Acta Histochemica 113 (2011) 682–702

Contents lists available at ScienceDirect

Acta Histochemica

journal homepage: www.elsevier.de/acthis

Review

Tailoring and histochemical application of fluorescent homo-dimeric styryl dyes using frozen sections: From peroxidase substrates to new cytochemical probes for mast cells, keratin, cartilage and nucleic acids

Reimar Krieg*, Annett Eitner, Karl-Jürgen Halbhuber

Institute of Anatomy/Anatomy II, Friedrich-Schiller-University Jena, Teichgraben 7, D-07743 Jena, Germany

ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 4 March 2011
Accepted 6 March 2011

Keywords:
Fluorescent cytochemical probes
Keratin
Mast cells
Cartilage
Nucleic acids
Peroxidatic activity
Fluorescence microscopy
Homo-dimeric styryl cyanines

ABSTRACT

Homo-dimers of styryl dyes were chemically tailored in order to become specific cytochemical probes for use in the life sciences. Histochemical applications using fixed cryotome sections are discussed. It is concluded, that homo-dimerization of specific styryl substrates of peroxidase (PO) by way of their covalent linkage, does not necessarily lead to improved detection sensitivity of endogenous and immuno-bound peroxidase (PO) activity. In general, these dimers act less specific towards PO activity than parent monomers. Synergetic interactions of the doubled basic dye compartments with cell constituents cause a pronounced staining of further targets at the cellular level. This behavior depends on the functional groups present in each dye compartment in a crucial manner. However, by way of chemical dye tailoring centering of these initially unwanted staining properties is possible leading to novel highly fluorescent stains for mast cells, nucleic acids, keratin and cartilage tissue. Structure/staining behavior-relationships of these stains are discussed.

Contents

Abbreviations: CARD, catalyzed reporter deposition; DMF, dimethylformamide; DMSO, dimethylsulfoxide; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethyl-2-sulphonic acid; HRP, horseradish peroxidase; PBS, phosphate-buffered saline; PIPES, piperazine-N,N′-bis(2-ethanesufonic acid); PO, peroxidase; S/N, signal to noise ratio; TBS, Tris-buffered saline (0.5 M Tris/HCl-buffer plus 6.3% NaCl, pH 7.2–7.4); THF, tetrahydrofuran; TLC, thin layer chromatography; TLM, transmission light microscopy; Tris, tris(hydroxymethyl)aminomethane; ε, extinction (in l/mol cm); E, absorbance (dimensionless, at 1 weight% measured); λ_{em}, fluorescence emission maximum (in nm); λ_{exc}, fluorescence excitation wavelength (in nm); λ_{max}, absorption maximum (in nm); mp, melting point (in °C); R_f, retention factor in TLC determined by dividing the distance traveled by the product through the total distance traveled by the solvent.

* Corresponding author. Tel.: +49 3641 938 566; fax: +49 3641 938 552.
E-mail address: Reimar.Krieg@mti.uni-jena.de (R. Krieg).

0065-1281/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.acthis.2011.03.003

Introduction

Small dyes, such as cyanines, have the advantage that they can easily be adapted to various applications. By way of structural modification of the chromophore portion of the cyanine, fluorescence properties can be tailored so that dyes absorb and emit light at wavelengths ranging from the UV over the visible to the near infrared region of the spectrum. Furthermore, they can be synthesized in many structural forms with a variety of functional groups arranged around the chromophore portion. Also, this structural versatility permits control over important factors such as solubility, bio-compatibility, and specificity. This versatility also allows the selection of approaches that do not perturb the function of the labelled compound and, most important, allows the introduction of targeting or selectivity enforcing auxiliary functional groups into intrinsically non-specific acting dyes.

In histochemistry, usually a dye stains a target by permanent binding modes. Thereby durability and accuracy of the spatial information of dye signaling can be achieved in three ways:

(1) through covalent binding,
(2) through non-covalent binding modes, and
(3) *in vivo* through specific dye accumulation and/or metabolization by cells or cell organelles conveyed by non-covalent interactions in the initially steps. This accumulation can than be accompanied by subsequent covalent conversion processes (e.g. scission of functional groups, metabolic degradation, cross-linking with proteins after metabolic activation as known, for example, from reactive dyes under the trade marks MitoTracker™ and LysoTracker™ (Haugland, 2002)).

Covalent labelling

In most bio-analytical applications, dyes are covalently attached to proteins or other materials to make these materials fluorescent so that they can be detected. Thus cyanine-conjugated fragments of DNA or RNA can be used to identify the presence of complementary nucleotide sequences in DNA or RNA such as Cy and Cy5. For improved detection sensitivity in sophisticated enzyme immunoassays (EIA) an antibody–enzyme conjugate is used. Finally, enzymatic conversion of a suitable substrate furnishes a specific dyestuff product in a catalytic-covalent way either though bond formation (e.g. tyramides used in the CARD-technique, Bobrow et al., 1992, 1989) or by bond scission (e.g. hydrolysable ELF™-enzyme substrates, Haugland, 2002).

Non-covalent binding modes

A variety of fluorescent dyes stain tissue constituents by way of non-covalent weak interactions. These interactions must be complementary and include: ionic, polar, π–π interactions, hydrophobic and lipophilic effects. Analogous to antigen–antibody interactions the resulting bond strength increases in a synergetic way with increasing number of interactions.

Particularly important applications involve dyes that bind to nucleic acids (Armitage, 2005). Frequently they exhibit large fluorescence enhancements upon binding caused by dye rigidization effects. Enhancement of binding strength by several orders of magnitude by utilizing dimers of intercalating dyes is a well proven approach. Thereby two intercalating dye moieties are connected covalently by alkyl linkers bearing quaternary amino groups as known from ethidium homo dimers. This principle is well proven for example in TOTO and YOYO homo dimers (Staerk et al., 1997; Glazer and Rye, 1992). A similar approach utilizes the introduction of affinity-modifying groups attached to intercalating dyes via a short alkyl spacer (Yarmoluk et al., 2001).

In living cells

In living cells additional challenges have to be considered including: the transport of the dye through outer and inner membrane barriers, differential accumulation into cell organelles, and metabolic dye degradation (Horobin et al., 2006; Horobin, 2001). Approaches such as the tailoring of fusion proteins which associate non-covalently with dyes may help to solve such problems (Gaietta et al., 2002; Zhang et al., 2002).

Recently we introduced covalently self-anchoring styryl derivatives as fluorescent substrates for the histochemical localization of peroxidase (PO) activity (Krieg et al., 2007, 2008; Krieg and Halbhuber, 2004). These substrates represent reactive dyes where the targeting functional group is part of the chromophore portion