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A sensitive protein-based sensor for quantifying histone acetylation levels

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ABSTRACT

H3K14ac (acetylation of lysine 14 of histone H3) is one of the most important epigenetic modifications in cells. Aberrant changes in H3K14ac are commonly found in various types of cancers and neurological disorders. Current detection approaches for histone modifications, however, require either tedious sample pre-treatments or lack the quantitative accuracy required for biochemical and biomedical applications. In this study, we engineered a protein sensor using the amino acid sequences derived from the bromodomain of human polybromo-1 (PB1). The protein sensor was conjugated to a fluorescent dye for sensitive detection of H3K14ac. Different detection conditions, such as additive concentrations and probe concentrations, were optimally selected by balancing signal strength (I_{Rel}) and signal-to-noise ratio (SNR). The protein sensor was verified using histone H3 peptides containing different H3K14 acetylation levels. The detection signal was found to be linearly dependent on acetylation levels of H3K14 ranging from 5% to 100%. The designed platform can be used for screening epigienetic drugs regulating H3K14 acetylation levels as well as monitoring H3K14 acetylation level of circulating nucleosomes for disease progression.

1. Introduction

Epigenetic modifications, such as DNA methylation and post-translational modification of histones, are heritable changes in chromatin that affect gene expression and contribute to determining cellular phenotype [1]. Post-translational modifications (PTMs) of histone proteins are an important category of epigenetic modifications. There are several types of histone PTMs, including acetylation, methylation and phosphorylation of lysines [2]. These modifications can act alone or synergistically to determine chromatin compactness and consequently regulate gene activity [3,4].

Lysine acetylation is one of the most common histone PTMs [5,6]. Aberrant changes in histone acetylation levels have established connections with carcinogenesis [7–9]. For example, ~64% of patients diagnosed with peripheral T-cell lymphoma have reduced H4 acetylation levels in their lymphoid tissues [10]. Similar observations were made in patients with gastric and prostate cancers [11,12]. Histone acetylation is emerging as a promising biomarker for early stage cancer detection (e.g., prostate and lung cancers) [9,13] and prognosis of neurodegenerative diseases (e.g., Alzheimer, Huntington, and Parkinson disease) [14,15]. Histone acetylation markers can be found in circulating body fluids, such as serum and plasma [16,17] and are thus considered non-invasive. Among different histone acetylation biomarkers, acetylation of H3, particularly acetylation of H3K14 (H3K14ac), is considered to be of great importance. Specifically, H3K14ac is associated with transcription activation by facilitating the recruitment of transcription factor TFIID for assembling pre-initiation complex [18]. H3K14ac is also known as a "master-regulator" that modulates other histone modifications, including, H3K9ac, H4K5ac and H4K16ac [19]. Accurate quantification of H3K14ac is thus of critical importance for applying histone acetylation biomarkers in clinic.

The acetylation level of histone proteins is regulated synergistically by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Specifically, HATs catalyze the transfer of an acetyl group from acetyl coenzyme A to the ε-amino group of a lysine [20]. There are five families of HATs, namely GNAT (Gcn5-related N-acetyltransferase), MYST (MOZ, Ybf2/Sas3, Sas2 and Tip60), p300/CBP, general transcription factor HATs (which includes the TFIID subunit TAF250), and the nuclear hormone-related HAT [21]. HDACs, on the other hand, remove the acetyl group from acetylated lysines in histones. HDACs can be generally categorized as NAD^+-dependent and zinc-dependent deacetylases [22]. Aberrant expression patterns of HATs and HDACs are commonly observed in disease tissues. For example, HDAC1 and HDAC2 are largely expressed in T-cell lymphoma and prostate cancer cell lines [23,24]. In mammalian cells, acetylation of H3K14 is regulated by the complex HBO1 and MOZ/MORF (acetylation) [25] and SIRT1

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(deacetylation) [26]. The activities of HAT and HDAC are typically measured by quantifying the acetylation level of histone proteins or peptides via *in vitro* acetyltransferase reactions [27].

There are several established research tools that are capable of monitoring histone acetylation levels and consequently characterizing the activities of HATs and HDACs. Specifically, mass spectroscopy and NMR have been used to quantify histone acetylation levels at close to single amino-acid resolutions [28,29]. Applications of these two approaches, however, are limited by the equipment availability and high cost. Immuno-based (antibody-based) techniques, such as chromatin immunoprecipitation (ChIP), blotting assays and surface plasmon resonance (SPR) have also been developed [30–33]. These techniques require the use of commercial antibodies (> $500 mg) with high affinity to a selected type of histone modification. Generally, immune-based assays can detect changes in acetylation levels of ~20% using ~5 μg of histones [34]. Immuno-based assays are commonly used in research laboratories because of their relatively short analysis time and the wide accessibility of down-stream analysis tools (e.g., spectrophotometer and fluorophotometer). Nevertheless, these assays have several major limitations, including high price of antibodies and not being able to detect neighboring modifications simultaneously (due to large size of antibodies (> 15 nm) [35]). There thus remains a pressing need to develop a sensitive probe for quantifying histone acetylation levels economically.

In this study, we engineered a protein probe based on the second bromodomain (BRD) of human polybromo-1(PB1) protein. This bromodomain, also known as PB1(2), can specifically bind to H3K14ac with a relatively high affinity [36]. The engineered probe contains a fluorescent tag (fluorescein) near its C-terminus and can be used to quantify H3K14 acetylation levels in the range of 5–100% using a fluorophotometer. Compared with conventional immuno-based assays, our probe is advantageous by its low production cost (~$50 per mg compared with > $500 per mg) and relatively high quantitative accuracy (5–100% using 100 ng of histone peptides). The engineered probe is expected to be useful for profiling acetylation patterns of histone proteins and monitoring acetylation level changes in circulating nucleosomes.

2. Materials and methods

2.1. Design and production of histone H3 acetyl-probes

Bromodomain (BRD) is a conserved structural motif that selectively binds to acetylated lysines [37,38]. BRDs contain ~110 amino acids and have a secondary structure consisting of four left-handed α-helices [39]. We engineered our protein probe based on the second BRD found in the human polybromo-1 protein, a subunit of PBAF (Polybromo, BRG1-associated factors) chromatin-remodeling complex [40]. This domain was selected because it was shown to have a high affinity (K_D ~1.9 ± 0.5 μM [41]) and selectivity toward H3K14ac [36,42]. A schematic illustration of the protein probe is shown in Fig. 1A. Specifically, the probe contains a PB1(2) domain, a

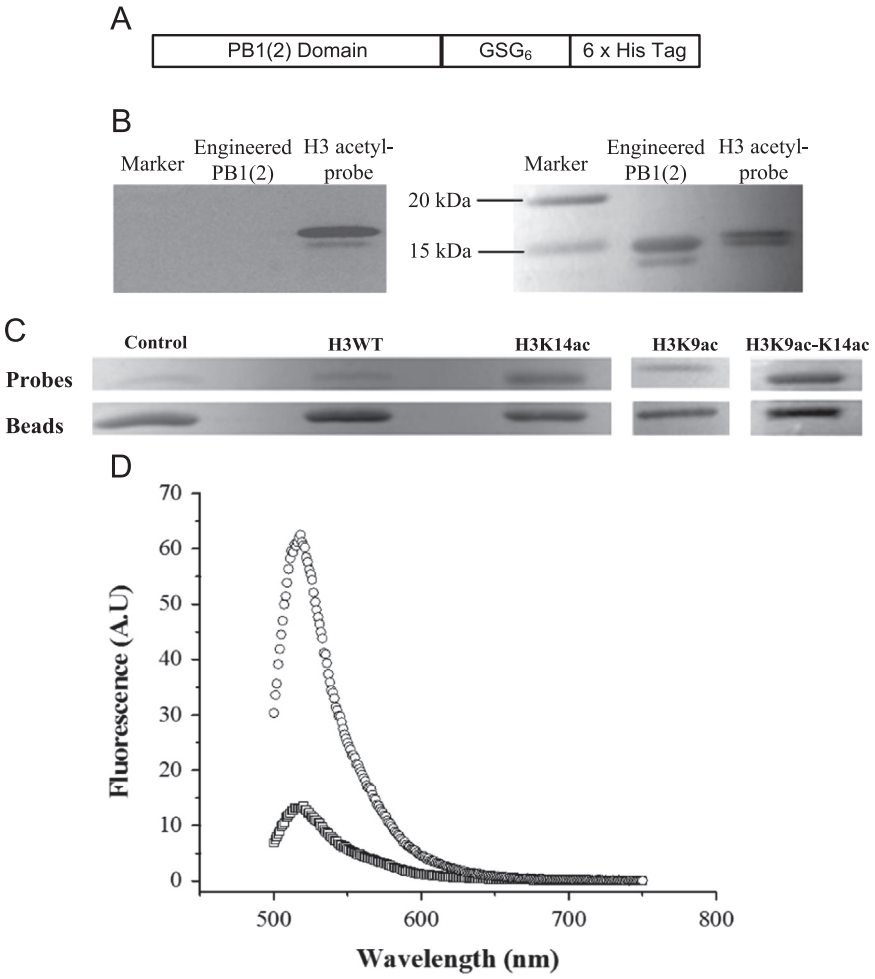

Fig. 1. (A) Schematic illustration of the designed protein probe. (B) SDS-PAGE of purified engineered protein and probe imaged before (left) and after (right) Coomassie blue staining. (C) SDS-PAGE of bound complex containing coated beads and engineered protein. (D) Fluorescence spectra for H3WT (□, 0% acetylation) and H3K14ac (○, 100% acetylation) peptides obtained using our fluorescence assay. A.U. : arbitrary unit.