Chinese Chemical Letters xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Chinese Chemical Letters

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

Communication

A proximity-induced covalent fluorescent probe for selective detection of bromodomain 4

Ling Feng[a,b], Mohit Chhabra[a], Wing Ho So[c], Qing Zhu[d], Jiang Xia[c,**], Hongyan Sun[a,b,*]

[a] Department of Chemistry and Center of Super-Diamond and Advanced Films (COSDAF), City University of Hong Kong, Hong Kong, China
[b] Key Laboratory of Biochip Technology, Biotech and Health Centre, Shenzhen Research Institute of City University of Hong Kong, Shenzhen 518057, China
[c] Department of Chemistry, The Chinese University of Hong Kong, Hong Kong, China
[d] College of Biotechnology and bioengineering, Zhejiang University of Technology, Hangzhou 310014, China

ARTICLE INFO

Article history:
Available online xxx

Keywords:
Peptide-based probe
Bromodomain
Fluorescent probe
Proximity effect
Peptides

ABSTRACT

Lysine acetylation is one of the most prevalent and important posttranslational modifications (PTMs) in proteins. The process can be recognized by bromodomains (BRDs), which are a class of protein-interaction modules involved in chromatin remodeling and transcriptional activation. The development of BRD fluorescent probes will be useful for monitoring the activity of BRDs in living cells as well as aiding inhibitor development. Herein we designed a peptide-based probe based on the proximity-induced protein conjugation reaction. The peptide-based probe is capable of covalently and selectively reacting with the unique cysteine residue in the bromodomain through proximity effect. Our experimental data showed that the probe displayed noticeable fluorescence response upon addition of BRD4(1). In-gel fluorescence scanning demonstrated that BRD4(1) can be covalently labelled by the probe. Moreover, the probe was shown to selectively detect BRD4(1) over other proteins. We envision that the probe developed in this study will provide a useful tool to further investigate the biological roles of BRDs.

Lysine acetylation is one of the most prevalent and important posttranslational modifications (PTM) with diverse biological functions. In the past years, research findings demonstrate that acetyl-lysine-mediated protein interactions are capable of regulating numerous cellular processes, *e.g.*, chromatin remodeling, DNA repair, cell metabolism and transcriptional activation [1]. For example, lysine acetylation on the histone proteins can neutralize their positive charge to attenuate the electrostatic interaction between the histones and DNAs. The process results in a more expanded chromatin structure that is linked with transcriptionally active genes. Acetylated lysine residues can also serve as the docking sites for reader proteins which could trigger protein-protein interactions [2].

Bromodomains (BRDs) comprise a family of proteins that selectively interact with acetylated lysine residues (Kac) [3]. BRD-containing proteins play an important role in regulating various cellular events including gene transcription, cell differentiation and cell cycle control. Malfunction of BRD-containing proteins are reported to be associated with a variety of diseases such as cancer, infectious diseases, diabetes, neurological diseases and others [4]. Many small molecule inhibitors have been developed to inhibit the interactions of acetylated lysine/BRDs. Some of them have become promising lead compounds for treating cancer. For example, two BET bromodomain inhibitors, JQ-1 and I-BET, were found to display effective anti-tumor activities against cancers such as NUT midline carcinoma (NMC) [5].

In light of the important roles of BRDs in biology, we seek to design fluorescent probes for detecting BRDs based on the proximity-induced protein conjugation reaction. Biocompatible site-specific covalent protein reactions, which stably tether the target protein with their inhibitors/substrates, greatly facilitate the study of post translational modifications [6], bioimaging of proteins [7] and the creation of protein conjugates for targeted therapeutics [8]. Cysteine mediated reaction is the classical method for proximity-induced protein reaction [9]. Through close examination of the X-ray structure of BRD4(1) [], we found that the BRD4(1) protein contains a unique cysteine residue Cys136 at the peptide binding site (Fig. 1), which points toward one of the acetylated lysine residue therefore allows a site-specific covalent probe to be designed (Fig. 1).

* Corresponding author at: Department of Chemistry and Center of Super-Diamond and Advanced Films (COSDAF), City University of Hong Kong, Hong Kong, China.
** Corresponding author.
E-mail addresses: jiangxia@cuhk.edu.hk (J. Xia), hongysun@cityu.edu.hk (H. Sun).

https://doi.org/10.1016/j.cclet.2018.05.031

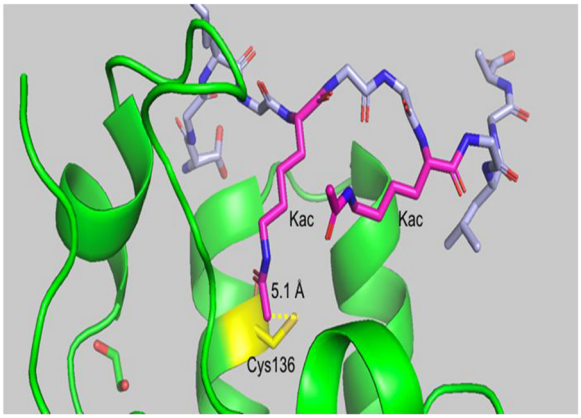

Fig. 1. Complex structure of BRD4 (1) bound with an acetylated peptide (SGRGKacGGKacGLGY) (PDB id 3uvw). The BRD4(1) domain is shown as cartoon in green, and the peptide ligand shown as ball-and-stick. Cys136 of BRD4(1) domain is highlighted in yellow, and two acetylated lysine residues in magenta. The distance between the sulfur atom of the thiol group and the methyl carbon atom of the acetyl group of acetylated lysine residue (underlined) is measured to be 5.1 Å.

As shown in Fig. 2, the probe consists of three parts: 1) a fluorophore that can detect BRD4 activity, 2) a recognition moiety that allows binding to the target protein, and 3) a cysteine reactive site that can form a covalent bond with the target protein. In our design, an environment-sensitive fluorophore NBD was chosen as the reporting unit. Environment-sensitive fluorophores can be highly sensitive to their surrounding environment [10]. The environment-sensitive dye NBD is only weakly fluorescent in water but it emits strong fluorescence in the visible range when transferred to a hydrophobic environment [11]. We envision protein-peptide interaction will bring NBD moiety into a hydrophobic environment of the protein, resulting in the increase of fluorescence emission. It therefore provides a convenient method for *in situ* monitoring of the protein-peptide interaction.

As reported in the previous research, the peptide SGRGKGGKacGLGY can bind to BRD4(1) efficiently [3a]. In this study we design the probes based on this peptide sequence. A total of two probes were designed with different cysteine-reactive groups to explore which functional group reacts better with BRD4 (1). The two probes are NBD-SGRGKacrylicGGKacGLGY (probe **1**) and SGRGKchloroGGKacGLGY (probe **2**). The two cysteine-reactive groups are acrylic amide moiety and chloroacetyl group, respectively (Fig. 2). To synthesize the probe, the peptide fragment was first assembled by solid-phase synthesis (Fig. S1 in Supporting information). Fmoc-Lys(Mtt)–COOH was introduced to covalently link the cysteine-reactive group. After the peptide assembly, the NBD fluorophore can be coupled to the N-terminus of the peptide. Subsequently the Mtt group of Fmoc-Lys(Mtt)–COOH was deprotected by diluting TFA and coupled with the two cysteine-reactive groups respectively. Finally, the peptide can be cleaved from the resin using TFA. The detail information was deposited in Supporting information.

The two peptide-based probes were first purified by HPLC and characterized by LC–MS (Figs. S2 and S3 in Supporting information). After confirming the purity and the identity of the peptide, we next carried out fluorescence study with BRD4(1). BRD4(1) protein was expressed and purified following the previous protocol [3]. After obtaining the pure protein, BRD4(1) protein was mixed with probes in PBS buffer and incubated for different time points. The fluorescence emission spectra were measured from 500 nm to 650 nm. As shown in Fig. 3A, the result shows that the fluorescence intensity of probe **1** increases with increasing time. It indicates that BRD4(1) can bind to the acrylic peptide efficiently and position the NBD of the probe in a hydrophobic environment, thus leading to the increase of fluorescence. However, in the case of the chloroacetyl-containing peptides, the result shows no obvious change in the fluorescence intensity of probe **2**, suggesting that the binding between the chloroacetyl-containing peptide and BRD4(1) protein is not strong enough to induce fluorescence increment (Figs. 3C and D).

In light of the above results, we chose probe **1** for subsequent protein labelling experiments. BRD4(1) was incubated with probe **1** in PBS buffer for different time points and then analyzed by SDS page experiments. As shown in Fig. 4, a distinct fluorescent band could be observed when BRD4(1) was incubated with probe **1** for 5

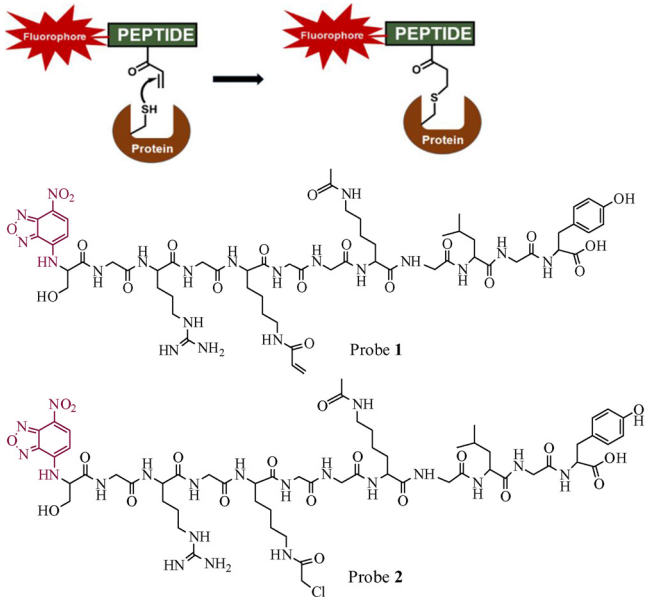

Fig. 2. Scheme of our probe design. The probe will interact with bromodomain and form a covalent bond through Michael addition reaction. Probe **1** contains an acrylic amide moiety. Probe **2** contains a chloroacetyl group.

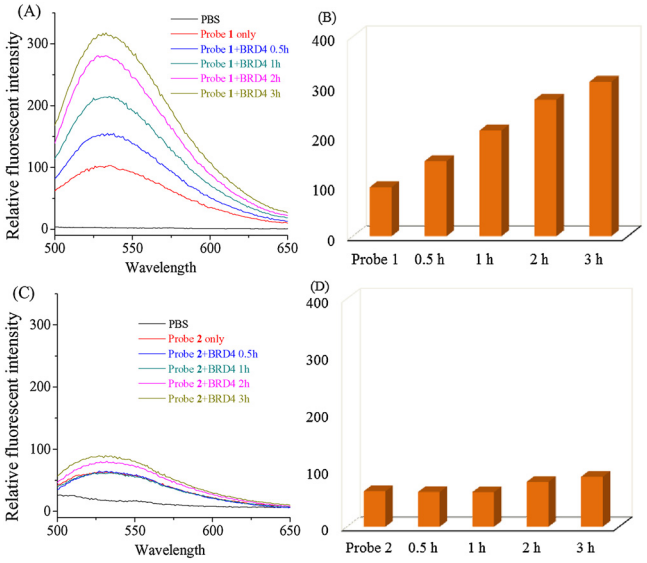

Fig. 3. Fluorescence measurement of probes incubated with BRD4(1) in PBS buffer. (A) and (B), time dependent experiment of probe **1** with BRD4(1); (C) and (D), time dependent experiment of probe **2** with BRD4(1). Ex: 460 nm.

Please cite this article in press as: L. Feng, et al., A proximity-induced covalent fluorescent probe for selective detection of bromodomain 4, Chin. Chem. Lett. (2018), https://doi.org/10.1016/j.cclet.2018.05.031