THE JOURNAL OF PHYSICAL CHEMISTRY B LETTERS

2007, *111,* 11877−11879
Published on Web 09/26/2007

Autoacetylation Induced Specific Structural Changes in Histone Acetyltransferase Domain of p300: Probed by Surface Enhanced Raman Spectroscopy

Mohammed Arif,[†] G. V. Pavan Kumar,[‡] Chandrabhas Narayana,[‡] and Tapas K. Kundu*[,†]

Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, and Light Scattering Laboratory, Chemistry and Physics of Materials Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O., Bangalore, India

Received: August 6, 2007; In Final Form: September 8, 2007

Reversible acetylation of histone and non-histone proteins plays an important role in the regulation of gene expression and cellular homeostasis. A balance between acetylation and deacetylation of these proteins are maintained by histone acetyltransferases (HATs) and histone deacetylases (HDACs). Among different HATs, p300/CBP is the most widely studied chromatin modifying enzymes. p300 is involved in several physiological processes like cell growth, regulation of gene expression, development, and tumor suppressor, and therefore its dysfunction causes different diseases. The autoacetylation of p300 is one of the key regulators of its catalytic activity. Mechanistically, autoacetylation induced structural changes in the p300 HAT domain acts as a master switch. In this report, we have shown that the natural HAT inhibitor garcinol could potently inhibit the autoacetylation activity. Furthermore, for the first time, we demonstrate that indeed autoacetylation induces structural changes in p300 HAT domain, as probed by surface-enhanced Raman scattering. Presumably, SERS will be a very useful tool to find out the structural changes in the other self-modifying enzymes like kinases and methyltransferases.

The acetylation and deacetylation of histone and non-histone proteins play a key role in the regulation of gene expression, cell growth, and differentiation and in maintaining cellular homeostasis in the eukaryotic cells.[1] A balance between acetylation and deacetylation of the proteins are brought about by two important classes of histone modifying enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs).[1,2] Among the HAT family, the p300/CBP group of HATs are most widely studied and have been identified as major enzymes that acetylate protein with a range of biological functions. The intrinsic histone acetyltransferase (HAT) activity of p300 plays important roles in the transcriptional coactivation of p53, p73, steroid hormone response, NFkB, the STATS, and GATA.[3,4] The dysfunction of p300/CBP HAT function has been implicated in several types of cancer, Huntington disease, cardiac hypertrophy, HIV, and inflammatory processes, which makes p300 as a novel target for therapeutics.[5] The HAT activity of p300 is regulated by several mechanisms, which include methylation and autoacetylation.[6−9] p300 HAT domain (p300HD) (Figure 1a) gets extensively autoacetylated, which is a highly cooperative process and is intermolecular in nature.[8] The autoacetylation of p300 dramatically enhances its activity[9] and thereby increases the HAT activity dependent signal via the acetylation of histone and non-histone proteins. Recently, the involvement of p300 autoacetylation has been documented in the transcriptional preinitiation complex formation.[10] From a convincing set of experiments, it has been suggested that the autoacetylation induces conformational change in the p300, and thereby it gets dissociated from the preinitiation site and enhances the binding of general transcription factor, TFIID. Although autoacetylation mediated structural alteration of p300 has been realized as one of the key mechanisms of action, the direct evidence for the conformational changes is yet to be demonstrated.

Recently, we have reported the SERS of the full-length p300 and also presented the complete vibrational spectral analysis of it.[11] We have successfully demonstrated the importance and use of the SERS study to understand the protein−small molecule interactions (inhibitors and activators).[12,13] These studies could have immense therapeutic importance. Here, we report the SERS study of the p300HD and hyperacetylated (autoacetylated) HAT domain in solution. This would be the first demonstration of the autoacetylation-mediated alteration of p300HD conformation, which is highly significant for its function.

To perform SERS of p300HD, it was adsorbed on the silver nanoparticles. The HAT and autoacetylation activities of p300HD incubated with silver nanoparticles were assayed by fluorography. As depicted in Figure 1b, the HAT activity of p300HD adsorbed on the silver nanosurface remained almost the same as compared to that of the mock-incubated enzyme (Figure 1b, lane 2 vs lane 4). Similarly, the autoacetylation activity of the p300HD was also not affected in the presence of silver nanoparticles (compare Figure 1c, lane 2 vs lane 6). Interestingly, we observe that the potent natural HAT inhibitor, garcinol,[14] could inhibit the HAT activity of p300HD (Figure 1b, lane 3 vs lanes 5 and 6) as well as the autoacetylation of p300HD (Figure 1c, lane 3 vs lanes 4 and 5). Presumably, the

* Corresponding author. E-mail: tapas@jncasr.ac.in.
[†] Transcription and Disease Laboratory, Molecular Biology and Genetics Unit.
[‡] Light Scattering Laboratory, Chemistry and Physics of Materials Unit.

10.1021/jp0762931 CCC: $37.00

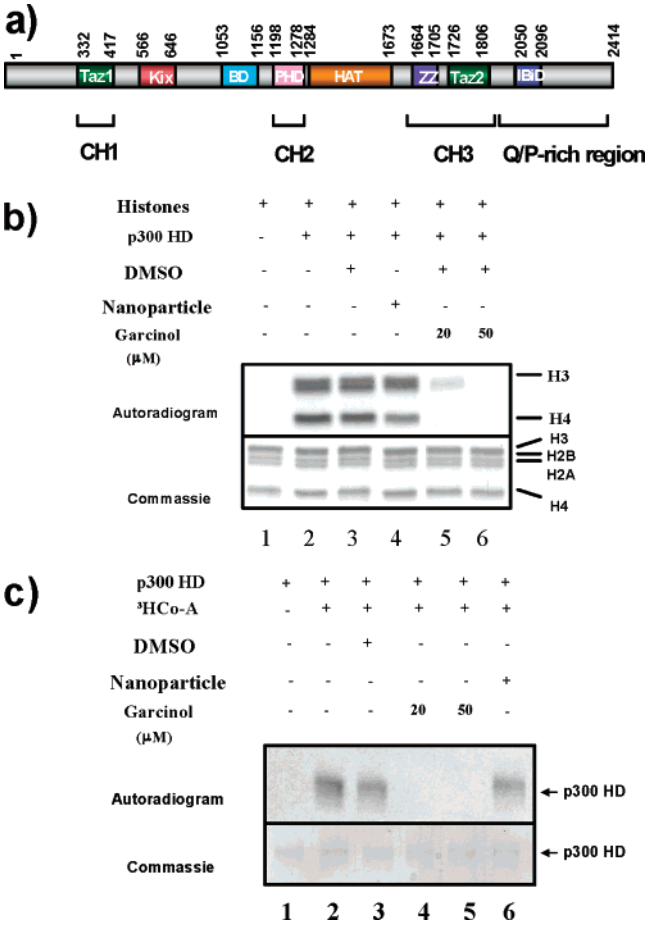

Figure 1. (a) Domain organization of full-length p300: Functional domains of p300 include CH1, CH2, CH3, KIX, Bromodomain (BD), HAT domain, and Q/P-rich region. (b) Effect of silver nanoparticle and garcinol on the p300HD catalytic activity: HAT assays were performed by using highly purified HeLa core histones in the absence (lane 1) and presence of p300HD (lane 2), the p300HD incubated with silver nanoparticle (lane 4); lane 3, histones with the HAT in presence of DMSO, lane 5 and 6; histones with the HAT and in the presence of 20 and 50 μM of garcinol, respectively. (c) Effect of silver nanoparticle and garcinol on the p300HD autoacetylation activity: Autoacetylation assays were performed using p300HD in the absence of [^{3}H] acetyl CoA (lane 1) and in the presence of [^{3}H] acetyl CoA with silver nanoparticle (lane 6), (lane 3); in the presence of DMSO as solvent control, lanes 4 and 5, HAT in the presence of 20 and 50 μM of garcinol, respectively. Reaction mixtures were resolved on SDS-PAGE and processed for fluorography.

p300 HAT inhibitory function of garcinol is exhibited through the inhibition of p300 autoacetylation and hence the substrate acetylation.

Further, to understand the structural details of p300HD in the absence of high-resolution crystal structure, we performed SERS of bacterially purified p300HD (see Supporting Information for protein purification). Figure 2 shows the SERS spectra of HAT domain in the range 400−1800 cm^{-1}. To confirm the amide band positions, we have performed SERS of the deuterated HAT domain. Upon deuteration of proteins, most of the hydrogen in the amide groups will be replaced by the heavier deuterium.[15] This will increase the reduced mass of the vibrating unit (oscillator), which is inversely proportional to the vibrational frequency. Therefore, one should expect a decrease (red-shift) in the amide vibrations. As expected, we observed a red-shift in amide bands (see Supporting Information). The shifts in the frequency for the amide I and II bands of p300HD were found to be 7 and 5 cm^{-1}, respectively.

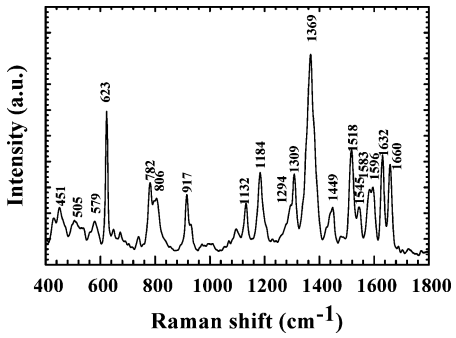

Figure 2. SERS of HAT domain.

TABLE 1: SERS Band Assignments of p300HAT Domain

SERS band (cm^{-1})	assignment
1660	amide I (α helix)
1632	amide I (random coil)
1596	Trp,Tyr, and/or Phe
1583	ν_{as}(COO$^-$), His, Trp
1545	Trp,Tyr, and/or Phe
1518	amide II and/or Trp
1449	δ(CH$_2$)
1369	ν_s(COO$^-$)
1303	ω(CH$_2$)
1294	amide III (α helix)
1184	Phe(ν_{9a})
1132	ν_{as}(C$_\alpha$CN)
917	ν(C−COO$^-$)
806	Tyr and/or ν_s(C−S−C)
782	Trp($_{W18}$)
623	Phe (ν_{6b})
579	Trp
505	ν(S−S)

To confirm that the SERS spectrum of the HAT domain is not contaminated with the spectra of the buffer, we have carried out the SERS of the neat buffer solution and the results are presented in Supporting Information. Table 1 shows the SERS band assignments of the HAT domain. Modes have been assigned in comparison to the SERS spectra of the parent protein p300[11] and using the standard amino acids and proteins band assignments. It is evident that the spectrum is dominated by SERS modes of ring-structure amino acids: tryptophan (Trp), tyrosine (Tyr), and phenylalanine (Phe), which are characteristic of SERS of any protein. To investigate the structural changes associated with the fully acetylated p300HD using SERS, we performed autoacetylation reactions (see Supporting Information). The autoacetylation was confirmed by mass spectrometry analysis. It was found that the p300HD got acetylated in all the available 37 sites as compared to recombinant p300HD, which has about 11 sites modified, as verified by mass spectrometry (Figure 3a, I and II). We have performed SERS of fully autoacetylated p300HD and compared it with normal, partially acetylated HAT domain. This is shown in Figure 3b (I and II). There is a marked change in the modes pertaining to symmetric stretching of COO$^-$ (1369 cm^{-1}), amide III (1294 cm^{-1}), ν_{9a} of Phe (1184 cm^{-1}), and asymmetric stretching of C$_\alpha$CN (1132 cm^{-1}). We observe not only a change in intensity of these modes but also some softening of modes (Figure 3b). Softening of the modes implies decrease in the Raman shift.[16] This observed softening of modes ($\sim$10 cm^{-1}), in fact, signifies bond weakening due to the interaction between various groups upon autoacetylation. Also, it was interesting to observe that some of the modes like 1449 and 1094 cm^{-1} that pertain to δ(CH$_2$) and proline (Pro) do not show any significant shifts after the autoacetylation. The possible reason for such an observation is

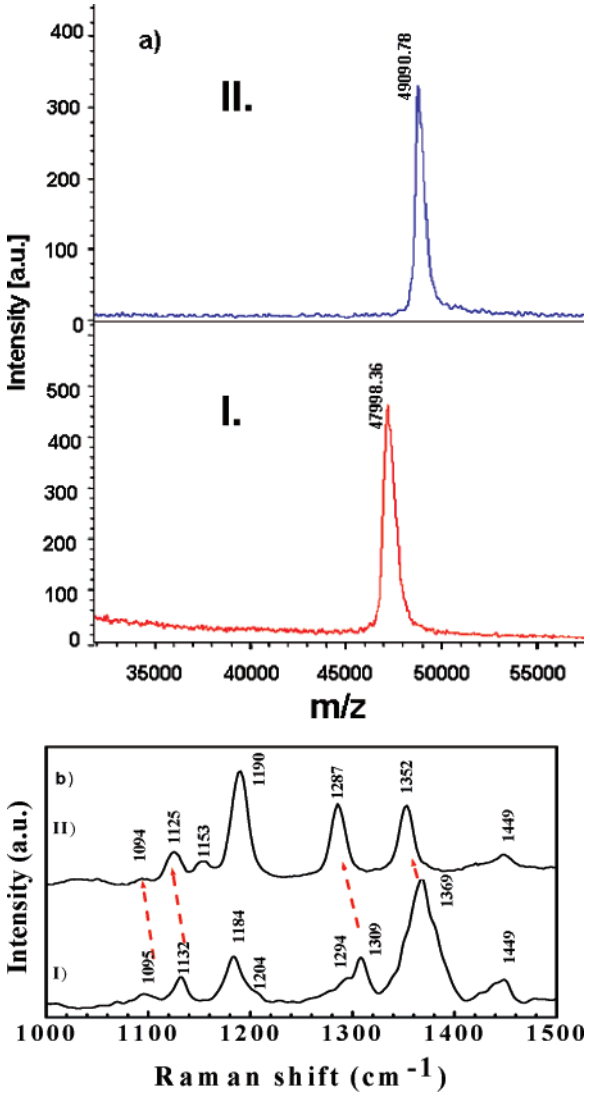

Figure 3. (a) MALDI-TOF mass spectroscopy of purified p300HD. The observed mass of recombinant protein is 47 998.36 Da and that of fully autoacetylated is 49 090.78 Da. The expected mass of p300HD is 47 536 Da. Because single acetyl modification is 42 AMU, so the recombinant p300HD has about 11 sites modified (I) and 37 in the fully autoacetylated p300HD (II). (b) SERS of (I) HAT domain compared with (II) autoacetylated HAT domain. The red arrows indicate the softening of some modes. The spectrum has been vertically shifted for clarity.

that, upon autoacetylation, there could be an increase in hydrogen bonding between groups. This could effect the bond strengths of various groups such as amides but would not effect groups like $\delta(CH_2)$.

Hence, these changes indicate structural reorganization of the HAT domain after the complete autoacetylation. It is to be noted that, unlike conventional Raman spectroscopy, SERS probes vibrational modes of groups that are in close proximity to the metal surface. This would be reflected as intensity changes due to the distance dependent electromagnetic enhancement and the surface selection rules, where the orientation of the molecule on the metal surface determines the enhancement of the vibrational modes. Therefore, all these conditions suffice to make SERS sensitive to probe the structural modifications of macromolecules near the surface of nanoparticles, as in the present case. Furthermore, these data also suggest that the SERS could be used to monitor the structural changes in the other self-modifying proteins like kinases, methyltransferases, and glycosyltransferases.[9]

In summary, this report establishes the structure of one of the most important domain of the master regulator p300. Remarkably, for the first time it also establishes that autoacetylation of p300 dramatically alters its structure, which is significantly important for the autoacetylation-mediated regulation of the p300 HAT activity, especially in the regulation of gene expression in humans.

Acknowledgment. We thank Dr. P. A. Cole for providing valuable reagent and Dr. K. Matelingu for the purification of garcinol. The Department of Biotechnology, Government of India and Jawaharlal Nehru for Advance Scientific Research supported this work. M.A. thanks CSIR for the Senior Research Fellowship. C.N. thanks JNCASR for financial support. We acknowledge the mass spectroscopy facility of the Molecular Biology and Genetics Unit, JNCASR.

Supporting Information Available: Protein purifications, histone acetyltransferase assay, p300 autoacetylation assay, synthesis of silver nanoparticles, autoacetylation of p300 HAT domain for SERS experiments and surface enhanced Raman spectroscopy experiment. The material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Roth, S. Y.; Denu, J. M.; Allis, C. D. *Annu. Rev. Biochem.* **2001**, *70*, 81−120.
(2) Berger, S. L. *Curr. Opin. Genet. Dev.* **2002**, *12*, 142−148.
(3) Goodman, R. H.; Smolik, S. *Genes Dev.* **2000**, *14*, 1553−1577.
(4) Costanzo, A.; Merlo. P.; Pediconi, N.; Fulco. M.; Sartorelli. V.; Cole, P. A.; Fontemaggi, G.; Fanciulli, M.; Schiltz, L.; Blandino, G.; Balsano, C.; Levrero, M. *Mol. Cell* **2002**, *9*, 175−186.
(5) Swaminathan, V.; Reddy, B. A.; Ruthrotha, S. B.; Sukanya, M. S.; Kundu, T. K. *Subcell Biochem.* **2007**, *41*, 397−428.
(6) Chevillard-Briet, M.; Trouche, D.; Vandel, L. *EMBO J.* **2002**, *21*, 5457−5466.
(7) Girdwood, D.; Bumpass, D.;Vaughan, O. A.; Thain, A.; Anderson, L. A.; Snowden, A. W.; Garcia-Wilson, E.; Perkins, N. D.; Hay, R. T. *Mol. Cell* **2003**, *11*, 1043−1054.
(8) Karanam, B.; Jiang, L.; Wang, L.; Kelleher, N. L.; Cole, P. A. *J. Biol. Chem.* **2006**, *281*, 40292−40301.
(9) Thompson, P. R.; Wang, D.; Wang, L.; Fulco, M.; Pediconi, N.; Zhang, D.; An, W.; Ge, Q.; Roeder, R. G.; Wong, J.; Levrero, M.; Sartorelli, V.; Cotter, R. J.; Cole, P. A. *Nat. Struct. Mol. Biol.* **2004**, *11*, 308−315.
(10) Black, J. C.; Choi, J. E.; Lombardo, S. R.; Carey, M. *Mol. Cell* **2006**, *23*, 809−818.
(11) Kumar, G. V. P.; Reddy, B. A.; Arif, M.; Kundu, T. K.; Narayana, C.; *J. Phys. Chem. B* **2006**, *110*, 16787−16792.
(12) Mantelingu, K.; Reddy, B. A.; Swaminathan, V.; Kishore, A. H.; Siddappa, N. B.; Kumar, G. V. P.; Natesh, N. G.; Roy, S.; Sadhale, P. P.; Ranga, U.; Narayana, C.; Kundu, T. K. *Chem. Biol.* **2007**, *14*, 645−657.
(13) Mantelingu, K.; Kishore, A. H.; Balasubramanyam, K.; Kumar, G. V. P.; Altaf, M.; Swamy, S. N.; Selvi, R.; Das, C.; Narayana, C.; Rangappa, K. S.; Kundu, T. K. *J. Phys. Chem. B* **2007**, *111*, 4527−4534.
(14) Balasubramanyam, K.; Altaf, M.; Varier, R. A.; Swaminathan, V.; Ravindran, A.; Sadhale, P. P.; Kundu, T. K. *J. Biol. Chem.* **2004**, *279*, 33716−33726.
(15) Tuma, R.; Prevelige, P. E., Jr.; Thomas, G. J., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9885.
(16) Fabriciova, G.; Sanchez-Cotez, S.; Garcia-Ramos, J. V.; Miskovsky, P. *Biopolymers* **2004**, *74*, 125−130.