

Structure of Peptidylarginine Deiminase Type 1; the First Example of a Monomeric Isozyme of PAD

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1 Introduction

Protein citrullination is a post-translational modification of arginine in which peptidylarginine is deiminated to generate peptidylcitrulline. This modification from the positively charged arginine to the neutral citrulline changes the molecular charge of the residue and the surrounding region, thereby altering not only the intermolecular interactions but also the intramolecular interactions of the target proteins. Citrullination is associated with the functioning of important biological processes. The citrullination is catalyzed by peptidylarginine deiminase (PAD; EC 3.5.3.15) in a Ca²⁺-dependent manner. In humans, 5 PAD isozymes (PAD1–PAD4 and PAD6) have been described. PAD isozymes show tissue-specific expression pattern, which is regulated in transcription level, and are responsible for citrullination of variety of substrate proteins. The most tolerant substrate specificity of PAD1 may account for the physiological significance of the non-redundant PAD isozymes. Considering the fact that citrullinated sites in the natural substrate proteins do not commonly share the specific consensus motif, it is likely that highly complex mechanism(s) underlie the molecular structure of each PAD isozyme. In order to elucidate the substrate-specificity of PAD1, we determined crystal structure of human PAD1 at 3.2 Å resolution [1]. During structural determination, we recognized that PAD1 molecules were arrayed in a different manner from that of the dimeric PAD2 and PAD4. In order to elucidate the functional biological unit, we measured the scattering of PAD1 solution using the small-angle X-ray scattering (SAXS) technique.

2 Experiment

The X-ray diffraction intensity data from PAD1 crystals were first conducted at BL-5A of Photon Factory. The higher resolution data were then collected at AR-NE-3A of PF Advanced Ring.

The small angle X-ray scattering data of PAD1 and PAD3 were collected at the beamline BL-10C of PF.

3 Results and Discussion

In the asymmetric unit of the PAD1 crystal, two PAD1 molecules were contained; however, the 3D arrangement of two molecules were completely different from what was found in the PAD2 or PAD4 homodimer. To determine the biological unit of PAD1, we conducted small-angle X-ray scattering analyses of PAD1, and compared with PAD3 in solution. From these structural analyses, we concluded that PAD1 exists as a monomer. This is the first example of the existence of a monomeric form among the PAD isozymes. We also found that the C-terminal catalytic domain of PAD1 is very similar to the substrate-bound PAD4, whereas the N-terminal IgG domain showed structural variety. Additionally, we conducted the reaction analyses of the partial peptides of S100A3, which is one of the substrates of PADs in human hair follicle. Our work implies that the monomer structure of PAD1 was related to its tolerant substrate specificity. It should be also noted that the present structure provides a structural basis for the substrate specificities of PAD isozymes [1].

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References

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