into a vial and acetone was evaporated. The concentration of probe in final samples was set to $5\ 10^{-6}$ mol L^{-1}. Model solvents and surfactants were introduced into the vial with the probe and the resulting solution was sonicated for 1 hour and stored during next 20 hours. Surfactants were in concentration above its critical micelle concentration (CMC) – c(SDS) = 50 mM (CMC_{SDS} = 8.3 mM), c (CTAB) = 10 mM (CMC_{CTAB} = 1.0 mM). Final concentration of DPPC was 10 mM.

The fluorescence emission spectra were monitored with a luminescence spectrophotometer (AMINCO-Bowman, Series 2) at 293.15 ± 0.1 K (Thermostat Grant). In most of all cases, the excitation and emission slit widths were set to 4 nm. Scan rate was set to 5 nm s^{-1}, and obtained spectra were corrected using Spectrophotometer Cary 50 (Varian, Inc.). Measurements were carried out in 1 cm quartz cuvette with stopper. Cuvette was washed in ethanol and water, to avoid sorption on cuvette wall. After some measurement series, cuvette were placed into acid hydrogen peroxide and stored through the night to remove all organic impurities. Next day cuvette was washed in organic solvents and at last in deionised water.

3 Results

Emission spectra of ANEPPS in environments with different polarity were obtained using luminescence spectrophotometer. With decreasing relative permittivity of the alcohols ANEPPS shows blue-shift of its emission maximum. On the other hand, in aqueous and acetone solution spectra exhibit no correlation with their relative permittivity.

In the case of surfactants and phospholipid, position of emission maximum decrease in line CTAB – SDS – DPPC.

Tab. 1.

	Emission max (nm)	F (a.u.)	Excitation max (nm)
CTAB	648	160	484
SDS	603	109	488
voda	600	1.3	478
MeOH	696	4.1	500
EtOH	679	8.9	495
PrOH	675	17.4	510
Aceton	686	14.0	490
Chloroform	655	3.21	540
Cyklohexan	N/A	N/A	N/A
DMSO	694	6.85	500

Figure 3 shows emission properties in phospholipids (DPPC) and surfactants aqueous solution (CTAB, SDS). Comparison of emission spectra in alcohols is shown in figure 4.

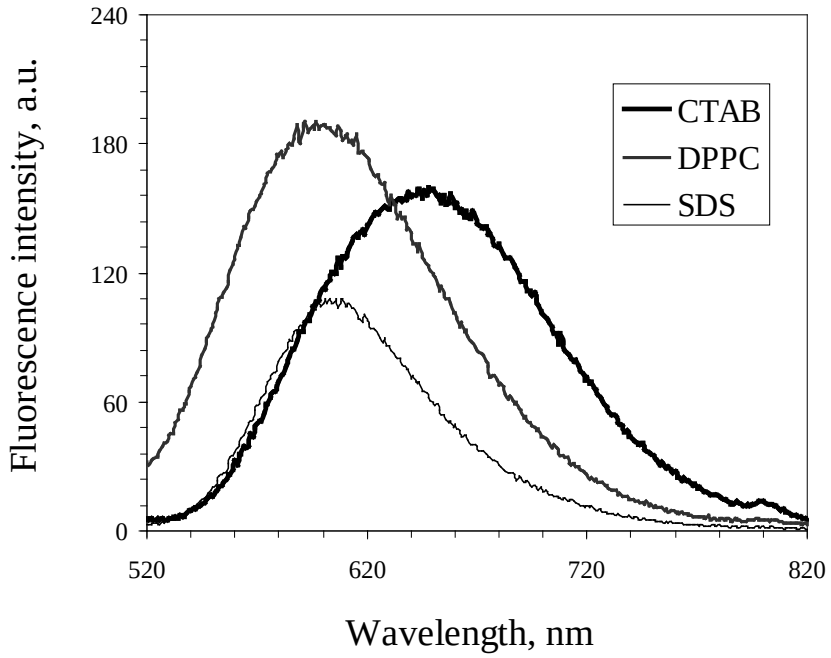

Fig. 3. Emission spectra of ANEPPS in different surfactant solutions – cationic CTAB, anionic SDS and phospholipid DPPC.

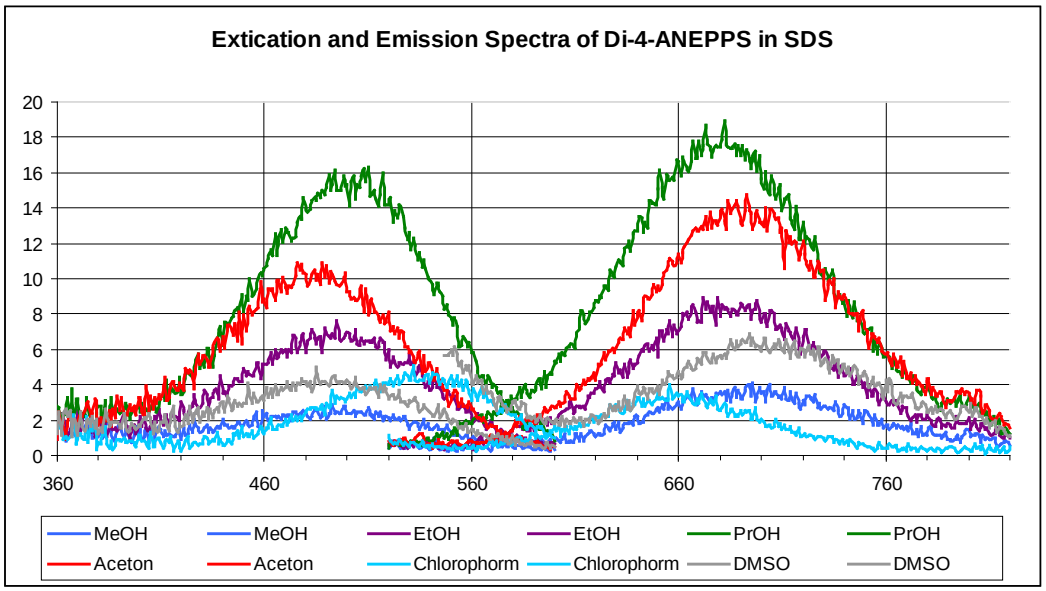

Fig. 4. Emission spectra of ANEPPS in different surfactant solutions – cationic CTAB, anionic SDS and phospholipid DPPC.

4 Discussion

It should be noted, that emission properties of ANEPPS in DPPC is similar in anionic SDS rather than in cationic CTAB. This fact suggests that relative position of ANEPPS in DPPC solution is analogous as in anionic SDS and that ANEPPS is localized close to negative charge of phosphatidyl group.

It is clear from figures 3 and 4 that the fluorescence intensity is much stronger when binding on phospholipids structures. The fluorescence is weak in aqua and aqua solutions.

ANEPPS show the highest solubility and the highest fluorescence quantum yield (realized as the fluorescence intensity F) in surfactants (CTAB, SDS). This should be related to its amphiphilic, a surfactant-like, structure. Dyes, or probes, with the amphiphilic structure can act as co-surfactants and contribute to aggregates stability.

Emission properties of ANEPPS in pure solvents were investigated. In a sequence of alcohols, with a decreasing relative permittivity, shifts of emission maximum to higher energy – lower wavelengths were found. This behavior is well known in a case of common fluorescence probe. Also in DMSO, with a relatively high relative permittivity, the ANEPPS has shown high wavelength of emission maximum.

In a case of chloroform, halogenated solvent with low relative permittivity, ANEPPS has shown the lowest emission wavelength. Water and acetone have been found out of relative permitivity order. In their case, a specific interaction with ANEPPS or hydrolysis of the dye should be taken into account. Lowest emission wavelengths of ANEPPS in surfactant solution were found in comparison of results between surfactants and pure solvents.

5 Conclusions

Emission properties of ANEPPS were determined in various environments. Results of this study compose a necessary step to further investigation of ANEPPS behavior in membrane of living cells. Also, the results describe fundamentals of various phenomena revealing during application of potential sensitive probes – namely in action potential recording in heart.

Acknowledgement

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