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A triterpene oleanolic acid conjugate with 3-hydroxyflavone derivative as a new membrane probe with two-color ratiometric response

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Abstract

We report on the synthesis by coupling of a triterpenoid oleanolic acid with 4'-diethylamino-3-hydroxyflavone (FE) to produce an environment-sensitive biomembrane probe with two-band ratiometric response in fluorescence emission. The synthesized compound (probe FOT) was tested in a series of model solvents and demonstrated the response to solvent polarity and intermolecular hydrogen bonding very similar to that of parent probe FE. Meantime when incorporated into lipid bilayer membranes, it showed new features differing in response between lipids of different surface charges as well as between glycerophospholipids and sphingomyelin.

Abbreviations: BrMe-FE, 6-Bromomethyl-4'-N,N-Diethylamino-3-hydroxyflavone; DOPC/PC, dioleoyl/phosphatidylcholine; DOPE/PE, dioleoyl/phosphatidylethanolamine; DOPG/PG, dioleoyl/phosphatidylglycerol; DOPS/PS, dioleoyl/phosphatidylserine; EYPC, egg yolk phosphatidylcholine; ESIPT, Excited-State Intramolecular Proton Transfer; F, 4'-dimethylamino-3-hydroxyflavone; FE, 4'-diethylamino-3-hydroxyflavone; FOT, 4'-N,N-diethylamino-3-hydroxyflavone-6-methylene)-3-hydroxyolean-12-en-28-oate; 3HC, 3-hydroxychromone; 3HF, 3-hydroxyflavone; ICT, Intramolecular Charge Transfer; MLV, multilamellar vesicles; SM, sphingomyelin.

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We observed that in the conditions of coexistence of rafts and non-raft structures the probe is excluded from the rafts.

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1. Introduction

Fluorescence probing is one of the major research tools in molecular and cellular biology, biotechnology and related areas. The probes are usually organic fluorophores that correlate optimal spectroscopic properties with optimal functional performance. Their spectroscopic properties involve the ability to generate strong and easily measurable signal reporting on different intermolecular interactions in the probe environment and on the dynamic processes in the studied system. Response can be obtained in different parameters characterizing fluorescence emission: positions of spectral bands, intensity, anisotropy and lifetime [1,2]. Regarding probing based on sensitivity to intermolecular interactions the dyes that respond by a spectral shift of a single fluorescence band as a function of solvent polarity due to dielectric relaxation mechanism dominate in both research and applications. Their disadvantage is associated with the fact that they often respond to factors unrelated to polarity (hydrogen bonding, excited state reactions). In addition, their spectral shifts are usually associated with variations of quantum yield and band width, which distorts the spectra and makes difficult the sensitive and convenient ratiometric detection.

It is a common perception that fluorescence probes exhibiting two bands in emission together with two-band ratiometric sensitivity should have a strong advantage over wavelength-shift probes in fluorescence sensing techniques [3]. Two bands in fluorescence emission can be obtained in aromatic dyes exhibiting intramolecular charge transfer (ICT) and possessing strong electron-donor and electron acceptor substituents [4,5]. In these cases emission from initially excited (commonly called locally excited, LE) and ICT states can be observed as two emission bands, so that the switching between them can be used in sensing [6,7]. But strong and often unpredictable quenching of one of the forms does not stimulate the development of this approach. Therefore, we started devising new fluorescence probes based on another excited-state reaction—Excited-State Intramolecular Proton Transfer (ESIPT) [3,8], which is known to produce emissive tautomer forms with dramatic Stokes shifts. Among all fluorophores exhibiting ESIPT known to us, only 3-Hydroxychromones (3HCs) and their derivatives 3-hydroxyflavones (3HFs) are the compounds, in which the observation of two separate bands in emission is not coupled to the presence of two or more ground-state or excited-state conformers or to a slow ESIPT reaction. In 3HCs the 3-hydroxyl group (proton-donor) and the 4-carbonyl group (proton-acceptor) are attached to a rigid skeleton and coupled by an intramolecular hydrogen bond. In 3HCs with electron-donor substituents the ESIPT reaction is faster than the emission, and the observation of two emission bands belonging to initially excited normal (N^*) form and reaction product tautomer (T^*) form is due to the rapid establishment of a dynamic

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