AR-NE3A/2020G681, 2022G031

X-ray crystallographic analysis of a scavenger receptor cysteine-rich domain (SRCRD) from Salivary scavenger and agglutinin (SALSA)

Changyu Zhang[1], Peng LU[1*] and Koji NAGATA[1,2]
[1] Department of Applied Biological Chemistry,
[2] Agricultural Bioinformatics Research Unit,
Graduate School of Agricultural and Life Sciences, The University of Tokyo,
1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan.

1 Introduction

Salivary scavenger and agglutinin (SALSA), a 300- to 400-kDa glycoprotein also as known as deleted in malignant brain tumors 1 (DMBT1) and salivary agglutinin (SAG), is a member of the scavenger receptor family, which was thought to be involved in the oral clearance of microorganisms due to its bacterial agglutination properties. It contains 14 SRCRDs separated by SRCR-interspersed domains (SIDs). Due to its ability to bind and agglutinate Streptococcus mutans, salivary agglutinin has been considered to play an important role in preventing dental caries.

As SALSA recognized a various of biological ligands, one of its main physiological activities is to mediate the adherence of *Streptococcus mutans* to teeth surface by binding to cell surface protein antigen c (PAc). The AVP region located at the morphological end of PAc protein was demonstrated to contain the binding affinity for SALSA. The ligand recognition by the SRCRD usually needs divalent metal ions, such as Ca^{2+}. Calcium induces secondary structural changes, increase the thermal stability and binding affinity to a Fe^{3+} binding protein, lactoferrin, which are essential for preventing the adherence of oral *Streptococci*. Most described ligand-binding interaction of SRCRDs was dependent on the presence of calcium ions.

2 Experiment

The DNA encoding SRCRD11 (1371-1489) from Deleted in malignant brain tumors 1 (DMBT1) protein was cloned into pET-32a expression vector. The verified recombinant plasmids were transformed into *Escherichia coli* strain SHuffle T7 cells. SRCRD protein was refolded using glutathione as a redox system and purified by ion exchange chromatography. The purified protein was then concentrated to ~10 mg/ml for crystallization.

Initial crystallization screening was performed Wizard I & II (Rigaku Reagents, Inc.), Crystal Screen HT™ (Hampton Research), and PEG Ion Screen™ (Hampton Research). The sitting-drop vapor diffusion method was adopted at 20℃ in 96-well VIOLAMO Protein Crystallization Plates (As One), and 0.5 μL protein solution and 0.5 μL reservoir solution were mixed in one drop to grow the crystals.

Crystals of SRCRD were obtained using a reservoir composition of 0.2 M Lithium citrate tribasic tetrahydrate and 20% w/v Polyethylene glycol 3,350 from PEG/ion kit. The crystals were picked up with Mounted CryoLoop and cryoprotected in reservoir solution containing 20% glycerol. Then the crystals were flash frozen in liquid nitrogen and transferred to unipuck. X-ray diffraction experiments for SRCRD crystals were performed on beamline AR-NE3A at Photon Factory. The data were indexed, integrated, and scaled using the XDS program. Initial structural models of SRCRD were obtained by molecular replacement with Morlep using the structure of GP340 SRCRD 8 (PDB 6SA5) as the search model. Further model building and refinement were performed with REFMAC5, Coot, and Phenix.

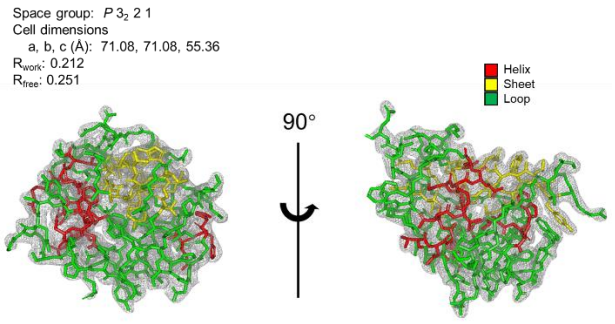

Fig.1 Crystal structure of SRCRD with Fo – Fc map coutoured at 1 σ.

3 Results and Discussion

The diffraction data from the SRCRD crystals were collected to ~1.8 Å (Fig. 1). The crystal structure of SRCRD from SALSA reveals a typical globular SRCR-fold. The fold contains one α-helix at the N-terminus and four β-strand at both N- and C-termini. The SRCRD contains eight cysteines that form four pairs of disulfide bonds, giving the SRCRD a complete and stable structure, which is evidence of the success of the refolding process. According to the surface charge distribution map of SRCRD, there is a region of negative charge on the surface of SRCRD, which is considered to be a binding site for calcium ions.

Acknowledgement

We would like to thank the beamline staff members Photon Factory AR-NE3A for their help with X-ray data collection and processing.

* porterlu@g.ecc.u-tokyo.ac.jp