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Crystal structures of sodium-bound annexin A4

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Introduction

Annexin A4 is a cytosolic protein, expressed in a variety of tissues including epithelial and secretory cells of the liver, brain, pancreas, intestine, adrenal medulla and kidney. In a previous study, we isolated and cloned a zymogen granule membrane associated protein of 36 kDa (ZAP36) from canine and rat pancreas [1]. ZAP36 is identical to annexin A4 and bound to the cytosolic side of zymogen granule membranes [1]. In the pancreatic acinar cells, zymogen granules secrete large amounts od digestive enzyme by regulated exocytosis in response to food intake. The characterization of ZG membrane-associated proteins is important to understand post-Golgi membrane dynamics such as sorting, exocytosis, and membrane fusion. In *in vitro* experiments, the interaction of annexin A4 with lipid vesicles is dependent on the concentration of Ca^{2+} ion[16,17] and is completely abolished at high concentrations of Na^+ ion.

Annexin A4 possesses four repeat domains with one Ca^{2+}-binding site in each domain, and exhibits Ca^{2+}-dependent phospholipid binding and Na^+-dependent dissociation. The tertiary structure of the Ca^{2+}-bound form has been resolved in previous studies and demonstrated that annexin A4 interacts with lipid vesicles through the associated Ca^{2+} ion. However, the relationship of Na^+ ion with the dissociation has been never described.

Our aim is to elucidate the atomic mechanism for the Na^+-dependent dissociation.

Results and discussion

Recombinant glutathione-S-transferase tagged annexin A4 was produced in *E. coli* cells harboring harboring a vector pGEX3 carrying rat annexin A4 gene [1]. The expressed proteins were purified and glutathione-S-transferase was removed with Factor Xa. We obtained two crystals from different crystallized conditions (0.1 M citrate, pH 5.9, 0.5 M $MgSO_4$, and 0.9 M Li_2SO_4 and 0.1 M citrate, pH 5.9, 0.5 M $(NH_4)_2SO_4$, and 0.9 M Li_2SO_4). Crystals were grown at 20 °C in hanging drops for 1 week. High-resolution diffraction data (1.58 and 1.35 Å) were obtained using a synchrotron-radiation source at the Photon Factory BL6A station (Tsukuba, Japan). These structures has been deposited in the RCSB Protein Data Bank under accession number 2zhi and 2zhj.

Although overall structures were almost same with that of Ca^{2+}-bound form, the structures possessed metal ion in the calcium binding sites. To identify the metal ion, we investigated on the metal ion using difference Fourier electron density maps, valence screening and atomic absorption spectroscopy. From these results, we determined the metal ion to be Na^+ ion. The structure of calcium binding site was compared with that of the Ca^{2+}-bound form (Figure 1). The Ca^{2+} ion is briefly located in closer to the molecular surface than the Na^+ ion. Alternatively, the Na^+ ion is buried in the deeper molecular interior than the Ca^{2+} ion.

Total standard enthalpy of hydration between Ca^{2+} ion and water molecules with a coordination number of six is about 3-fold lower than that of Na^+ ion and water molecules with the same coordination number. The lower enthalpy means that the coordination geometry of the Ca^{2+}-bound structure is more stable than that of the Na^+-bound structure. The more stable coordination geometry means that Ca^{2+} ion would be preferable to Na^+ ion for the interaction with lipid vesicles. Thus, the Na^+-bound annexin A4 is thermodynamically easier to release from lipid vesicles.

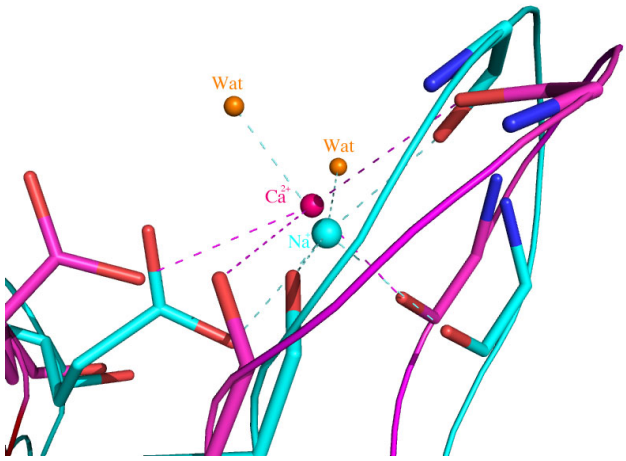

Figure 1: Structural comparison of calcium binding site between the Na^+-bound (cyan: PDB code 1zhi) and Ca^{2+}-bound forms (magenta: PDB code 1i4a). These structures were illustrated with PyMOL.

Reference

[1] S.-I. Fukuoka et al., Biochim. Biophys. Acta 1575, 148-152 (2002).

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