

STILBENE RELATED DYES AS NOVEL VERSATILE PROBES FOR FLUORESCENCE MICROSCOPICAL LOCALIZATION OF PEROXIDASE ACTIVITY

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Encouraged by development of light excitation and measuring techniques and the commercial availability of highly sensitive equipment, luminescent labels represent the most sensitive and worthwhile detection tools to date. In contrast to a wide variety of well established chromogenic techniques fluorescent labels for detection of peroxidase (PO) are confined only to a few substrates. Thus novel fluorescent substrates of PO derived from heterocyclic 4-hydroxy styrenes have been developed to become an important analytical tool in life sciences for detection of endogenous and exogenous targets at the long time scale. Excellent localization, high staining sensitivity and exceptionally low background staining were achieved by optimizing the substrate by way of chemical synthesis. Structure/staining_behavior-relationships are discussed. Multiple step-by-step anchoring of enzymatically activated substrate intermediates to surrounding cell constituents, related to the catalyzed reporter deposition (CARD) technique, is discussed. In contrast to tyramine-fluorochrome conjugates, as employed in the CARD amplification, the separation between reporting and anchoring function is eliminated. As important consequence, a new fluorochrome with altered spectral properties is yielded after enzymatic cross linking of the applied substrate. Because spectral properties and anchoring capability are interdependent and are crucially influenced by the cellular environment, the overall staining capability of 4-hydroxy styryl derivatives (benzothiazolium, quinolinium and pyridinium salts) was histochemically screened in a semi-empirical approach and leading structures were manifested. In conclusion, a solid and reliable staining performance is achieved with alkyl chains of short or medium length at the positively charged nitrogen while polar end groups evoke quite different effects towards the staining specificity and photo stability. The in some cases outstanding performance of substrates with polar groups (except H-donating groups) at the N-alkyl chain initiated the investigation of novel dimers of two individual non-conjugated and by an alkyl chain spaced dye compartments. Both individual parts act thereby as individual fluorescent substrates independently. By means of modification the spacer length and the heterocycle staining preferences for PO, nuclei or mast cells could be achieved. A computer aided geometry consideration indicated that the relative spatial alignment of both dye compartments (in terms of flexibility/conformation and molecular shape/configuration) effect the observed staining behavior.

In conclusion, catalytic cross linking of heterocyclic 4-OH-styryl derivatives as fluorescent substrates of PO is a promising approach which complements the on fluorochrome-labeled tyramine based CARD technique. This novel approach features an exceptional low background staining, provides a broad excitation and emission range of fluorescence and an outstanding spatial resolution of specific fluorescence signaling. Histochemical and immunohistochemical applications are presented using conventional and confocal fluorescence microscopes with different excitation sources.