USE OF THE RED-EDGE EXCITATION EFFECT FOR INVESTIGATION OF DIELECTRIC INTERACTIONS IN BIOMEMBRANES

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Dipole moments of the fluorescent probes 1-phenylnaphthylamine (1-AN) and 1-anilinonaphthalene-8-sulfonate (1,8-ANS) are measured using electro-optical absorption and emission methods. Dipole moments in the ground and excited states were measured in cyclohexane and dioxane. It is shown that the charge distributions in the 1-AN and 1,8-ANS molecules differ substantially. The spectral dependence of the electro-optical coefficients suggests that the absorption spectrum of 1,8-ANS is due to a superposition of (at least two) electronic transitions. It is found that spectra of 1-AN in erythrocyte ghosts are inhomogeneously broadened. The above effect makes it possible to selectively excite probe molecules localized at different sites of a membrane. Dielectric interactions (described by the local dielectric constant) are investigated in human erythrocyte membranes. It is found that the dielectric constant of erythrocyte membranes varies from 6.79 ± 0.8 to 17.6 ± 3.5 depending on the excitation frequency and, therefore, on the localization of the probe.

Key words: fluorescent probe, dipole moment, electro-optical method, dielectric constant, human erythrocyte membranes.

Aminonaphthalene fluorescent probes, especially 1-phenylnaphthylamine (1-AN) and 1-anilinonaphthalene-8-sulfonate (1,8-ANS) are widely used in investigation of proteins, membranes, and other biological systems. These probes are also of interest from the viewpoint of investigation of absorption and fluorescence mechanisms, since their characteristics depend substantially on the properties of the medium. For example, the fluorescence quantum yield of 1,8-ANS in water is only 0.004, whereas in butanol is equals 0.66 [1].

Due to the high sensitivity of their fluorescence to the properties of the medium, 1-AN and 1,8-ANS molecules can be used for determination of the micropolarity at their localization sites, e.g., in membranes. Biological membrans consist of a lipid bilayer and proteins bound to the bilayer. Electric interaction forces between proteins, their segments, and lipid regions determine the functional properties of the membrane. Since the potential of intermolecular interactions can vary, depending on the dielectric constant, by one to two orders of magnitude, information on the local dielectric constant of the membrane is necessary for development of the concept of electrostatic interactions between membrane proteins.

The best known method of evaluation of the dielectric constant in complex macromolecules is based on the use of equations describing the dependence of the position of absorption and fluorescence spectra of the probe on the dielectric constant and refractive index of the medium. These equations also include dipole moments of the probe in different electronic states. Thus, if the dipole moments of the probe in the corresponding electronic states are known, the local dielectric constant can be determined from the position of the absorption and fluorescence spectra.

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In the present work, dipole moments of 1-AN and 1,8-ANS probes in the equilibrium ground state and the Franck–Condon and equilibrium excited states are determined using electrooptical absorption and emission methods. In addition, the spectral inhomogeneity of 1-AN molecules in membranes is revealed, and local dielectric properties of human erythrocyte ghosts are investigated using the red-edge excitation method.

Electro-optical absorption and emission methods of measurement of molecular dipole moments. The high sensitivity of the fluorescence parameters of 1-AN and 1,8-ANS to the structure and dynamics of their environment is due to the substantial change in the electric dipole moment of the molecule upon its transition to an excited state. Electro-optical absorption and emission methods make it possible to obtain direct information on the magnitude and direction of dipole moments and the polarizability of molecules.\[2-4]\]

Compared to the classical method of dielectric losses used for measurement of the dipole moment in the ground state, the electro-optical method has an undeniable advantage, since it makes it possible to carry out measurements in solutions at very low concentrations. This is important in investigations of nonpolar solvents, because the solubility of molecules with a constant dipole moment in nonpolar solvents is usually very low. As compared to frequently used variants of the method of spectral shifts, an obvious advantage of the electro-optical method consists in the possibility of evaluation of dipole moments in a single solvent in both equilibrium and nonequilibrium electronic states. This is very important from the viewpoint of investigations of various intra- and intermolecular charge transfer reactions whose probability depends on the properties of the environment. In addition, as opposed to the method of spectral shifts, measurements by the electro-optical method do not require evaluation of the Onsager sphere radius, which is a fundamental obstacle in the case of nonspherical probe molecules.

Based on Liptay’s formalism\[2\], the effect of an external electric field \(E_f\) on the molar extinction coefficient \(K(\nu)\) can be described by the quantity \(L\) given by the following expression:

\[
L = L(\nu, \chi) = \frac{K^E(\nu, \chi) - K(\nu)}{K(\nu)} E_f^2,
\]

where \(K^E\) is the molar extinction coefficient in the presence of the external electric field and \(\chi\) is the angle between the direction of the vector \(E_f\) and the polarization direction of the incident light.

For a homogeneously broadened absorption band, the quantity \(L\) is described by the expression:

\[
L = Dr + \frac{1}{6} Es + Frt + Gst + Hru + Isu,
\]

where the parameters \(r\) and \(s\) are determined by the angle \(\chi\), and the quantities \(t\) and \(u\) depend on the first and second derivatives of the absorption spectrum:

\[
r = \frac{(2 - \cos^2 \chi)}{5}, \quad s = \frac{(3 \cos^2 \chi - 1)}{5}, \quad t = \frac{1}{hc} K(\nu)^{-1} \frac{dK(\nu)}{d\nu},
\]

\[
u = \frac{1}{2hc^2} K(\nu)^{-1} \frac{d^2K(\nu)}{d\nu^2}.
\]

In the case of aminonaphthalene molecules, effects connected with the electronic polarizability and the polarizability of transitions, which are substantially smaller than the effect of constant dipole moments, can be neglected. With this assumption taken into account, the coefficients \(D, E, F, G, H,\) and \(I\) are as follows:

\[
D = 0 \quad \text{within the framework of the above assumptions},
\]

\[
E = \frac{1}{kT} f_c^2 \left[ 3 \left( m_a \mu_g - \mu_g^2 \right) \right],
\]

\[
F = \frac{1}{kT} f_c^2 \mu_g \Delta \mu,
\]

\[
G = \frac{1}{kT} f_c^2 \left( m_a \mu_g \right) \left( m_a \Delta \mu \right),
\]

\[
H = f_c^2 \left( \Delta \mu \right)^2,
\]

\[
I = f_c^2 \left( m_a \Delta \mu \right)^2.
\]