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An acrylated isocyanonaphthalene based solvatochromic click reagent: Optical and biolabeling properties and quantum chemical modeling

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ABSTRACT

The optical and biolabeling properties of a novel molecule 1-(2-acryloyloxy-3-chloro-prop-1-yl)-amino-5-isocyanonaphthalene (ACAIN) is reported. In addition to being a real solvatochromic fluorophore it reacts quantitatively and rapidly with simple thiols in a thiol-ene click reaction. DFT calculations revealed a dark nonfluorescent state of ACAIN due to a close energy triplet state where electron transition can happen between the acrylic double bond and the aromatic core through an intramolecular hydrogen bond between the NH and C=O moieties. The hydrothiolation reaction is accompanied by a 1.5–19 fold increase in fluorescence intensity depending on the solvent used owing to the saturation of the acrylic group. The quantum yield and reactivity of the molecules were found to be largely dependent on the substituent of the acryl moiety.

The biolabeling properties were investigated in detail by fluorometry and electrospray ionization (ESI) mass spectrometry using cysteine, KAC as a simple tripeptide and BSA as a model protein.

1. Introduction

Over the past few decades, interest in the thiol-Michael addition reaction has increased dramatically, since it proceeds with high yield, stereoselectivity, rate, and is thermodynamically favored [1–4]. Thiol-ene additions can be extremely useful in the detection, localization, qualification and quantitation of intracellular thiols such as cysteine, glutathione or the free cysteine units of complex peptides [5]. The development of fluorescent probes that react through catalyzed (by base or nucleophile) Michael additions resulting in anti-Markovnikov addition products [6,7] has been in the focus in the last decades. The application of the reactive flourophore is more favorable when the base molecule in non/less fluorescent and the thiol addition is accompanied by a substantial increase in fluorescent intensity. Such fluorophores include pyrazolines [5] BODIPY [8], α,β-unsaturated ketones [9] and nitroolefin-based coumarin [10].

Solvatochromic dyes are important members of smart materials. The wavelength (color) and intensity of their emitted fluorescent light is affected by the environment, particularly by its polarity. This feature can be applied advantageously in molecular biology, especially as biolabeling dyes for fluorescence microscopy, where the different parts of biomolecules can be easily differentiated from each other by the various colors of the emitted fluorescent light caused by the polarity changes around the fluorophores. This phenomenon is also suitable for the determination of the structure of biomacromolecules (eg. peptides, or locate binding sites of enzymes), or even for following their interactions by observing the local changes [14].

Acryl(ate)s and methacryl(ate)s are easily reacted with thiols and can also be easily attached to different solvatochromic fluorophores. A good example is Acrylodan, a Prodan derivative bearing a reactive acryl moiety, which has a high affinity to thiols, therefore selectively labels proteins with free thiol groups [15,16] and has many important applications in bioanalytics [17–20]. After reacting with the –SH group of biomolecules the conjugation of the acryloyl group with the close aromatic ring in Acrylodan will cease and

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fluorescence intensity increases dramatically. This phenomenon is useful in differentiating the unreacted dye from the labelled biomolecules and can be used in the construction of molecular switches. This intensity increase after the reaction does not necessarily require conjugation, it can also happen by the help of inter- or intramolecular H-bonding [21–24].

In addition to biolabeling, thiol-ene reactions can be preferable routes for the synthesis of various types of polymers, such as end functionalized polymers, dendrimers, furthermore several controlled radical polymerization techniques have also been developed based on this technique [11–13]. When a reactive fluorophore is used in the construction of smart networks, larger amounts are needed and consequently price and availability becomes serious issue. Most of these fluorophores are of complex structure or based on a pricey and hard to prepare backbone as is the case with Acrylodan. Therefore, there is a constant need for click reagents that are easily prepared from cheap and common starting materials using simple reactions, while the resulting fluorophore is solvatochromic, reacts completely with thiols, and has a dark and highly fluorescent state.

In this paper we report the design and preparation of a reactive solvatochromic dye (ACAIN) (and its methylated and chlorinated derivatives) by the modification of our recently developed molecule, 1-amino-5-isocyanonaphthalene (ICAN) [25] with only easily available reagents such as epichlorohydrin and acryloyl chloride. The acryl group reacts with thiols through a click reaction rapidly, followed by a significant increase of the emitted fluorescent light intensity because of the saturation of the double bond. ACAIN showed high affinity to thiol groups on a wide range of free thiol-containing molecules without the use of any catalyst. The kinetics of its biolabeling properties were investigated in detail using cysteine, lysyl-alanyl-cysteine (KAC), and bovine serum albumin (BSA) by fluorometry and electrospray ionization (ESI) mass spectrometry. Based on density functional (DFT) calculations a model was developed for the description of the unique fluorescent behavior that can be useful in the development of fluorescent molecular switches. The resulting solvatochromic, highly fluorescent bioconjugate products can be particularly useful in pharmaceutical chemistry and fluorescence microscopy.

2. Results and discussion

The acryl group cannot be attached directly to the amino group of ICAN without disrupting its electron donating properties and as a consequence flawing the solvatochromic properties of the molecule. The attachment should be carried out using a spacer, favorably a short chain hydrocarbon. Epichlorohydrin offers an easy alternative because after the opening of the epoxy ring with the amine a secondary hydroxyl group is formed which in turn is easily reacted with acryloyl chloride as is presented in Scheme 1. The reaction was also carried out using methacryloyl-chloride and 2-choloroacrylic acid to investigate the change in the optical properties when an electron donating (Me) and an electron withdrawing (Cl) group is present on the acryl moiety.

To test the solvatochromic properties of ACAIN and its derivatives UV–Vis and steady-state fluorescence measurements were carried out. It should be noted, however, that this paper focuses on ACAIN, therefore its properties are presented in the main article, while the corresponding data for MACAIN and CACAIN are presented in the supporting information.

The UV–Vis spectrum of ACAIN recorded in THF shows a diffuse band between $\lambda = 300$ nm and 400 nm with a peak value of 366 nm (spectrum No. 1. in Fig. 1).

The peak position showed only a slight variation between $\lambda_{max} = 356–373$ nm depending on the solvent used. The extinction coefficient corresponding to this peak varies between $\varepsilon = 5700\ M^{-1}\ cm^{-1}$ in water and $\varepsilon = 9100\ M^{-1}\ cm^{-1}$ in dichloromethane. The double peak structure of the band is characteristic for the ICAN derivatives containing free NH-hydrogen [33] and is most probably due to the superposition of the NH bending vibrational transition onto the absorption spectrum. It should be noted that the experimental UV–Vis spectra are in very good agreement with the calculated spectra (Fig. S22 in the Supporting Information (SI)). Emission spectra were recorded in solvents of different polarity. The results are summarized in Table 1 for ACAIN and for MACAIN and CACAIN in TableS1(a)-S1(b) in the SI.

As can be seen from the data of Table 1. ACAIN has visible emission in every solvent investigated. Characteristically for solvatochromic behavior the Stokes shifts increase with solvent polarity, that is bathochromic shift of the emitted light with increasing solvent polarity was found. The lowest emission wavelength was observed in hexane with $\lambda_{em,max} = 423$ nm, the largest in water $\lambda_{em,max} = 502$ nm and that of the THF solution can be found in between them at $\lambda_{em,max} = 460$ nm as is presented in Fig. 1. Surprisingly low quantum yields were observed in nonpolar solvents for all three compounds compared to the values of the starting ICAN molecule, which has the strongest emission in such solvents. According to the data of Table S1 the introduction of an electron donating methyl group to the 2 position of the acryl moiety of ACAIN increases the quantum yields in almost every solvent by ~10–20%. On the other hand, the introduction of the electron withdrawing chlorine atom to the same position drastically lowers the QY values, that is the QYs do not exceed 10% even in polar solvents in the case of CACAIN. The explanation for these phenomena will be presented later.

The solvent-dependent behavior of ACAIN was quantitatively described by the most recent Catalán model [44] according to Equation (1).

$$Y = Y_o + aSA + bSB + sSP + tSdP, \quad (1)$$

where Y_0 is the property of the substance of interest (e.g., emission maximum and Stokes shift) in the absence of solvent, for example, in the gas phase. SA is the quantitative empirical measure of the ability of bulk solvent to act as a hydrogen-bond donor towards a solute. SB is the quantitative empirical measure of the ability of a bulk solvent to act as a hydrogen-bond acceptor or electron-pair donor towards a solute, forming a solute-to-solvent hydrogen bond or a solvent-to-solute coordinative bond, respectively. SP and SdP are the solvent polarizability and dipolarity parameters, respectively, determined using reference dye molecules. a, b, s and t are the corresponding coefficients and their inclusion in the equation indicates the dependence of the property under investigation upon the respective solvent parameter.

The Catalán coefficients for the emission wavenumber at the maximum ($\bar{\nu}_{em,max}$) and the Stokes shifts ($\Delta\bar{\nu}$) were obtained by multilinear regression analysis and are summarized in Table 2. The corresponding SA, SB, SP and SdP values of the solvents along with the plots of the measured values of $\bar{\nu}_{em,max}$ and $\Delta\bar{\nu}$ *versus* their calculated values according to Eq. (1) for ACAIN (and its derivatives) are presented in the Supporting Information as Table S4 and Fig. S9. According to the data ACAIN, MACAIN and CACAIN can be considered as real solvatochromic dyes.

As seen from the data of Table 2 solvent polarity has the largest effect on the solvatochromic behavior but both the H-bond donating and accepting capabilities of ACAIN are also pronounced (large a and b parameters) because of the presence of the free NH hydrogen (H-bond donor) and the isonitrile group (H-bond acceptor).