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Differential binding modes of the bromodomains of CREB-binding protein (CBP) and p300 with acetylated MyoD

Lanlan Wei^{a,b,1}, Nuttara Jamonnak^a, Jeremy Choy^{a,2}, Zhenghe Wang^c, and Weiping Zheng^{a,*}

^a Department of Chemistry, University of Akron, 109 East Buchtel Commons, Akron, OH 44325, USA

^b Department of Microbiology, Harbin Medical University, Harbin 150086, Heilongjiang, P.R. China

^c Department of Genetics and Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44106, USA

Abstract

The recruitment of the bromodomains of CREB-binding protein (CBP) and p300 by the acetylated myogenic transcription factor MyoD was previously shown to be critical for the enhanced MyoD transcriptional activity following acetylation at its Lys⁹⁹ and Lys¹⁰² positions. However, the modes of binding interactions of the bromodomains of CBP and p300 with acetylated MyoD have not been well-characterized. In the current study, by employing a panel of MyoD peptides encompassing the 99 and 102 positions, we showed that Lys⁹⁹ monoacetylation and Lys⁹⁹/Lys¹⁰² double acetylation defined the critical binding interfaces with the bromodomains of CBP and p300, respectively. This also represented the first identification of a recognition motif for the p300 bromodomain and revelation of the differential recognition motifs for the bromodomains of CBP and p300. This information could be exploited for developing novel tools for structural and functional studies of the highly homologous CBP and p300 transcriptional coactivators.

Keywords

Acetylated MyoD; Bromodomain; CBP; p300; Binding

Reversible lysine *N*^ε-acetylation on proteins has been increasingly recognized to play critical roles in regulating multiple pivotal cellular processes such as gene transcription, cell-cycle progression, and metabolism [1–6]. This functional significance could be derived from the reversible lysine acetylation itself which is coordinated by protein acetyltransferases and protein deacetylases, as well as the recruitment of bromodomain-containing proteins following lysine acetylation. Bromodomain represents a family of acetyl-lysine binding protein modules that contain ~110 amino acid residues and are present in many chromatin-associated proteins including transcription coactivators (e.g. CBP, p300, PCAF, and GCN5), transcription factor TAF_{II}250, the BET family of nuclear proteins (e.g. Brd2, Brd4, and Bdf1), and ATP-dependent chromatin-remodeling complexes (e.g. SWI/SNF) [7,8]. Lysine acetylation was first observed on histone proteins, but an increasing list of non-histone proteins, such as various transcription factors, HIV Tat protein, alpha-tubulin, and acetyl-coenzyme A synthetases, were also found to be acetylated [1–6].

* Corresponding author. E-mail address: wzhang@uakron.edu (W. Zheng).

¹Present address: Department of Molecular Genetics and Microbiology, The University of New Mexico School of Medicine, Albuquerque, NM 87131, USA.

²Undergraduate summer research student from Brown University, Providence, RI 02912, USA.

MyoD is a muscle-specific transcription factor essential for muscle cell terminal differentiation and muscle cell programming [9]. Its transcriptional activity has been demonstrated to be enhanced by specific lysine acetylation at the 99, 102, and 104 positions, catalyzed by the acetyltransferase activity of transcriptional coactivators CBP, p300, and PCAF [10–13]. It has also been shown that the recruitment of the bromodomains of CBP and p300 by acetylated MyoD is essential for the observed enhanced transcriptional activity following acetylation at the Lys⁹⁹ and Lys¹⁰² positions of MyoD [14]. However, the modes of binding interactions of the bromodomains of CBP and p300 with acetylated MyoD have not been well-characterized. Even though CBP and p300 are highly homologous to each other with 91% sequence identity for human proteins, and were initially suggested to be functionally equivalent [15–17], their differential functional roles have been demonstrated in different cellular processes [18–21]. Furthermore, while the CBP bromodomain was shown to be able to bind to a Lys³⁸² monoacetylated peptide derived from the human tumor suppressor protein p53, the p300 bromodomain was unable to do so [8,22]. These previously documented differences between the bromodomains of CBP and p300 and between these two proteins themselves further prompted us to examine the possible differential modes of binding interactions between acetylated MyoD and the bromodomains of CBP and p300.

In the current study, using a panel of MyoD peptides encompassing the 99 and 102 positions (Fig. 1), glutathione-S-transferase (GST)-fused bromodomains of CBP and p300, and an *in vitro* peptide–bromodomain binding assay, we have examined the individual and collective contributions of acetylation at the Lys⁹⁹ and Lys¹⁰² positions to the binding interactions with the bromodomains of CBP and p300.

Materials and methods

Peptide synthesis and purification

All peptides were synthesized on a PS3 peptide synthesizer (Protein Technologies Inc., Tucson, AZ, USA) based on the Fmoc chemistry strategy [23]. Fmoc- ϵ -amino-caproic acid and all the other Fmoc-protected amino acids and resins were purchased from Novabiochem. While four equivalents of Fmoc-protected amino acids were used in 1-h coupling reactions, 2 equivalents of Biotin-NHS (Calbiochem) were used in 2-h double coupling reactions to couple biotin to the N-terminal ϵ -amino-caproyl in each peptide. All the peptides were cleaved from the resin by reagent K (83.6% (v/v) trifluoroacetic acid, 5.9% (v/v) phenol, 4.2% (v/v) double deionized water (ddH₂O), 4.2% (v/v) thioanisole, 2.1% (v/v) ethanedithiol), precipitated in cold diethyl ether, and purified by reversed-phase high pressure liquid chromatography (HPLC) on a preparative C18 column (100 Å, 2.14 × 25 cm), eluting with a gradient of ddH₂O containing 0.05% (v/v) of trifluoroacetic acid and acetonitrile containing 0.05% (v/v) of trifluoroacetic acid. The pooled HPLC fractions were stripped of acetonitrile and lyophilized to give all peptides as puffy white solids. Peptide purity (>95%) was verified by reversed-phase HPLC on an analytical C18 column (100 Å, 0.46 × 25 cm), and their molecular weights were confirmed by either matrix assisted laser desorption ionization-time of flight (MALDI-TOF) or electrospray ionization (ESI) mass spectrometric analysis.

Expression and purification of GST-CBP (1082–1197) and GST-p300 (1047–1157)

Following the transformation of pGEX4T-3-CBP (1082–1197) (derived by subcloning from pGEX2T-CBP (1079–1457) which is a kind gift from Prof. Annick Harel-Bellan) or pGEX2TKx-p300 (1047–1157) (a kind gift from Prof. W. Lee Kraus) that define, respectively, the human CBP bromodomain [22,24] or the human p300 bromodomain [24,25] into the *Escherichia coli* strain BL21-CodonPlus (DE3)-RIL, one of the resulting colonies was used to inoculate a 50-mL Luria Broth containing 100 µg/mL of ampicillin, and the culture was grown at 37 °C for 20 h. This culture was subsequently used to inoculate a 450-mL Luria Broth also

containing 100 µg/mL of ampicillin, and the culture was grown at 37 °C until the optical density (OD) at 600 nm reached 0.8–1.0. Fresh 1.0M isopropyl-1-thio-β-D-galactopyranoside (IPTG) was then added to the culture to a final concentration of 0.1 mM, and the culture was grown at 25 °C for an additional 20 h (or at 30 °C for an additional 1 h for the expression of GST-p300 (1047–1157)). The cells were harvested by centrifugation at 6000 rpm and 4 °C for 10 min, and the pellet was resuspended in 10 mL of the cold lysis buffer (10 mM Tris·HCl (pH 7.5), 800 mM NaCl, 2 mM EDTA, 0.8% (v/v) Triton X-100, 10% (v/v) glycerol). Resuspended cells were lysed by double passage through a French pressure cell at 700 psi and centrifuged at 12,000 rpm and 4 °C for 30 min to remove cell debris. The supernatant was incubated with rotation on a Nutator at 4 °C for 4 h with 35 mg of the glutathione-agarose (that had been pre-swollen in 10 mL of ddH₂O, equilibrated with 10 mL of the lysis buffer, and pelleted by centrifugation at 1400 rpm and 4 °C for 20 s with the supernatant being discarded). This was followed by centrifugation at 1400 rpm and 4 °C for 20 s. The pellet was washed four times, each time with 10 mL of the lysis buffer, and centrifuged at 1400 rpm and 4 °C for 20 s with the supernatant being discarded. The GST fusion protein was eluted off of the washed pellet with 4 × 1 mL of the elution buffer containing 5 mM glutathione in the lysis buffer (pH 7.5). The pooled fractions were dialyzed against the peptide–bromodomain binding assay buffer (50 mM Tris·HCl (pH 7.5), 50 mM NaCl), concentrated by ultrafiltration, and used in the peptide–bromodomain binding assay (*vide infra*). The protein concentration was determined by the Bradford assay.

In vitro peptide–bromodomain binding assay

Part of the following assay conditions was derived from Ref. [22]. A homogeneous mixture consisting of 16 µL of GST-CBP (1082–1197) (or GST-p300 (1047–1157)) (25 µM in binding buffer), 4 µL of immunoglobulin G (IgG) (SIGMA (catalog # M5534), in 10 mM PBS (pH 7.4), 1% BSA, and 0.1% sodium azide), and 20 µL of a MyoD peptide (200 µM in binding buffer) was incubated with rotation on a Nutator at room temperature for 1 h. At the same time, an agarose mixture was prepared by mixing streptavidin-agarose (50% slurry from Novagen, catalog # 69203-3) and protein A-agarose (50% slurry from Calbiochem, catalog # IP02) at a ratio of 8:1 (v/v), followed by washing with the binding buffer (twice, each with a bed volume of the agarose). 90 µL of a 50% slurry (in binding buffer) of thus prepared agarose mixture was then added to the above homogenous mixture of a GST-bromodomain, a MyoD peptide, and IgG. The resulting mixture was incubated with rotation at room temperature for an additional 45 min. After centrifugation at 3000 rpm and 4 °C for 5 min, the pellet was washed with 3 × 200 µL of the wash buffer containing 50 mM Tris·HCl (pH 7.5), 0.9M (or 1.2 M) KCl, 0.1% (v/v) NP-40, and centrifuged at 3000 rpm and 4 °C for 5 min, with the supernatant being discarded. Of note, 1.2M was also the highest KCl concentration used by Polesskaya et al. in their comparative assessment of the binding interactions of acetylated and non-acetylated MyoD proteins with the bromodomain of CBP [14]. To the washed pellet was added 40 µL of a 1× SDS gel-loading buffer. The mixture was vortexed vigorously, boiled for 10 min, centrifuged, and 30 µL of it was loaded onto a 10% SDS–PAGE gel. The bands for IgG and GST-bromodomain that were retained on the agarose mixture were visualized with “blue silver” staining [26]. Briefly, after electrophoresis, a protein gel was treated with a destaining solution (50% (v/v) methanol, 10% (v/v) glacial acetic acid, and 40% (v/v) ddH₂O) for 2 h before the gel was treated overnight with the colloidal “blue silver” aqueous solution that contained 1.2% Coomassie Blue G-250, 10% (NH₄)₂SO₄, 10% H₃PO₄, and 20% methanol. The stained gel was then washed with ddH₂O, dried, and subjected to densitometry analysis to determine the adjusted band intensities for IgG and GST-bromodomain.

Densitometry analysis

The ImageJ software (version 1.37) available from U.S. National Institutes of Health was used [27]. Briefly, a dried SDS–PAGE gel was scanned and saved as a JPEG image, and the adjusted band intensities for IgG and GST-bromodomain were determined.

Results and discussion

Design of MyoD peptides

Fig. 1 shows the MyoD peptides used in the current study. All peptides were biotinylated for use in the *in vitro* peptide–bromodomain binding assay (*vide supra*). The sequence (amino acid residues 93–107) of human MyoD [28] that is located between the basic helix–loop–helix (bHLH) domain and the cysteine–histidine (C/H) rich region was used as a template for constructing the following MyoD peptides (“un” stands for “unacetylated”, “Ac” stands for “acetylated”): **K^{un}**, an unacetylated negative control peptide; **K^{99Ac}**, the peptide monoacetylated at Lys⁹⁹; **K^{102Ac}**, the peptide monoacetylated at Lys¹⁰²; and **K^{99Ac,102Ac}**, the peptide diacetylated at Lys⁹⁹ and Lys¹⁰². In addition, **sK^{un}** (“s” stands for “shorter”) that was based on a shorter MyoD sequence (96–107) was included in our study as a second unacetylated negative control peptide. Together with **K^{un}**, the inclusion of **sK^{un}** in our study could help to ensure the maximal removal of non-specific binding interaction between a peptide and a bromodomain.

In vitro peptide–bromodomain binding assay

Fig. 2 shows a schematic diagram for the *in vitro* binding assay that was used in the current study to quantify the binding interaction between a MyoD peptide and a bromodomain. This binding assay was a derivative version of that originally developed by Mujtaba et al. [22], with the following two modifications. First, as compared to the original assay format, we included IgG to be incubated with a GST-bromodomain and a biotinylated peptide at the first step of the assay, and used a mixture of protein A-agarose and streptavidin-agarose, instead of streptavidin-agarose only, to capture simultaneously biotinylated species and IgG from the incubation mixture. It is worth noting that inclusion of protein A-agarose and IgG had no interference with the bromodomain-peptide binding (unpublished observation). Second, as compared to the original assay format, instead of using Western blotting with anti-GST antibody to detect and quantify the peptide-bound GST-bromodomain at the last step of the assay, we used an improved Coomassie blue staining (“blue silver” staining [26]), which is easier and quicker than the Western blotting approach. One unique feature of the *in vitro* binding assay used in the current study is that the possible amount variation for the IgG protein retained on the protein A-agarose beads would reflect a parallel amount variation for the streptavidin-agarose beads remaining in tubes following repetitive manipulations during washing. Therefore, the amount of the peptide-bound GST-bromodomain could be normalized against the amount of IgG following the densitometry analysis of a single SDS–PAGE gel, affording the true relative binding affinities of a set of peptides for a single bromodomain.

Acetylated MyoD peptides differentially bind to the bromodomains of CBP and p300

Each of the MyoD peptides shown in Fig. 1 was initially evaluated for its binding interaction with the GST-bromodomain of CBP (i.e. GST-CBP (1082–1197), see Materials and methods). As shown in Fig. 3, when a wash buffer containing 0.9 M of KCl was used, peptides **K^{99Ac}** and **K^{102Ac}** were observed to have comparable binding affinities for GST-CBP (1082–1197), but a slightly lower binding affinity was observed for peptide **K^{99Ac,102Ac}**. Peptide **K^{un}** was observed to have a binding affinity that was twice that of peptide **sK^{un}**. When the KCl concentration in a wash buffer was raised to 1.2 M, comparable binding affinities were observed for the following four peptides, i.e. **sK^{un}**, **K^{un}**, **K^{102Ac}**, and **K^{99Ac,102Ac}**. However, their

binding affinities were all lower than that of peptide **K^{99Ac}**. The observed change in the relative binding affinities (i.e. from twofold difference to comparable) of the two negative control peptides (i.e. **sK^{un}** and **K^{un}**) when the KCl concentration was raised from 0.9 M to 1.2 M suggested that non-specific binding interactions between peptides and GST-CBP (1082–1197) had been reduced to a minimum. Therefore, the relative binding affinities of acetylated peptides observed under 1.2 M KCl wash condition, rather than those observed under 0.9 M KCl wash condition, should reflect their relative extents of specific binding to GST-CBP (1082–1197).

Our observations thus suggested that (i) acetylation at Lys⁹⁹ plays a more significant role than that at Lys¹⁰² in recruiting the CBP bromodomain, which is consistent with a previous observation by Polesskaya et al. [14] in which MyoD protein and its mutants were used instead of MyoD peptides and (ii) double acetylation at Lys⁹⁹ and Lys¹⁰² attenuates the effect of monoacetylation at Lys⁹⁹.

Following the binding experiments with CBP bromodomain, each of the MyoD peptides shown in Fig. 1 was further evaluated for its binding interaction with the GST-bromodomain of p300 (i.e. GST-p300 (1047–1157), see Materials and methods). As shown in Fig. 3, when a wash buffer containing 0.9 M of KCl was used, peptide **K^{99Ac,102Ac}** was observed to have a higher binding affinity for GST-p300 (1047–1157) than both peptides **K^{99Ac}** and **K^{102Ac}**. When the KCl concentration in a wash buffer was raised to 1.2 M, a nearly same pattern of peptide binding to GST-p300 (1047–1157) was observed. Again, non-specific binding interactions between peptides and GST-p300 (1047–1157) appeared to have been reduced to a minimum when KCl concentrations were raised from 0.9 to 1.2 M, as suggested by the observed relative binding affinities of the two negative controls (i.e. peptides **sK^{un}** and **K^{un}**) that were approaching each other following [KCl] increase from 0.9 to 1.2 M.

These observations thus suggested that neither Lys⁹⁹ monoacetylation nor Lys¹⁰² monoacetylation plays an important role in recruiting the p300 bromodomain, rather, it is the double acetylation at Lys⁹⁹ and Lys¹⁰² that promotes the binding to and recruitment of the p300 bromodomain. This pattern of recognition between the p300 bromodomain and the acetylated MyoD peptides is in stark contrast with that between the CBP bromodomain and the same peptides. While there has been documented examination of the molecular determinants for the binding of the CBP bromodomain to an acetyl-lysine-containing target sequence, the corresponding examination for the p300 bromodomain has been lacking [7,8, 22]. Our results reported here may thus further suggest that, as a general rule, p300 bromodomain is unable to bind to a target sequence with only one acetylated Lys residue, but it is able to bind to a target sequence with two or perhaps more acetylated Lys residues with appropriate relative positioning. If this is true, it could explain the incapability of p300 bromodomain to bind to a p53 peptide harboring a monoacetylation at Lys³⁸² [8,22].

In summary, by employing a panel of MyoD peptides encompassing the 99 and 102 positions, we have shown that Lys⁹⁹ monoacetylation and Lys⁹⁹/Lys¹⁰² double acetylation define the critical binding interfaces with the bromodomains of CBP and p300 transcriptional coactivators, respectively. This is not only the first revelation of the differential recognition motifs for the bromodomains of CBP and p300, but also the very first identification of a recognition motif for the p300 bromodomain. Peptide **K^{99Ac,102Ac}** could thus be exploited for structural studies with the p300 bromodomain which are currently un-explored but are expected to be able to further our understanding of how this important protein module found in the critical transcriptional coactivator p300 recognizes its target sequences.

Our findings in the current study could also be exploited for developing novel tools (e.g. compounds capable of selectively blocking the binding interactions of the bromodomains of CBP and p300 with their target motifs) for a comparative functional examination of the highly

homologous CBP and p300 in various biological processes. In this regard, peptides **K^{99Ac}** and **K^{99Ac,102Ac}** as well as their derivatives replacing acetyl-lysine with a non-hydrolyzable analog, such as *N*^ε-methanesulfonyl-lysine that we developed recently [29], could serve as the lead compounds.

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References

1. Yang XJ, Seto E. HATs and HDACs: from structure, function and regulation to novel strategies for therapy and prevention. *Oncogene* 2007;26:5310–5318. [PubMed: 17694074]
2. Glozak MA, Seto E. Histone deacetylases and cancer. *Oncogene* 2007;26:5420–5432. [PubMed: 17694083]
3. Hodawadekar SC, Marmorstein R. Chemistry of acetyl transfer by histone modifying enzymes: structure, mechanism and implications for effector design. *Oncogene* 2007;26:5528–5540. [PubMed: 17694092]
4. Batta K, Das C, Gadad S, Shandilya J, Kundu TK. Reversible acetylation of non histone proteins: role in cellular function and disease. *Subcell Biochem* 2007;41:193–212. [PubMed: 17484129]
5. Shahbazian MD, Grunstein M. Functions of site-specific histone acetylation and deacetylation. *Annu Rev Biochem* 2007;76:75–100. [PubMed: 17362198]
6. Saunders LR, Verdin E. Sirtuins: critical regulators at the crossroads between cancer and aging. *Oncogene* 2007;26:5489–5504. [PubMed: 17694089]
7. Mujtaba S, Zeng L, Zhou MM. Structure and acetyl-lysine recognition of the bromodomain. *Oncogene* 2007;26:5521–5527. [PubMed: 17694091]
8. Yang XJ. Lysine acetylation and the bromodomain: a new partnership for signaling. *Bioessays* 2004;26:1076–1087. [PubMed: 15382140]
9. Berkes CA, Tapscott SJ. MyoD and the transcriptional control of myogenesis. *Semin Cell Dev Biol* 2005;16:585–595. [PubMed: 16099183]
10. Polesskaya A, Duquet A, Naguibneva I, Weise C, Vervisch A, Bengal E, Hucho F, Robin P, Harel-Bellan A. CREB-binding protein/p300 activates MyoD by acetylation. *J Biol Chem* 2000;275:34359–34364. [PubMed: 10944526]
11. Polesskaya A, Harel-Bellan A. Acetylation of MyoD by p300 requires more than its histone acetyltransferase domain. *J Biol Chem* 2001;276:44502–44503. [PubMed: 11577095]
12. Duquet A, Polesskaya A, Cuvelier S, Ait-Si-Ali S, Hery P, Pritchard LL, Gerard M, Harel-Bellan A. Acetylation is important for MyoD function in adult mice. *EMBO Rep* 2006;7:1140–1146. [PubMed: 17028574]
13. Sartorelli V, Puri PL, Hamamori Y, Ogryzko V, Chung G, Nakatani Y, Wang JY, Kedes L. Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol Cell* 1999;4:725–734. [PubMed: 10619020]
14. Polesskaya A, Naguibneva I, Duquet A, Bengal E, Robin P, Harel-Bellan A. Interaction between acetylated MyoD and the bromodomain of CBP and/or p300. *Mol Cell Biol* 2001;21:5312–5320. [PubMed: 11463815]
15. Arany Z, Sellers WR, Livingston DM, Eckner R. E1A-associated p300 and CREB-associated CBP belong to a conserved family of coactivators. *Cell* 1994;77:799–800. [PubMed: 8004670]

16. Arany Z, Newsome D, Oldread E, Livingston DM, Eckner R. A family of transcriptional adaptor proteins targeted by the E1A oncoprotein. *Nature* 1995;374:81–84. [PubMed: 7870178]
17. Lundblad JR, Kwok RPS, Lurance ME, Harter ML, Goodman RH. Adenoviral E1A-associated protein p300 as a functional homologue of the transcriptional co-activator CBP. *Nature* 1995;374:85–88. [PubMed: 7870179]
18. Fonte C, Trousson A, Grenier J, Schumacher M, Massaad C. Opposite effects of CBP and p300 in glucocorticoid signaling in astrocytes. *J Steroid Biochem Mol Biol* 2007;104:220–227. [PubMed: 17475479]
19. Kasper LH, Fukuyama T, Biesen MA, Boussouar F, Tong C, de Pauw A, Murray PJ, van Deursen JM, Brindle PK. Conditional knockout mice reveal distinct functions for the global transcriptional coactivators CBP and p300 in T-cell development. *Mol Cell Biol* 2006;26:789–809. [PubMed: 16428436]
20. Ma H, Nguyen C, Lee KS, Kahn M. Differential roles for the coactivators CBP and p300 on TCF/beta-catenin-mediated survivin gene expression. *Oncogene* 2005;24:3619–3631. [PubMed: 15782138]
21. Kalkhoven E. CBP and p300: HATs for different occasions. *Biochem Pharmacol* 2004;68:1145–1155. [PubMed: 15313412]
22. Mujtaba S, He Y, Zeng L, Yan S, Plotnikova O, Sachchidanand, Sanchez R, Zeleznik-Le NJ, Ronai Z, Zhou MM. Structural mechanism of the bromodomain of the coactivator CBP in p53 transcriptional activation. *Mol Cell* 2004;13:251–263. [PubMed: 14759370]
23. Wellings DA, Atherton E. Standard Fmoc protocols. *Methods Enzymol* 1997;289:44–67. [PubMed: 9353717]
24. Jeanmougin F, Wurtz JM, Le Douarin BP, Chambon P, Losson R. The bromodomain revisited. *Trends Biochem Sci* 1997;22:151–153. [PubMed: 9175470]
25. Manning ET, Ikehara T, Ito T, Kadonaga JT, Kraus WL. p300 forms a stable, template-committed complex with chromatin: role for the bromodomain. *Mol Cell Biol* 2001;21:3876–3887. [PubMed: 11359896]
26. Candiano G, Bruschi M, Musante L, Santucci L, Ghiggeri GM, Carnemolla B, Orecchia P, Zardi L, Righetti PG. Blue silver: a very sensitive colloidal Coomassie G-250 staining for proteome analysis. *Electrophoresis* 2004;25:1327–1333. [PubMed: 15174055]
27. Rasband, WS. Image J. U.S. National Institutes of Health; Bethesda, Maryland, USA: 1997–2007. Available from: <<http://rsb.info.nih.gov/ij/>>
28. Pearson-White SH. Human MyoD: cDNA and deduced amino acid sequence. *Nucleic Acids Res* 1991;19:1148. [PubMed: 1850513]
29. Jamonnak N, Fatkins DG, Wei L, Zheng W. *N*(epsilon)-methanesulfonyl-lysine as a non-hydrolyzable functional surrogate for *N*(epsilon)-acetyl-lysine. *Org Biomol Chem* 2007;5:892–896. [PubMed: 17340003]

sK^{un}: Biotin-Ahx-W⁹⁶-A-C-**K⁹⁹**-A-C-**K¹⁰²**-R-K-T-T-N¹⁰⁷-COOH
K^{un}: Biotin-Ahx-C⁹³-L-L-W-A-C-**K⁹⁹**-A-C-**K¹⁰²**-R-K-T-T-N¹⁰⁷-COOH
K^{99Ac}: Biotin-Ahx-C⁹³-L-L-W-A-C-**AcK⁹⁹**-A-C-**K¹⁰²**-R-K-T-T-N¹⁰⁷-COOH
K^{102Ac}: Biotin-Ahx-C⁹³-L-L-W-A-C-**K⁹⁹**-A-C-**AcK¹⁰²**-R-K-T-T-N¹⁰⁷-COOH
K^{99Ac,102Ac}: Biotin-Ahx-C⁹³-L-L-W-A-C-**AcK⁹⁹**-A-C-**AcK¹⁰²**-R-K-T-T-N¹⁰⁷-COOH

Fig. 1.

Biotinylated MyoD peptides used in the current study. AcK, *N*^ε-acetyl-lysine; Ahx, ε-amino-caproic acid.

Fig. 2.

A schematic diagram for the *in vitro* peptide-bromodomain binding assay used in the current study.

Fig. 3. SDS-PAGE and densitometry analyses indicating the relative binding affinities of MyoD peptides for the bromodomains of CBP and p300 under two different salt washing conditions. A bar graph based on densitometry analysis is shown below each representative protein gel image. GST-Brd band intensities were normalized based on band intensities for the internal control IgG. Each bar in a bar graph represents the average GST-Brd band intensity measured for a duplicate sample within each single binding experiment. Bar sK^{un} represents the average intensity for the GST-Brd bands in lanes 1 and 2 (peptide sK^{un} was used in the experiment), bar K^{un} for the GST-Brd bands in lanes 3 and 4 (peptide K^{un} was used in the experiment), bar K^{99Ac} for the GST-Brd bands in lanes 5 and 6 (peptide K^{99Ac} was used in the experiment), bar K^{102Ac} for the GST-Brd bands in lanes 7 and 8 (peptide K^{102Ac} was used in the experiment), and bar K^{99Ac,102Ac} for the GST-Brd bands in lanes 9 and 10 (peptide K^{99Ac,102Ac} was used in the experiment). The same binding experiment was repeated two more times, and a pattern

of GST-Brd band intensities consistent with that shown in this figure was observed. Exactly the same assay conditions were employed to assess the binding interactions between a GST-Brd (of CBP or p300) and peptides shown in Fig. 1.