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Expression, purification and characterization of individual bromodomains from human Polybromo-1

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Abstract

Computational analysis reveals six tandem bromodomains within the amino-terminal region of the human Polybromo-1 protein, a required subunit of the Polybromo, BRG1-associated factors chromatin remodeling complex. Bromodomains are acetyl-lysine binding modules found in many chromatin binding proteins and histone acetyltransferases. Recent *in vivo* studies suggest that bromodomains can both discriminate the presence of an acetyl group on a lysine side chain and locate the acetyl-lysine within a histone protein. Together, this implies that multiple bromodomains may be able to function cooperatively and recognize a specific acetylation pattern to localize remodeling complexes to specific chromatin sites. Here, the cloning, expression and bioactivity of recombinant bromodomains from the human Polybromo-1 protein is described. Individual bromodomains from Polybromo-1 were cloned from human cDNA into a pET30b expression vector enabling effective one-step purification by affinity chromatography. Due to complications, including the high number of rare codons found in the coding regions and the tendency of individually expressed domains to aggregate and misfold, bacterial expression was only achieved using a cell strain containing rare eukaryotic tRNAs. Fluorescence-based bioactivity assays were performed to determine if native binding features were retained. The present cloning, expression, and purification procedure enabled the preparation of large quantity and high yields of biologically active recombinant bromodomains from human Polybromo-1 for *in vitro* structure and function studies. This is the first report of recombinant active form of bromodomains obtained from PB1.

Keywords: Bromodomain; Polybromo; Histone-binding protein

Bromodomains represent a family of evolutionarily conserved protein modules roughly 100 amino acids in length, originally found in proteins associated with chromatin and nuclear histone acetyltransferases [1–3]. While it has been suggested from genetic studies that bromodomains play an important role in chromatin remodeling [3–5], it was only after the discovery that biological function was dependent upon acetyl-lysine binding did their key role in transcription begin to emerge [1]. This new finding suggests an important posttranslational mechanism for regulating protein–protein interactions via lysine acetylation, and has broad implications for acetyl-histone/bromodomain directed assembly of multi-protein complexes at specific chromosomal sites, as observed for SAGA [6], SWI/SNF [7], and RSC [8].

The human Polybromo-1 (PB1)[1] protein was recently identified as a unique subunit of Polybromo, BRG1-associated factors (PBAF) chromatin-remodeling complex, which is required for localization of the PBAF complex at the kinetochores during mitosis [9]. The 1634-amino acid PB1 protein contains six tandem bromodomains (BD), two bromo-adjacent homology domains (BAH), and a high-mobility group (HMG) [10] (Fig. 1). The fact that BDs bind acetylated histones [1], BAH domains are protein-interaction modules [11,12], and HMGs have been shown to bind nucleosomal DNA [13,14], the PB1 protein may serve as an

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[1] *Abbreviations used:* PB1, Polybromo-1; BD, bromodomain; BAH, bromo-adjacent homology domains; HMG, high-mobility group; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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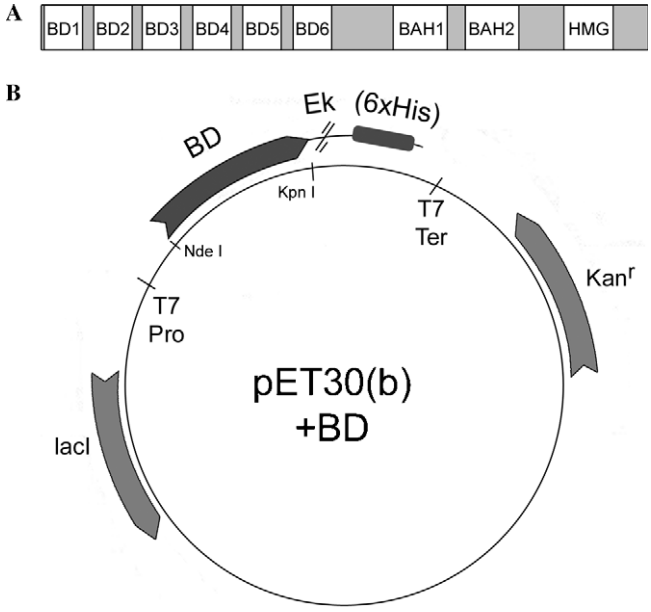

Fig. 1. Schematic representation of the PB1 expression construct. (A) The 1634-amino acid PB1 protein contains six tandem bromodomains, two bromo-adjacent homology domains, and a high-mobility group. (B) Parent vector pET30(b) has a bromodomain inserted upstream of an enterokinase (Ek) protease site and a hexa-histidine (6×His) tag. The T7 promoter (Pro) and terminator (Ter) sites are shown along with the Kan and lacI genes.

important PBAF subunit coordinating several roles central to the function of most known chromatin remodeling complexes; *targeting* chromatin sites and *recruiting* specific effector proteins to control genetic functions [15–17]. Interestingly, the PB1 subunit may target and anchor PBAF at specific chromatin sites via direct interaction of its six tandem BDs with acetylated histone proteins.

The role of PB1 in the PBAF complex is still unclear, although it does exhibit a striking relationship with several Rsc proteins. Computational analysis of *Saccharomyces* Genome Database indicates yeast proteins Rsc1, Rsc2 and Rsc4, three members of the nucleosome remodeling complex RSC, have features conserved in PB1 [18]. All three Rsc proteins have two BDs, whereas Rsc1 and Rsc2 also have a C-terminal BAH domain, totaling the six BDs and two BAH domains observed in PB1. Because Rsc1, Rsc2 and Rsc4 share multiple conserved structural motifs showing high sequence homology to PB1, it has been suggested that PB1 may be a product of gene fusions [9] and consequently confer a comparable set of biochemical features in human PBAF as the three Rsc proteins provide in yeast RSC.

In vivo studies suggest that individual BDs target only a few of the many possible histone acetylation sites [19–21]. This implies that the cumulative effect of tandem BDs is the selective recognition of specific histone acetylation patterns. In spite of the important biological role of histone post-translational modifications, no quantitative analysis of specific binding interactions has yet been performed. This lack of quantitative information is a direct reflection of the challenges associated with attaining sufficient amounts of highly purified BDs that exhibit native structural features and biological activity. Here, optimized conditions to overcome such technical barriers are described. Furthermore, bioactivity of the purified proteins is rigorously examined using fluorescence anisotropy to give solution-phase dynamics of the acetylation dependence of bromodomain–histone complex formation.

Materials and methods

Construction of bromodomain expression system

Recombinant bromodomains from the PB1 protein were generated using methods adapted from standard recombinant DNA techniques [22]. The cDNA for PB1 used here was obtained from the Horikawa Lab [10] and represents the most commonly observed splice variant that retains all six BDs. The pET30(b) vector (Novagen) was used as the parent vector for construction of the expression system (Fig. 1). Sequence alignments using the PB1 sequence (GenBank Accession No.: AF225871) were used to determine the coding region expected to represent fully functional BDs (Fig. 2) and for the design of PCR primers. Gene regions corresponding to individual BDs were PCR amplified using specific primers, which introduced the restriction enzyme sites Nde I (5′-CATATG-3′) for BDs 1–5 or Ase I (5′-ATTAAT-3′) for BD6 on the sense primer and Kpn I (5′-GGTACC-3′) on the antisense primer for subcloning into pET30(b). This construct places an enterokinase cleavage site after the carboxy terminus of the bromodomain for removal of the hexa-histidine tag. Primers for the amino-terminal bromodomain (BD1) 5′-GGTGGTCATATGCC AAGCAGGAAAAGGAGGA-3′ and 5′-GCTGCTGGT ACCATTGTCTTGCCCATCTTC-3′ correspond to amino acids 31 through 170 in the native sequence. The second bromodomain (BD2) was amplified using the forward primer 5′-GGTGGTCATATGGATGGGCAAGACAAT-CAGG-3′ and reverse primer 5′-GCTGCCGGTACC TGAGCCATATTGAAGTGCCAT-3′ corresponding to amino acids 165 through 322 in the native sequence. Primers for BD3 5′-GGTGGTCATATGGCACTTCAATAT GGCTCA -3′ and 5′-GCTGCTGGTACCGGTGGCTG AAGAGATCATGC-3′ correspond to amino acids 315 through 473 in the native sequence. Primers for BD4 5′- G GTGGTCATATGAGCATGATCTCTTCAGCCAC-3′ and 5′-TCCTCCGGTACCGAGTTTGGGAGAAGCC ATGTC-3′ correspond to amino acids 466 through 608 in the native sequence. Primers for BD5 5′- GGTGGTCATA TGGCTTCTCCCAAACTCAA-3′ and 5′-GCTGCTGG TACCCTCTTGAATCAGCAAAGTCACATT-3′ correspond to amino acids 602 through 749 in the native sequence. Primers for BD6 5′- GGTGGTATTAATATGC CAAATGTGACTTTGCTGATTC-3′ and 5′-GCTGCT GGTACCTGTGGTATAGCTGAGTGCCG-3′ correspond to amino acids 740 through 864 in the native sequence. The PCR product was cloned into the parent vec-