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Does bromodomain flexibility influence histone recognition?

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ABSTRACT

Bromodomains are protein modules that selectively recognize histones by binding to acetylated lysines. Here, we have carried out multiple molecular dynamics simulations of 20 human bromodomains to investigate the flexibility of their binding site. Some bromodomains show alternative side chain orientations of three evolutionarily conserved residues: the Asn involved in acetyl-lysine binding and two conserved aromatic residues. Furthermore, for the BAZ2B and CREBBP bromodomains we observe occlusion of the binding site which is coupled to the displacement of the two aromatic residues. In contrast to available structures, the simulations reveal large variability of the binding site accessibility. The simulations suggest that the flexibility of the bromodomain binding site and presence of self-occluded metastable states influence the recognition of acetyl-lysine on histone tails.

1. Introduction

Bromodomains, protein modules of about 110 residues, recognize acetylated lysine side chains mainly in histones and are thus involved in transcriptional regulation [1,2]. In the human genome there are 46 proteins with a total of 61 different bromodomains, with up to six bromodomains per protein [3]. Due to the potential role of bromodomains in tumors and inflammation [4–6], large scale structural studies have been carried out with the ultimate goal to facilitate the discovery of small-molecule inhibitors able to interfere in the process of reading acetylated lysine [7]. Since 1999, when the first three-dimensional structure of a human bromodomain was solved [8], the crystal and/or solution structures of more than 40 human bromodomains have been reported [9,10]. All available structures show a conserved four-helix bundle topology (Fig. 1) in which the ZA-loop and BC-loop connect the first two α helices (called Z and A) and last two α helices (called B and C), respectively [10,11]. The acetyl-lysine binding site is very similar in all structures of bromodomains [10]. The BC-loop contributes the evolutionary conserved Asn side chain [12] which acts as hydrogen bond donor to the acetylated lysine side chain. Moreover, two conserved Tyr residues [13] are present in the ZA-loop and BC-loop, respectively (Fig. S1). In striking contrast to the abundance of available three-dimensional structures, there are only few computational studies on bromodomains [14–16]. In particular, it seems that the dynamic properties of human apo bromodomains have not been investigated yet by atomistic simulations. Here, we have studied the binding site flexibility of 20 bromodomains (covering seven of the eight families of human bromodomains) by explicit solvent MD simulations. The simulation results reveal a surprisingly high heterogeneity of the plasticity and accessibility of the acetyl-lysine binding site.

2. Results and discussion

A sequence alignment of the 20 simulated bromodomains with indication of important structural features and investigated key residues is given in Fig. S1. Family memberships are listed in Table S-I. The time series of root mean square deviation (RMSD) of the C_α atoms show that the overall fold of the simulated bromodomains is stable over the 1-μs time scale and 310 K temperature of the MD runs (Fig. S2). Most of the fluctuations are localized in the ZA-loop, BC-loop, and the termini (Fig. S3). The ZA-loop and BC-loop are involved in binding the histone tail whereas the termini are located far away from the acetyl-lysine binding site so that their motion can be neglected. Importantly, we observe multiple events of reversible rotation of the side chains investigated in the present study along the 0.5- to 1-μs time scale of the MD runs (Figs. S4 and S5). These rotations take place on a time scale about one order of magnitude smaller than the length of the simulations so that the following analysis is not marred by statistical errors.

We first investigated the flexibility of the conserved Asn which is directly involved in acetyl-lysine binding (i.e., Asn1944 in BAZ2B, Asn1168 in CREBBP, Asn1604 in TAF1(2), etc.). For most of the bromodomains studied here, there are many events of reversible

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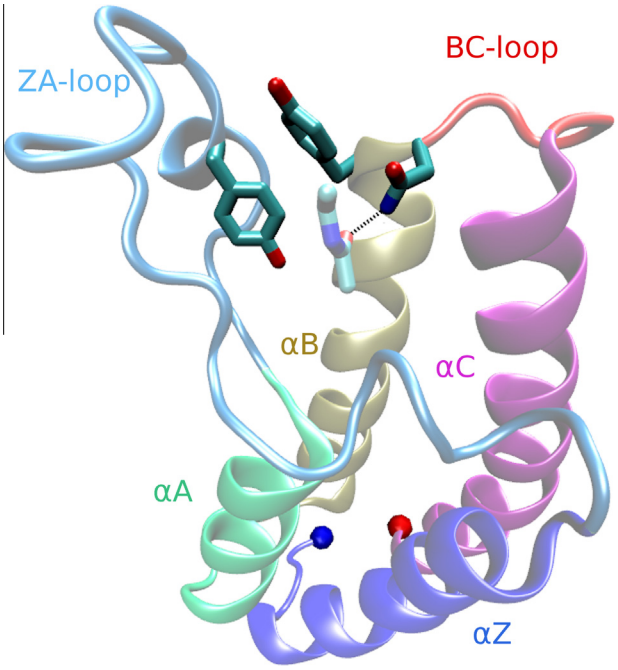

Fig. 1. Ribbon illustration of the crystal structure of the complex between CREBBP and acetyl-lysine (PDB code 3P1C). Each of the four α-helices and two binding site loops is displayed with a different color. The side chains of the conserved Tyr1125 of the ZA-loop, and Tyr1167-Asn1168 of the BC-loop are emphasized (sticks) together with the side chain of acetyl-lysine (sticks, light colors). The N-terminus and C-terminus are shown with a blue and red sphere, respectively.

rotations of the side chain amide of the conserved Asn (Figs. S4 and S5). The rotation (of about 180 degrees) around the χ_2 angle of the conserved Asn switches between an orientation in which the side chain $-NH_2$ group caps the C-terminal turn of α helix B (by donating a hydrogen bond to the backbone carbonyl of the n-4 upstream residue, e.g., the hydrogen bond between the Asn1168 side chain and the carbonyl group of residue 1164 in CREBBP) to a completely solvent exposed orientation of the $-NH_2$ group (Fig. 2, top). Note that the former orientation is observed in most (13 of 17) X-ray structures of the apo bromodomains used in this study (three of them have Thr (PHIP(2) and WDR9(2)) or Tyr (ASH1L) instead of the conserved Asn), as well as in the X-ray structures of BRD4(1) with three different diacetylated peptides (PDB codes 3UVW, 3UVY, and 3UW9) and in the CREBBP/acetyl-lysine complex (PDB code 3P1C). Interestingly, in seven of the 17 bromodomains the Asn $-NH_2$ group is oriented towards the solvent during more than one third of the simulation time (Figs. S4 and S5). These bromodomains are: TAF1L(2) (90% of the snapshots with solvent-exposed orientation of the Asn $-NH_2$ group), TAF1(2) (81%), TAF1(1) (68%), GCN5L2 (44%), ATAD2 (43%), BAZ2B (39%), and PB1(2) (38%). For TAF1L(2), TAF1(2), and TAF1(1) (all belonging to a single clade of family VII in the bromodomain phylogenetic tree [10]) the solvent exposed orientation of the Asn -NH_2 group is stabilized by a water-bridged hydrogen bond between the Asn side chain carbonyl and the hydroxyl group of the Ser in position n-4 upstream (Fig. 2, bottom). It has to be noted that most bromodomains have Ala or Cys at position n-4 upstream of the conserved Asn so that these side chains cannot form a (water-bridged) hydrogen bond. Note also that an in-depth analysis of the water molecules in the

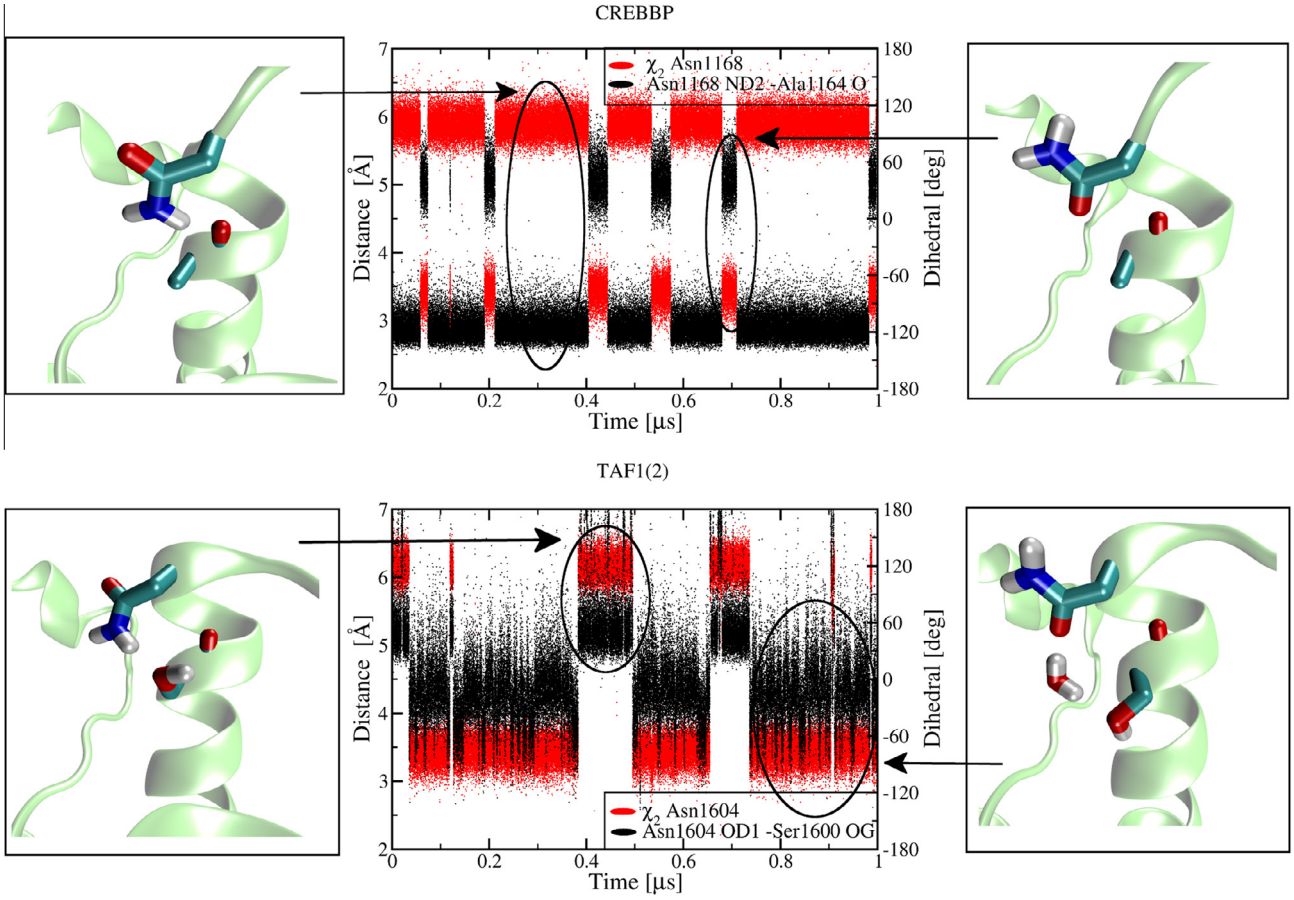

Fig. 2. Rotational flexibility of the conserved Asn side chain as observed in MD simulations of CREBBP (top) and TAF1(2) (bottom). (Middle) Time series of Asn χ_2 angle of conserved Asn (red) and distance between Asn side chain and residue n-4 upstream (black). (Left and right) Representative MD snapshots.