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Refined Crystal Structures of Human Ca^{2+}/Zn^{2+}- binding S100A3 Protein Characterized by Two Disulphide Bridges

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1 Introduction

The S100 protein family constitutes the largest subgroup of the EF-hand type of Ca^{2+}-binding protein family. Among more than 20 members of this protein family, S100A3 has the highest cysteine content (10 out of 101 amino acids) and an exceptionally high affinity for Zn^{2+} ion (K_d = 1.5-11 μM). This Ca^{2+} and Zn^{2+}-binding protein is highly expressed in differentiating cuticular cells in the human hair follicle and organized into the mature cuticles. We previously reported that natural occurring S100A3 protein was post-translationally citrullinated in hair follicles. Peptidylcitrulline residues brought by peptidylarginine deiminases (PAD) have been considered to decrease the net positive charge of peptidylarginine residues in the substrate protein, thereby changing its structure owing to alterations of the intra- and inter-molecular ionic interaction. This irreversible post-translational modification has recently been implicated in several physiological and pathological processes and also associated with human diseases including cutaneous disorders. Among the five known human PADs (types I-IV and VI), type III isozyme (PAD3), which is dominantly expressed in hair follicles, converts a symmetric pair of Arg51 residues on dimeric S100A3 to citrullines in the presence of Ca^{2+}. This specific citrullination of S100A3 causes assembly of a Ca^{2+}-bound homotetramer and the Ca^{2+}-ions could transfer to PAD enzymes in turn. S100A3 is postulated to act as Ca^{2+}-modulating protein during its dimer-tetramer transition within the hair cuticular cells.

Although characterization of the Zn^{2+}- and Cu^{2+}-binding sites was initially hampered due to the absence of a conserved motif, recent analyses of the crystal and solution structures have revealed their unequivocal locations. Regarding the high affinity Zn^{2+}-binding site in S100A3, a structural model of a dinuclear thiolate-bridged Zn^{2+}-cluster coordination in the C-terminal cysteine-rich domain was proposed based on spectrophotometry. A putative single Zn^{2+}-binding site was also reported to be partially preformed in the helix IV terminal region based on the previously reported crystal structure of metal-free S100A3 dimer (PDB code 1KSO). However, the exact location of the Zn^{2+}-binding site was not determined.

2 Experiment

Purified proteins were concentrated by centrifugal filtration for crystallization. To attempt disulphide bond refolding within Ca^{2+}/Zn^{2+} bound forms (PR-treatment), $CaCl_2$ and $ZnSO_4$ were added to the protein solution to 2 equimolar with S100A3 monomer. Crystallization was carried out using the hanging-drop vapour diffusion method at 20°C. Crystals suitable for high-resolution structure determination for were obtained using 2.4 M $(NH_4)_2SO_4$ as a reservoir solution within a few days.

3 Results and Discussion

We identified two previously undocumented disulphide bridges: one is between Cys30 in the N-terminal *pseudo*-EF-hand and Cys68 in the C-terminal EF-hand (SS1), and another attaches Cys99 located in the C-terminal coil structure to Cys81 in helix IV (SS2). Mutational disruption of SS1 (C30A+C68A) abolished the Ca^{2+}-binding property of S100A3 and retarded citrullination of Arg51 by peptidylarginine deiminase type III (PAD3), while SS2 disruption inversely increased both Ca^{2+}-affinity and PAD3 reactivity *in vitro*. Similar backbone structures of WT, R51A and C30A+C68A indicated that neither Arg51 conversion by PAD3 nor SS1 alter the overall dimer conformation. SS1 renders the hinge region flexible and the helix III reorientation, those are essential for its Ca^{2+}-binding properties, whereas SS2 structurally shelters Arg51 in the metal-free form. A model of the tetrahedral coordination of a Zn^{2+} ion by $(Cys)_3His_1$ residues that is compatible with SS2 formation in S100A3 is proposed.

References

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