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Structural study of isozymes of peptidylarginine deiminases and its related proteins in hair follicle

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We are studying the structures of peptidylarginine deiminases (PADs) type I and type III (PAD1 and PAD3) to understand the substrate specificities and for structure-based drug design of isozyme-selective inhibitors. We are also studying their substrate S100A3 which is highly expressed in hair cuticuler cell.

1 Introduction

PAD (peptidylarginine deiminase) which is an enzyme that converts arginine residue to citrulline in target proteins under the condition that the Ca^{2+} exists. It's becoming clear that citrullination by this enzyme is involved in various pathophysiological roles. Five PAD isozymes in mammals have been described; those are PAD1, PAD2, PAD3, PAD4, and PAD6. Understanding of the mechanisms of the different substrate-specificities of each isozyme is useful for the treatment of disease in which a PAD isozyme participates.

Also, PAD1, PAD2 and PAD3 exist in hair follicle. S100A3 protein (hereafter S100A3), one of calcium-binding proteins, is thought to be a substrate of these PAD isozymes. When arginines in S100A3 are citrullinated, S100A3 converts its molecular structure from dimer to tetramer. It is also known that Ca^{2+} and Zn^{2+}-binding affinities are increased upon tetramerization. We are also interested in understanding the mechanism of these phenomena.

In order to develop the isozyme-specific chemical compounds and to elucidate the substrate-specificities and S100A3 molecular structural changes and Ca^{2+}/Zn^{2+}-binding mechanism, we tried to determine the structure of PAD1, PAD3 and Ca^{2+}/Zn^{2+}-binding S100A3 tetramer at atomic level by X ray crystallography.

2 Experiment

We reported the structure of the Ca^{2+}-bound PAD1, previously [1, 2]. The structure was His-tagged at its N-terminal amino acid. In this work, we digested the His-tag by molecular biological technique, because the N-terminal tail structure was very different conformation from that of the other isozymes. PAD1 was crystallized with hanging drop vapor diffusion method using the solutions containing 0.1 M MES (pH 6.5) and 12% (w/v) PEG20000 or 0.1 M Tris-HCl (pH 8.5) and 25% (w/v) PEG8000 as reservoir solutions.

We also crystallized PAD3 to obtain the structure at atomic level resolution. Actually, we obtained the PAD3 crystals 5 years ago [3], but the crystal was very unstable for Ca^{2+}-soaking. Thus, we searched new crystallization conditions. We crystallized PAD3 with hanging drop vapor diffusion method using 0.1 M Tris-HCl (pH 8.5), 20% (w/v) PEG4000 and 0.2 M $CaCl_2$ as a reservoir solution.

3 Results and Discussion

We obtained rod shape crystals of PAD1 after cleaving the N-terminal His-tag (Fig. 1). However, the crystals diffracted up to ~6 Å resolution. We have to improve the crystal quality and purification procedure.

We also obtained the Ca^{2+}-bound PAD3 crystals (Fig. 2) and determined the structure. PAD3 contains 5 Ca^{2+} ions (Fig. 3) in its structure, but the active site was disordered. This is different aspect from the PAD4 structure earlier reported [4].

We are now searching the other crystallization conditions. We have been also trying to form complexes with their inhibitors and substrate.

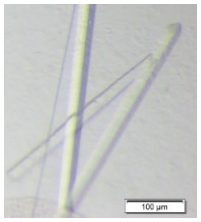

Fig. 1 Crystals of PAD1.

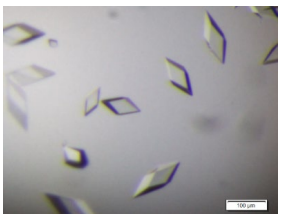

Fig. 2 Crystals of His-tagged Ca^{2+}-bound PAD3.

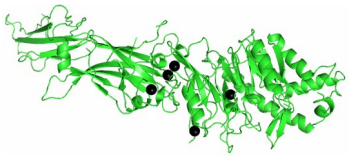

Fig. 3 Ca^{2+}-bound PAD3 structure. Black balls represent Ca^{2+}.

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