Biological Science

10C/2007G003

An Oblate Shape of Ca^{2+} (and Zn^{2+})-bound S100A3 Homotetramer

Kenji KIZAWA[1], Yuji JINBO[2], Takafumi INOUE[1], Yoshinobu IZUMI[2]*
[1]Basic Research Laboratory, Kanebo Cosmetics Inc., Odawara 250-0002, Japan
[2]Graduate School of Science & Eng., Yamagata Univ., Yonezawa, Yamagata 992-8510, Japan

Introduction

S100A3, of which Arg-51 is specifically converted to citrulline by Ca^{2+}-dependent peptidylarginine deiminases (type III), assembles a homotetramer within hair cuticular cells [1]. Its Cys-rich C-terminal domain reported to bind two Zn^{2+} ions [2], however its structural and functional in the Ca^{2+}-dependent tetramerization is still unknown. This study aimed to elucidate Ca^{2+} (and Zn^{2+})-bound tetrameric architecture in solution by small-angle X-ray scattering (SAXS) analyses.

Materials and methods

Dimer to tetramer ratio of S100A3 and R51A (a mutated protein analogous to the citrullinated form), and overall shape in Ca^{2+} (and Zn^{2+})-containing solution were evaluated using the SAXS measurement at BL-10C [3].

Results and discussion

Mol fraction of S100A3/R51A tetramer was calculated from M_w determined by SAXS data with the Guinier approximation. S100A3/R51A dimers converted to tetramers up to ~70% even at 100 mM Ca^{2+}. Fluorescent titration revealed that affinities to either Ca^{2+} or Zn^{2+} of S100A3 were mutually increased by the concurrent heterotrophic ion. In the presence of Zn^{2+}, R51A more efficiently assembled a homotetramer with 1mM Ca^{2+} [4].

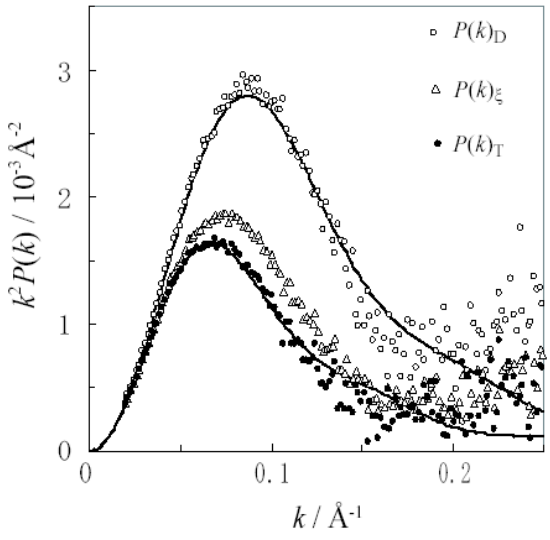

Fig. 1. Kratky plot, $k^2P(k)$ vs. k, for R51A $P(k)_D$ and $P(k)_\xi$ are particle scattering functions for dimer and the mixture in 30 mM $CaCl_2$. $P(k)_T$ for the tetramer was calculated from $P(k)_D$ and $P(k)_\xi$. Solid lines are theoretical values for an oblate model: ν = 0.44 for $P(k)_D$ and ν = 0.39 for $P(k)_T$

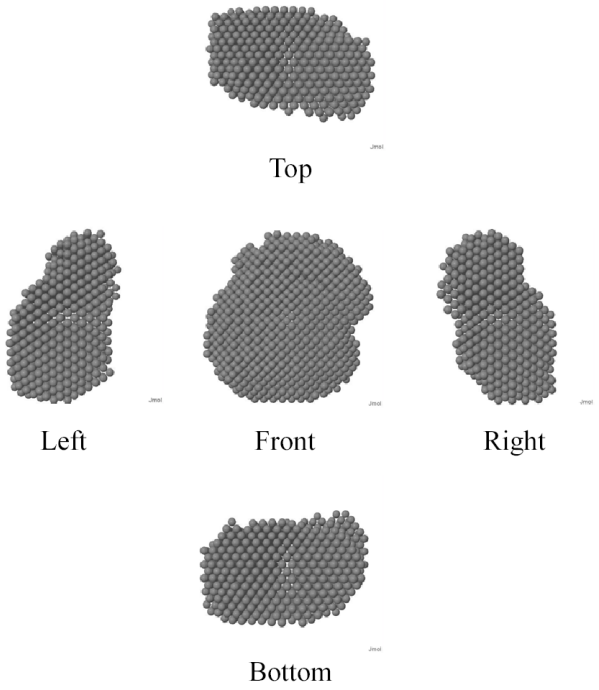

Fig. 2. Overall shape of Ca^{2+}-bound R51A homotetramer generated by DAMMIN program (D_{max} = 78 Å)

Kratky plots of the SAXS data of apo-R51A dimer and Ca^{2+} (and Zn^{2+})-bound R51A tetramer are all reproduced using an oblate model fairly compared with a theoretical curve calculated by the ellipsoidal rotation (Fig. 1). Overall shapes generated by the program DAMMIN (Fig. 2) support our prediction that two S100A3 dimers tightly associate *via* exposed broad hydrophobic clefts, which is similar to target protein recognition by responsible S100 proteins. Four S100A3 elements reorient their helix III within the tetramer upon Ca^{2+}-binding. Molecular modeling by assembling known crystal structures of analogous Ca^{2+}-bound S100 proteins (*e.g.*, Protein Data Bank Code 1K9K for S100A6) is in progress. Our finding suggests that the S100A3 tetramer plays important roles in the presumable Ca^{2+}/Zn^{2+}-homeostasis of the hair cuticular maturation.

References

[1] K. Kizawa et al., J. Biol. Chem. 283, 5004 (2008)
[2]G. Fritz et al., Biochim.Biophys.Acta. 1448, 264 (1998)
[3] T. Ueki et al., Biophys. Chem. 23, 115(1985)
[4] Y. Jinbo et al., 26th PF Symposium abstr. pp 72 (2009)
* yizumi@yz.yamagata-u.ac.jp