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Development of zwitterionic near-infrared emitting fluorophores

FACULTY RESEARCH GRANT REPORT

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B. Restatement of problem researched or creative activity

The main goal of the research project is to develop zwitterionic near-infrared (NIR) emitting fluorophores for detection of proteins and DNA in samples. Our hypothesis is if suitable donors and acceptors are connected by extended π-electron conjugation, charge transfer states can be generated at specific pH, and low energy excitation of electrons to the new low-lying states can be achieved in the near-infrared region.

C. Brief review of the research procedure utilized

A zwitterionic NIR fluorophore was designed via donor-acceptor (D-A) network of extended conjugation. First, 3-ethyl-1,1,2-trimethyl-1H-benzo[e]indol-3-ium was prepared by a SN^2 reaction between 1,1,2-trimethyl-1H-benzo[e]indole and ethyl iodide in acetonitrile solvent. Then, a phenolate donor and 3-ethyl-1,1,2-trimethyl-1H-benzo[e]indol-3-ium acceptor was attached by Knoevenagel condensation under refluxing condition. Fluorophore was purified by silica gel chromatography and recrystallization techniques. The yield of the final condensation reaction was 55%. The fluorophore was characterized by infrared (IR) and NMR spectroscopy. IR spectra displayed signals indicating presence of alkene stretching frequencies. Broad signal corresponding to –OH group was also observed at ~3309 cm^{-1}. In the ^1H NMR spectrum, signal at 3.82 ppm confirmed presence of –OMe group attached to a phenyl moiety. In DMSO broad downfield shifted signals in the range of 9.5-11.0 ppm confirmed presence of phenolic –OH groups. Selectivity to all-trans isomer of Knoevenagel condensation was very high within the limits of the NMR detection. Presence of vinylic hydrogens was confirmed by calculating coupling constant, and it was found to be 16 Hz. Upon excitation with suitable wavelength of light, donor to acceptor excitation took place, resulting charge transfer states. It was further stabilized in polar solvents such as methanol, DMSO, water, etc. The excited state energy was mostly lost due to the non-

radiative decay via multiple rotational motions. It was recovered in highly viscous solvent and with addition of protein BSA and HSA. The output signal was quantitative to the amount of fluorophore-protein complex in the experimental concentration range. The strong association affinity of the fluorophore toward the protein was attributed to the van der Waals and hydrophobic interactions.

D. Summary of findings

Due to polar nature of the donor and acceptor moieties, the fluorophore was highly soluble in polar solvents. For all the spectrometric experiments a dimethyl sulfoxide stock of the fluorophore was diluted in 0.1 M phosphate buffer (pH = 7.5) solution. The final dimethyl sulfoxide content in buffer was 1%, and the concentration of the fluorophore was maintained at 10 µM, unless otherwise stated.

The fluorophore exhibited moderate fluorescence in dimethyl sulfoxide, dimethylformamide, and acetone; very weak emission in nonpolar solvents such as toluene and hexane and marginal emission in deionized water. All the emission bands were broad and structure-less, which is characteristic of ICT type electronic interactions. Emission intensity and λ_{max} were very sensitive to the polarity of the solvents, indicating solvatochromism. As the dielectric constant of the solvents increased the emission maxima shifted toward lower energy, suggesting positive solvatochromism. The lowest energy charge transfer emission recorded was in DMSO (λ_{max}^{em} ~ 630 nm). In water, emission maxima was at ~ 625 nm with lowest intensity, most likely due to loss of excited state energy through additional decay channel facilitated by polar protic water through hydrogen bonds. However, in viscous liquid such as ethylene glycol and glycerol, multi-fold enhancement of intensity was recorded.

The fluorescence enhancement within the viscous microenvironment prompted us to investigate the complexation of the fluorophore with HSA and BSA in buffer (1 mM, pH = 7.4). A linear relationship between the concentration of the protein and the fluorescence intensity of the fluorophore was observed, suggesting a 1:1 complex formation. From the linear relationship and the Benesi-Hildebrand plot, binding affinity (K_a) was calculated in phosphate buffer.

E. Conclusions and recommendations

In conclusion, a NIR emitting zwitterionic fluorophore was designed, synthesized and characterized. The fluorophore exhibited excellent photophysical properties and high binding affinity with select proteins. Therefore, it can used as an extrinsic probe for detection of target biomarker in biological samples. We are currently investigating specificity of the probe for a protein by drug displacement assays.

We believe that the donor-π-acceptor fluorophores based on pH-controllable phenol derivatives will provide a new promising platform for tuning absorption and emission profiles of new NIR probes for biosensing and cellular imaging.