Long-wavelength, pH-Sensitive Benzodipyrrolenine-Squaraine Dye with pKa in the Acidic pH Range

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Introduction

We have developed a pH-sensitive squaraine dye containing a benzodipyrrolenine moiety. In addition this dye features a sulfonic acid and a pendant carboxylic functional group. The presence of the sulfo-group aids solubility in aqueous media and reduces probe aggregation in solution, while the carboxy-function which can be easily converted into an N-hydroxysuccinimidyl (NHS) ester facilitates bioconjugation.

Spectral Properties of Free Dye

Between pH 5 and 9 this dye exists in a mono-protonated form with an absorption maximum at 648 nm and an emission maximum at 668 nm (Table 1). The di-protonated form obtained in acidic media is also fluorescent. Remarkably, only a small blue-shift (4 nm) of the absorption maximum and a noticeable increase of the extinction coefficient (ε) is observed under strong acidic conditions (pH 3). The quantum yield (Q.Y.) of the mono-protonated dye increases three-fold for the di-protonated form. The deprotonated dye molecule which is formed in alkaline media shows no detectable fluorescence.

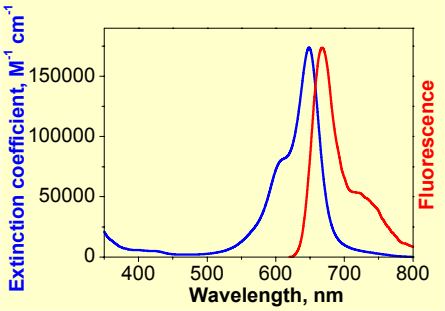

Fig. 1. Absorption and emission spectrum of benzodipyrrolenine-squaraine dye in phosphate buffer (pH 7.4)

Spectral Properties upon Covalent and Non-covalent Interaction with Protein

Upon non-covalent interaction with BSA at neutral pH the absorption and emission maxima of the probe are red-shifted by 19 nm and 7 nm, respectively, while the quantum yield increases from 4 to 12% (Table 2).

Table 2. Spectral characteristics of covalent conjugates and non-covalent complexes of the dye with BSA in phosphate buffer (pH 7.4)

Sample	D/P	λ_{max} (Abs), nm	λ_{max} (Em), nm	Quantum Yield, %	Fluorescence Lifetime, ns
Free dye	-	648	668	4	0.22
BSA complex	-	667	675	12	-
BSA conjugate	0.22	666	678	9.0	1.32
BSA conjugate	0.42	666	679	8.5	1.27
BSA conjugate	0.58	665	678	5.3	1.23
BSA conjugate	0.94	664	678	1.6	0.99

The long-wavelength absorption peak gradually decreases with increasing pH and disappears at pH 12.5. At the same time a new band at 510 nm appears.

Due to the presence of two distinct protonation centers in the molecule this dye shows two pKa values: the pKa of 3.5 and 10.8 (based on absorption) and 3.8 and 10.9 (from the emission) are almost the same and reveal similar charge distribution in the excited and the ground state.

The new dye was also covalently labelled to BSA. The BSA conjugates of the dye show a less pronounced red-shift and reduced quantum yields compared to the non-covalently attached dyes.

The dye was also found to be useful for staining of cells in biological imaging applications.

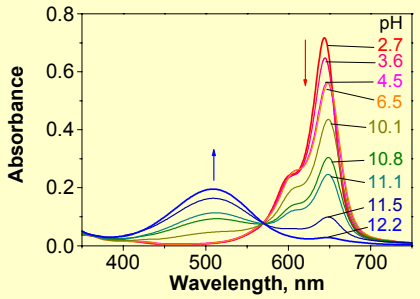

Fig. 2. Absorption spectra of benzodipyrrolenine-squaraine dye as a function of pH

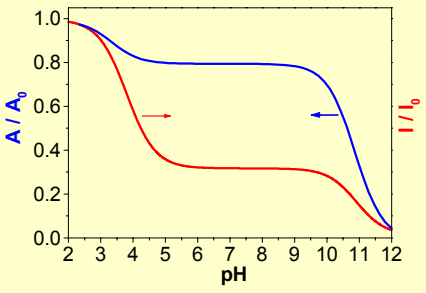

Fig. 3. pH-titration curves: normalized absorption (A / A_0) and normalized fluorescence intensity (I / I_0) as a function of pH

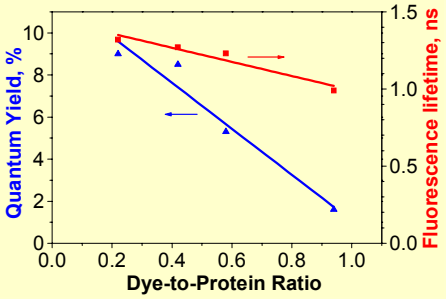

Fig. 4. Fluorescence quantum yield and mean lifetime *vs.* dye-to-protein ratio of Dye — BSA conjugates in phosphate buffer (pH 7.4)

Table 1. Spectral properties of the dye at different pH

pH	λ_{max} (Abs), nm	ε $M^{-1}cm^{-1}$	λ_{max} (Em) nm	Quantum Yield %
3.0	644	220,000	667	12
7.4	648	174,000	668	4
12.0	650 510	12,000 61,000	non-fluorescent	

Conclusion

The novel, long-wavelength, pH-sensitive cyanine dye with pKa's at 3.5 and 10.9, exhibits a high extinction coefficient and a 300% quantum yield increase upon complete protonation. The dye shows potential as a pH probe to detect acidic organelles in eukaryotic cells.

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