Rational design, synthesis and spectroscopic and photophysical properties of a visible-light-excitable, ratiometric, fluorescent near-neutral pH indicator based on BODIPY.

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Kolekcja

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Streszczenie

A visible-light-excitable, ratiometric, brightly fluorescent pH indicator for measurements in the pH range 5-7 has been designed and synthesized by conjugatively linking the BODIPY fluorophore at the 3-position to the pH-sensitive ligand imidazole through an ethenyl bridge. The probe is available as cell membrane permeable methyl ester 8-(4-carbomethoxyphenyl)-4,4-difluoro-3-[2-(1H-imidazol-4-yl)ethenyl]-1,5,7-trimethyl-3a,4a-diaza-4-bora-s-indacene (I) and corresponding water-soluble sodium carboxylate, sodium 8-(4-carboxylatophenyl)-4,4-difluoro-3-[2-(1H-imidazol-4-yl)ethenyl]-1,5,7-trimethyl-3a,4a-diaza-4-bora-s-indacene (II). The fluorescence quantum yield Φ(f) of ester I is very high (0.8-1.0) in the organic solvents tested. The fluorescence lifetime (ca. 4 ns) of I in organic solvents with varying polarity/polarizability (from cyclohexane to acetonitrile) is independent of the solvent with a fluorescence rate constant k(f) of 2.4×10(8) s(-1). Probe I is readily loaded in the cytosol of live cells, where its high fluorescence intensity remains nearly constant over an extended time period. Water-soluble indicator II exhibits two acid-base equilibria in aqueous solution, characterized by pK(a) values of 6.0 and 12.6. The Φ(f) value of II in aqueous solution is high: 0.6 for the cationic and anionic forms of the imidazole ligand, and 0.8 for neutral imidazole. On protonation-deprotonation in the near-neutral pH range, UV/Vis absorption and fluorescence spectral shifts along with isosbestic and pseudo-isoemissive points are observed. This dual-excitation and dual-emission pH indicator emits intense green-yellow fluorescence at lower pH and intense orange fluorescence at higher pH. The influence of ionic strength and buffer concentration on the absorbance and steady-state fluorescence of II has also been investigated. The apparent pK(a) of the near-neutral acid-base equilibrium determined by spectrophotometric and fluorometric titration is nearly

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independent of the added buffer and salt concentration. In aqueous solution in the absence of buffer and in the pH range 5.20-7.45, dual exponential fluorescence decays are obtained with decay time τ(1)=4.3 ns for the cationic and τ(2)=3.3 ns for the neutral form of II. The excited-state proton exchange of II at near-neutral pH becomes reversible on addition of phosphate (H(2)PO(4)(-)/HPO(4)(2-)) buffer, and a pH-dependent change of the fluorescence decay times is induced. Global compartmental analysis of fluorescence decay traces collected as a function of pH and phosphate buffer concentration was used to recover values of the deactivation rate constants of the excited cationic (k(01)=2.4×10(8) s(-1)) and neutral (k(02)=3.0×10(8) s(-1)) forms of II.

Słowa kluczowe

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