Bioorganic & Medicinal Chemistry Letters 23 (2013) 2230–2233

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Fluorogenic 'click-on' dendrimer reporter for rapid profiling of cell proliferation

Sascha N. Goonewardena [a,b], Pascale R. Leroueil [a], Colleen Gemborys [a], Pawan Tahiliani [a], Sarah Emery [a], James R. Baker Jr. [a,*], Hong Zong [a,*]

[a] Michigan Nanotechnology Institute for Medicine and Biological Sciences, 4608 Chemistry, 930 N. University Ave., University of Michigan, Ann Arbor, MI 48109, United States
[b] Division of Cardiovascular Medicine, Department of Internal Medicine, University of Michigan, United States

ARTICLE INFO

Article history:
Received 24 September 2012
Revised 9 January 2013
Accepted 16 January 2013
Available online 29 January 2013

Keywords:
Fluorogenic reporter
Dendrimer
Coumarin
Click chemistry

ABSTRACT

The application of small molecule fluorescent reporters to monitor biological systems is limited by their poor water solubility and background fluorescence of these reporters. Herein, we describe the synthesis and testing of a fluorogenic 'click' dendrimer reporter to monitor cellular processes. The reporter system consists of a polyamidoamine (PAMAM) dendrimer conjugated with 3-azido-7-hydroxy coumarin. After the copper(I)-catalyzed azide–alkyne cycloaddition reaction ('click' reaction) with alkyne-derivatized target molecules, the natively non-fluorescent construct has a strong enhancement in fluorescence. This fluorogenic dendrimer reporter can be used to efficiently monitor biological processes and the specificity afforded by the 'click' reaction greatly reduces background noise and enhances assay flexibility. We used this fluorogenic dendrimer reporter to monitor incorporation of 5-ethynyl-2′-deoxyuridine (EdU) into newly synthesized DNA, as a surrogate marker of cellular proliferation. We anticipate that this new class of fluorogenic reporter can be used to monitor a wide array of molecules and lends itself to high-throughput profiling of biological systems.

Published by Elsevier Ltd.

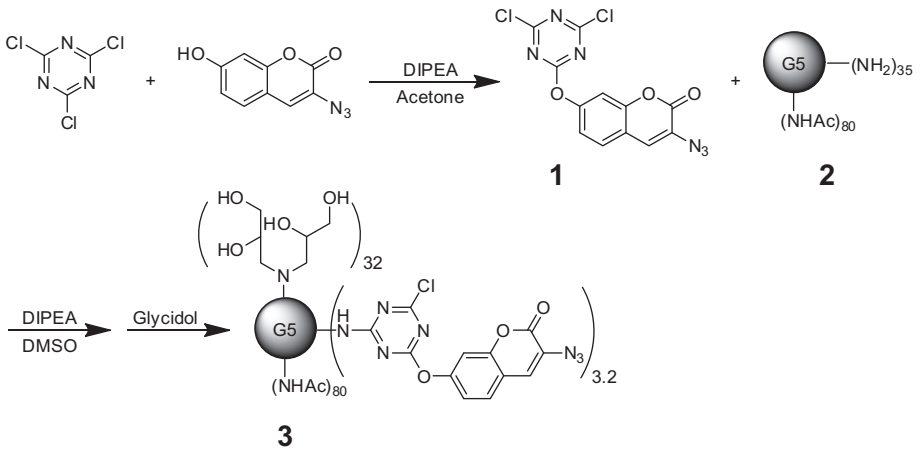
Understanding dynamic cellular processes requires the ability to track biomacromolecules and small molecule metabolites in complex biological environments including cells and animal models. Traditionally, molecules have been tracked using genetically encoded fluorescent labels like GFP or with exogenous antibody reporters.[1–3] While these methodologies have greatly enhanced our ability to probe biological systems, these approaches require the creation of bulky fusion proteins and antibody reporters that often perturb cellular processes or have inherent limitations in their efficiency.[4]

Monitoring biomolecules in living systems using bioorthogonal chemical reporters has drawn much attention lately.[4–9] The introduction of these reporter tags rely on their selective and fast reaction, under physiological conditions, and inertness to the surrounding biological functionalities. Copper(I)-catalyzed azide–alkyne cycloaddition reaction ('click' reaction) developed by Sharpless and co-workers has provided investigators with a powerful, chemoselective tool to conjugate chemical reporters that overcomes many of the existing reporter limitations.[10,11] 'click' fluorescent reporters have been used to label a wide spectrum of molecules including nucleotides, amino acids, lipids, and monosaccharides.[12] These 'click' fluorescent reporters utilize a unique chemical handle (azide or alkyne reactive group) that has been introduced onto the target molecule using the cells own biosynthetic machinery. The target molecule is detected by the highly selective ligation of a 'click' fluorescent reporter to the molecule's chemical handle. Because of the unobtrusive nature of the azide and alkyne handles, they can be easily incorporated into target molecules without perturbing the targets biological function. Additionally, because these reactive groups are only rarely present in biological systems, the 'click' reaction is highly specific and is well suited for monitoring of cellular processes such as DNA synthesis, protein translation, and post-translational modifications.[4,13–15]

Although the use of bioorthogonal molecular profiling using 'click' fluorescent reporters has greatly expanded, the existing 'click' fluorescent reporters still have certain limitations including poor water solubility and high background noise.[12,16] Because typical fluorescent reporters fluoresce continuously and do not predictably change their emission properties after they have been conjugated, the unreacted reporter contributes to background noise and requires extensive purification steps limiting assay throughput. An alternative strategy that is not limited by the need for extensive purifications or background noise is to use fluorogenic chemical reporters, whose emission wavelength or intensity changes after conjugation to its target molecule.[17] The unique properties of these fluorogenic reporters provide the opportunity

* Corresponding authors. Tel.: +1 734 615 0623; fax: +1 734 615 0621 (H.Z.).
E-mail addresses: jbakerjr@med.umich.edu (J.R. Baker), hzong@med.umich.edu (H. Zong).

0960-894X/$ - see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.bmcl.2013.01.061



Scheme 1. Synthesis of G5 dendrimer functionalized with 3-azido-7-hydroxy coumarin.

to enhance signal-to-noise and to reduce the purification steps associated with existing fluorescent reporters. While this approach has obvious benefits, it has not been widely used because of the limited availability of fluorogenic reporters, their poor water solubility, and the lack of chemoselectivity in biological systems.

Recently, 'click-on' fluorogenic reporters have been introduced that take advantage of the electronic changes associated with the formation of the triazole ring during the 'click' reaction. The 3-azido coumarin small molecule, an example of a 'click-on' fluorogenic reporter, has been developed and shown utility in labeling a range of molecules.[18] The fluorescence properties of coumarin are strongly influenced by substituents at the 3- and 7-position. The introduction of an azido moiety at the 3-position quenches the coumarin fluorescence due to the electron donating properties of the azido group. After the 'click' reaction, the formation of the triazole ring delocalizes the electrons which restores the fluorescence of the coumarin.[16,19] These types of 'click-on' fluorogenic reporters have significant potential in labeling applications but are still limited by their poor water solubility and incomplete fluorescence quenching in biological matrices. To address these limitations while preserving the functionality of the 'click-on' flourogenic reporter, we developed a polymer reporter consisting of a 3-azido coumarin conjugated to a generation 5 (G5) polyamidoamine (PAMAM) dendrimer.[20] Dendrimers have been extensively utilized as drug delivery platforms by virtue of their well-defined structure, size and shape, low toxicity and immunogenicity, and the possession of a large number of reactive surface functional groups.[20–23] We hypothesized that by conjugating the 'click-on' fluorogenic reporter to the dendrimer, we could improve its water solubility while retaining its fluorescence quenching properties in biological systems. Using this 'click-on' dendrimer reporter, we demonstrate that we can track alkyne-functionalized small molecules to monitor DNA synthesis and cell proliferation. Herein, we demonstrate that the synthetic macromolecular reporter has several advantages over small molecule chemical reporters including (1) improved aqueous solubility, (2) the ability to tune signal-to-noise with reduced background fluorescence in biological matrices, and (3) enhanced assay throughput owing to the reductions in purifications steps.

Our fluorogenic dendrimer reporter is composed of a partially acetylated G5 PAMAM dendrimer,[24] a fluorogenic 3-azido-7-hydroxy coumarin dye, and a triazine ring linker (Scheme 1). The synthetic strategy is comprised of three parts: (1) conjugation of 3-azido-7-hydroxy coumarin to 2,4,6-trichloro-1,3,5-triazine at 0 °C, triazine-coumarin **1**; (2) G5 dendrimer was partially acetylated (70%) with acetic anhydride, G5-NHAc-NH_2 **2**; (3) conjugation of triazine-coumarin to the partially acetylated G5 dendrimer at room temperature, G5-Coumarin **3**. The ease of displacement of the chlorine atoms in 2,4,6-trichloro-1,3,5-triazine by various nucleophiles in a controlled manner makes this reagent useful to serve as a cross-linker between G5 dendrimer and 3-azido-7-hydroxy coumarin. After reacting with 2,4,6-trichloro-1,3,5-triazine, the triazine-coumarin dye **1** can be ligated to any macromolecule possessing a free amino group by simply mixing them together at room temperature. The final conjugate was characterized by analytical HPLC, NMR, and MALDI (Supplementary data).

The fluorogenic properties of the small molecule and dendrimer coumarin reporters were first evaluated by performing the 'click' reaction with a representative alkyne-derivatized molecule, 3-(4-(prop-2-yn-1-yloxy)phenyl)propanoic acid at room temperature for 12 h (Supplementary Scheme S1). As mentioned earlier, azido-coumarin itself shows very weak fluorescence and the 'click' product shows strong fluorescence. Indeed, the strong fluorescence was observed and both small molecule and dendrimer 'click-on' products showed similar fluorescence properties (λ_{max} Em 470 nm). However, after attaching to the dendrimer, the absorbance of azidocoumarin has red-shifted 20 nm (λ_{max} Ex 340 vs 360 nm) (data

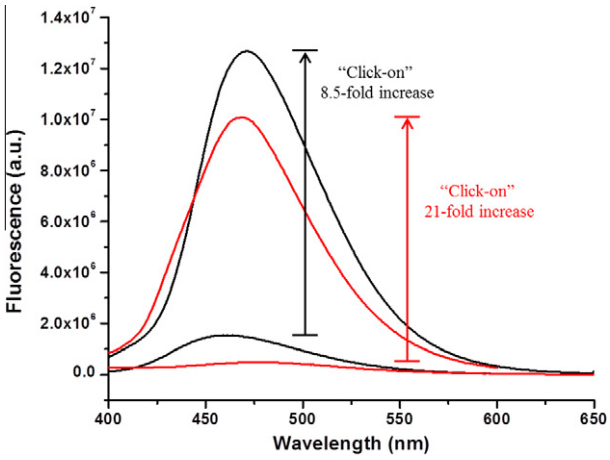


Figure 1. Emission spectra of coumarin reporters after 'click' reaction: G5-Coumarin **3** and G5-Coumarin-Click (red) (λ_{ex} = 360 nm); 3-azido-7-hydroxy-coumarin and 3-azido-7-hydroxy-coumarin-Click (black) (λ_{ex} = 340 nm). G5-Coumarin **3** and 3-azido-7-hydroxy-coumarin were compared at same coumarin molar concentrations.