

Recognition of the N-Terminal Histone H2A and H3 Peptides by Peptidylarginine Deiminase V

Yuji Hidaka*, Mayumi Watase, Hironori Matsubayashi, and Masatoshi Saiki

School of Science and Engineering, Kinki University, Higashi-osaka, Osaka 577-8502, Japan
E-mail: yuji@life.kindai.ac.jp

Introduction

Peptidylarginine deiminase 5 (PAD5), which is the same protein as PAD4, catalyzes the citrullination reaction of an Arg residue to yield a citrulline residue (Cit) in peptides and proteins, such as histone and eukaryotic translation initiation factor 4G1 [1-3]. Ca²⁺-dependent citrullination of the Arg residues of histone by PAD5 has come into focus as a novel post-translational modification linked to transcriptional regulation in eukaryotes [4,5]. Recently, it has been reported that citrullination of cellular proteins by PAD5 might cause rheumatoid arthritis [6]. However, the target proteins that are related to rheumatoid arthritis, are not well known. We previously reported that PAD5 citrullinated histone proteins *in vivo* [1,2,7]. Therefore, we assumed that histone citrullination is related to rheumatoid arthritis. However, the citrullination mechanism of histone subunits by PAD5 is yet to be studied in detail. In order to investigate the effect of post-translational modifications, such as acetylation on PAD5 recognition, a series of the N-terminal peptides of histone H2A and H3 was prepared, as shown in Fig. 1, its citrullination reaction by PAD5 was examined, and the citrullination sites *in vitro* by MALDI-TOF/MS were determined.

H2A-N20:	SGRGKQGGKARAKAKTRSSR
Ac-H2A-N20:	Ac-SGRGKQGGKARAKAKTRSSR
H3-N20:	ARTKQTARKSTGGKAPRKQL
Ac-H3-N20:	Ac-ARTKQTARKSTGGKAPRKQL

Fig. 1 Primary structures of the N-terminal synthetic peptides of histone H2A and H3. H2A-N20 and H3-N20 consists of the N-terminal 20 amino acid residues of H2A and H3, respectively. Ac-H2A-N20 and Ac-H3-N20 represent the N-terminal acetylated peptides.

Results and Discussion

PAD5 recognizes the Arg3 residue in histone H2A as well as the Arg3 residue in histone H4 *in vivo* [7,8]. In addition, it has been reported that the Arg17 residue in histone H3 was predominantly citrullinated and the Arg8 was also partially citrullinated *in vivo* [9]. In these studies, the N-terminal acetylated H2A and H4 and the N-terminal non-acetylated H3 were examined with respect to PAD5 recognition since the N-terminal Ser residue in histone H2A is predominantly acetylated *in vivo*, but the N-terminal Ala residue in histone H3 is not acetylated *in vivo*. However, the N-terminal acetylation effect of histone subunits on PAD5 recognition is yet to be studied. In

addition, the N-terminal non-acetylated H2A was also reported and the N-terminal analysis of histone H3 has not been studied in detail. Therefore, in order to investigate the N-terminal acetylation effects of histone H2A and H3 on PAD4 recognition, a series of N-terminal peptides of H2A and H3 was prepared by solid phase peptide synthesis, purified by RP-HPLC and treated with PAD5.

When non-acetylated H2A-N20 was treated with PAD5 *in vitro*, two peaks were observed on an HPLC profile, as shown in Fig. 2. Peaks 1 and 2 corresponded to an original H2A-N20 peptide and to a product containing one citrulline residue, respectively, which was confirmed by MALDI-TOF/MS analyses.

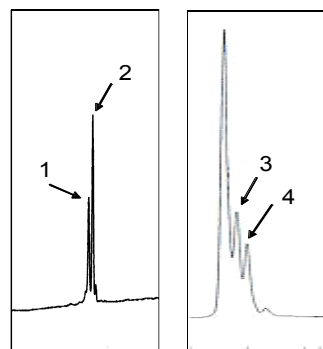


Fig. 2 HPLC Profiles of PAD5-treated H2A-N20 (left) and Ac-H3-N20 (right).

To determine the citrullination site in the product (peak 2 in Fig. 2), the peptide was treated with an enzyme, Arg-C, in the presence of H₂¹⁸O and purified by RP-HPLC. We employed H₂¹⁸O to determine the citrullination site. Citrullination increases only one mass unit from the original peptide with the Arg residue. To identify the citrullination site easily, H₂¹⁸O was employed, since the ¹⁸O-labeled citrullinated peptide possesses three mass units that are different from the original peptide with the Arg residue. MALDI-TOF/MS analyses of the Arg-C digests of the citrullinated peptides indicated that the Arg3 residue in non-acetylated H2A-N20 was citrullinated along with Ac-H2A-N20 [10]. This result suggests that the acetylation at the N-terminal amino group of histone H2A is not significant for PAD5 recognition. However, a minor product was also observed on the HPLC profile (data not shown). PAD5 recognizes the Arg3 residue of histone H2A, which is localized at the second position of the type II β-turn structure [11]. The Arg3 residue is closely localized at the N-terminal moiety. Therefore, one can speculate that

the N-terminal acetyl group of histone H2A stabilizes the β -turn structure for PAD5 recognition and the de-acetylation caused destabilization of the β -turn structure at the N-terminal moiety, resulting in a loss of specific recognition.

Next, the N-terminal acetylation effect of histone H3 was also examined with respect to PAD5 recognition. Ac-H3-N20 was treated with PAD5, and the products were separated by RP-HPLC. Two major products were observed in the HPLC profile, as shown in Fig. 2. Surprisingly, the N-terminal acetylation of histone H3 dramatically affected the PAD5 recognition. It has been reported that the N-terminal histone H3 peptide was citrullinated at the Arg17 and Arg8 *in vitro*, as well as in *in vivo*, experiments using histone H3 [9, 10]. However, MALDI-TOF/MS analyses indicated that Ac-H3-N20 was predominantly citrullinated at the Arg2 residue *in vitro* in our experiments (peak 3 in Fig. 2). In addition to Arg2 residue, Arg8 residue was also citrullinated (peak 4 in Fig. 2). Previously, we reported that PAD5 specifically recognized the Arg residue, which was localized in the type II β -turn structure in the crystal structure of the complex of the H2A peptide and PAD5 (PAD4), as described above. The type II β -turn structure consists of four amino acid residues, and needs a -Arg-Gly- sequence in the turn moiety. However, the N-terminal H3 peptide does not possess the -Arg-Gly- sequence, which affects the specificity of PAD5 recognition. Therefore, PAD5 recognizes not only the Arg17 residue but also the Arg8 residue of histone H3 *in vitro*. The crystal structures of the complex of the histone H3 peptides and PAD5 were also reported. However, it was difficult to estimate the difference between the turn structures of Arg17 and Arg2 with respect to PAD5 recognition. In addition, it seemed that the N-terminal moiety of the H3 peptide did not possess the specific turn structure in the crystal structure. Therefore, one can speculate that the acetylation of the N-terminal Ala residue (Ac-H3-N20) induced the β -turn formation at the N-terminal moiety of histone H3, resulting in the induction of a dramatic effect on PAD5 recognition. This result also suggests that Arg17 originally may not be a suitable substrate of PAD5 since the -Pro-Arg-Lys-Gln-moiety cannot form the type II β -turn structure [11, 12].

To obtain the structural information of PAD5 recognition, CD measurements of the synthetic peptides were performed. In the aqueous solution, the specific secondary structures were not observed for the synthetic histone peptides. However, the CD spectra of the peptides did not correspond to that of the random-coiled structure. The CD spectrum of H2A-N20 was almost the same as that of Ac-H2A-N20 as well as H3-N20 and Ac-H3-N20. These results indicate that acetylation does not affect the secondary structure. To estimate the structure under hydrophobic circumstance, such as the binding pocket of PAD5, CD measurements of the synthetic histone peptides were also performed in 30% TFE. The CD spectrum of H2A-N20 in 30% TFE was almost identical to that in aqueous solutions, indicating that the secondary structure of the histone N-terminal peptide did not change under the hydrophobic conditions. CD spectra of the other synthetic peptides also showed identical results to that of H2A-N20. Further structural analysis is needed to elucidate the structure function relationship concerning the N-terminal acetylation.

In conclusion, N-terminal acetylation is important for PAD5 recognition of histone H2A and H3. The de-acetylation of H2A loosened the PAD5 recognition,

resulting in citrullination at the Arg3 and another Arg residue of the N-terminal H2A peptide *in vitro*. In addition, the acetylation of H3 caused a dramatic effect in PAD5 recognition; PAD5 predominantly citrullinated the Arg2 residue of Ac-H3-N20. This result suggests that N-terminal acetylation causes a critical structural change in the N-terminal H3 peptide *in vivo*. These observations provide insight into the recognition mechanism of PAD5 *in vivo*.

Acknowledgments

We gratefully thank Prof. Aimoto and Prof. Kawakami (Osaka University) for use of HF apparatus. This work was supported in part by Grant-in-Aid for Scientific Research No. 16510160 from the Ministry of Education, Science and Culture.

References

1. Hagiwara, T., Nakashima, K., Hirano, H., Senshu, T. and Yamada, M. (2002) *Biochem. Biophys. Res. Commun.*, **290**, 979-983.
2. Nakashima, K., Hagiwara, T. and Yamada, M. (2002) *J. Biol. Chem.*, **277**, 49562-49568.
3. Okazaki, Y., Suzuki, A., Sawada, T., Ohtake-Yamanaka, M., Inoue, T., Hasebe, T., Yamada, R., and Yamamoto, K. (2006) *Biochem Biophys Res Commun.*, **34**, 94-100.
4. Arita, K., Hashimoto, H., Shimizu, T., Nakashima, K., Yamada, M., and Sato, M. (2004) *Nature Struct. Mol. Biol.* **11**, 777-783
5. Cuthbert, G.L., Daujat, S., Snowden, A.W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P.D., Tempst, P., Bannister, A.J. and Kouzarides, T. (2004) *Cell* **118**, 545-553.
6. Suzuki, A., Yamada, R., Chang, X., Tokuhiko, S., Sawada, T., Suzuki, M., Nagasaki, M., Nakayama-Hamada, M., Kawaida, R., Ono, M., Ohtsuki, M., Furukawa, H., Yoshino, S., Yukioka, M., Tohma, S., Matsubara, T., Wakitani, S., Teshima, R., Nishioka, Y., Sekine, A., Iida, A., Takahashi, A., Tsunoda, T., Nakamura, Y. and Yamamoto, K. (2003) *Nat. Genet.*, **34**, 395-402.
7. Hagiwara, T., Hidaka, Y. and Yamada, M. (2005) *Biochemistry*, **44**, 5827-5834.
8. Hidaka, Y., Hagiwara, T. and Yamada, M. (2005) *FEBS Lett.*, **579**, 4088-4092.
9. Wang, Y., Wysocka, J., Sayegh, J., Lee, Y.H., Perlin, J.R., Leonelli, L., Sonbuchner, L.S., McDonald, C.H., Cook, R.G., Dou, Y., Roeder, R.G., Clarke, S., Stallcup, M.R., Allis, C.D., and Coonrod, S.A. (2004) *Science*, **306**, 279-283.
10. Watase, M. and Hidaka, Y. (2006) *Peptide Science 2005*, 189-190.
11. Arita, K., Shimizu, T., Hashimoto, H., Hidaka, Y., Yamada, M., and Sato, M. (2006) *Proc Natl Acad Sci U S A.*, **103**, 5291-5296.
12. Matsubayashi, H., Watase, M., and Hidaka, Y. (2006) *Peptide Science 2006*, 192.