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SAXS analyses of S100A3: Masking effect of Arg-51 on its homotetramerization

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Introduction

S100A3 is an EF-hand type Ca^{2+}-binding protein highly expressed in the maturing cuticular cells within the hair follicle [1, 2]. We previously reported that Arg-51 in S100A3 is specifically converted to citrulline by Ca^{2+}-dependent peptidylarginine deiminases (type III) [3]. Mutational substitution of Arg-51 to alanine (R51A), as well as citrullinated S100A3, promotes its homotetramer assembly. This study aimed to confirm our hypothetic proto-type model of the Ca^{2+}-bound S100A3 tetramer (Fig. 1), which deduced from the location of Cit-51 in a known crystal structure of apo-dimeric S100A3 (1KSO) [4]; and to elucidate precise assembling manner by small-angle X-ray scattering (SAXS) analyses.

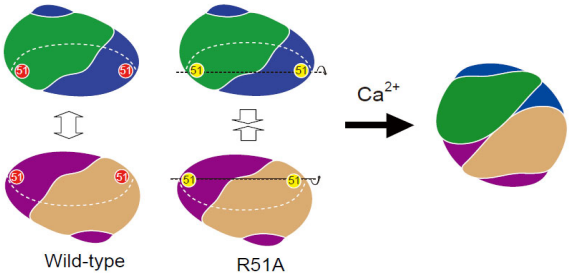

Fig.1. Hypothetic effect of Arg-51 neutralization on the Ca^{2+}-dependent S100A3 tetramerization.

Materials and methods

Wild type and mutant (R51A) of human S100A3 were expressed in Sf-21 cells by inoculating the recombinant baculoviruses. Recombinant proteins were purified as previously reported for the natural product [3] with slight modification. SAXS measurements of the purified proteins (100 - 400 μM) were performed at BL-10C [5].

Results and discussion

Changes in the size and shape of S100A3 could be directly monitored by the SAXS technique. SAXS data were acquired under various Ca^{2+} concentrations. *R*g value of wild-type protein (360 μM) evaluated by Guinier approximation increased remarkably around 10 mM $CaCl_2$, whereas that of R51A arose around 3 mM $CaCl_2$ (Fig. 2). *R*g values at infinite dilution of apo-form were 19.1Å for wild type and 20.3Å for R51A; while those of Ca^{2+}-bound form were 24.9Å for wild type and 24.4Å for R51A. Estimated molecular weight was 1.9-fold for wild type and 1.6-fold for R51A, respectively. Changes of their single Trp fluorescence were observed within the similar range of Ca^{2+} concentrations (Fig.2, inset). These results suggest that S100A3 tetramerization is coupled with the Ca^{2+}-induced conformational change.

Preliminary analysis of a prolate model revealed a long-shaped structure of apo-dimeric form of R51A compared to that of wild-type (Fig. 3). This suggests that masking of Arg-51 may be unfold the C-terminal regions, which is not resolved in a crystal structure (1KSO) [4]. Further acquisition of SAXS data is in progress for modeling of more precise solution structures of dimeric and tetrameric S100A3 by fitting analogous S100 3D-structures.

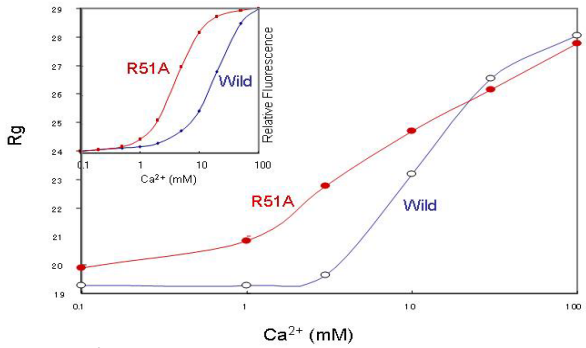

Fig. 2. Ca^{2+}-induced changes in *R*g of S100A3 consistent with its fluorescent change (inset).

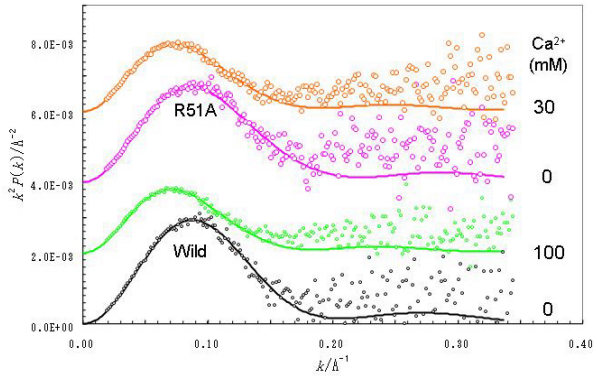

Fig. 3. Kratky plots for S100A3. Wild type: 0mM (black); 100mM (green), R51A: 0mM (violet); 30mM (orange).

References

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