CONJUGATED OLIGOELECTROLYTES AS A FLUORESCENT DYES FOR STAINING CELL MEMBRANES

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ABSTRACT

Bioimaging is one of the methods, which allow for multi-dimensional detection, visualization and characterization of the biological structures and processes in real time. Optical imaging methods based on the bioluminescence and photoluminescence phenomenon. Depending on the duration of the photoluminescence, when the emission disappears immediately after the excitation agent it is fluorescence, if lasts longer it is phosphorescence. The physical nature of the occurring phenomena is the absorption of energy by electrons, their transition to higher energy levels and the return to the basic state with the emission of energy by radiation. Fluorescent imaging is an important interdisciplinary field bridging the research from organic chemistry, physical chemistry and cell biology. Important parameters of fluorescent probes include: characteristic spectra of excitation and emission, lifetime and quantum efficiency of emitted fluorescence, quenching and photobleaching of fluorochromes, as well as biocompatibility and low cytotoxicity. To the wide range of existing external fluorescence probes include fluorescent proteins, small organic molecules and nanoparticles, including quantum dots. However, due to the limitations of the available dyes, a new, functional flurophores are still sought. During the last years Conjugated Polyelectrolytes (CPEs) and their shorter analogues, Conjugated Oligoelectrolytes (COEs) have emerged as a bioimaging tools. Both belong to the class of specialized molecules, with ´-conjugated molecular structures, which are responsible for the optical activity properties. In addition, the hydrophilic side groups provide their compatibility with polar solvents.

Within this dissertation the biological properties of conjugated oligoelectrolytes based on the phenylenevinylene (PV-COEs) and distyrylnaphthalene core (SN-COEs) as membrane-specific fluorescent dyes were tested. The first class of tested PV-COEs consists three cationic derivatives: three-ring $DSBN^+$, four-ring $DSSN^+$, five-ring $COE1\text{-}5C^+$ with ionic ammonium groups and one four-ring, anionic $COE1\text{-}4C^-$ with carboxyl groups. The second class of tested compounds includes the newly synthesized distyrylnaphthalene derivatives (DSNN): trimethylammonium derivative ($DSNN\text{-}NMe_3^+$), phosphonate derivative

(DSNN-P), morpholine derivative (DSNN-Mor), hydroxyethyl derivative (DSNN-DEA), phosphonate potassium salt (DSNN-POK), amino derivative (DSNN-NH_2) and pyridinium derivative (DSNN-Py^+). The applied research methodology included testing of the biological stability of COEs at the cellular level and their cytotoxicity to the mammalian cell lines. Cytotoxicity test results show that all tested COEs can be applied as fluorescent probes in selected human and animal cell lines at effective 1εM concentration. Fluorescence and confocal microscopy observations confirm the suitability of tested COEs as dyes for different cell types and their localization in intracellular membrane components. In addition, the intracellular fluorescence intensity of PV-COEs and SN-COEs was analysed by fluorescence activated cell sorting (FACS) and photoluminescence spectroscopy.

In conclusion, PV-COEs and SN-COEs have interesting biological properties. The applied research techniques allow for confirmation the application of these compounds in the staining of intracellular membrane structures of eukaryotic cells.