Searching for crystallization method of a highly flexible domain

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Abstract

X-ray crystallography is a powerful tool to solve protein structures. However, it is difficult to obtain protein crystals in some cases. Previous studies suggested that fusion partners and protein complex formation can help protein crystallization. In my study, I utilize the protruding domains of nodavirus *Macrobrachium rosenbergii* (MrPd, residues 246-371), *Penaeus vannamei* (PvPd, residues 250-368), *Grouper nervous necrosis virus* (GNNVPd residues 214-338), the shell domains of nodavirus *Penaeus vannamei* (PvSd residues 38-250) and glutathione-S-transferase (GST) as fusion partners. WW domain-containing oxidoreductase (WWOX) is a regulatory and signaling protein that mediates specific protein-protein interactions. The WW domain (residues 16-90) is an important protein-protein interaction binding site of WWOX. Nevertheless, the structure of human WW domain has not been determined. To determine the structure of the WW domain, we prepared the expression plasmids and transformed to BL21 to produce MrPd-WW, PvPd-WW, WW-GNNVPd, WW-PvSd and GST-WW recombinant proteins. MrPd-WW, PvPd-WW, WW-GNNVPd and WW-PvSd are fused with small ubiquitin-related modifier (SUMO) His-tag to increase folding accuracy and solubility. All SUMO His-tag recombinant proteins were purified with nickel affinity chromatography. Then SUMO His-tag was removed and the high purity recombinant proteins were collected for crystallization condition screening.

Among the recombinant proteins that were used to screen protein crystallizations, MrPd-WW and GST-WW protein samples have been crystalized in individual condition. The refined MrPd-WW structure showed MrPd-WW crystals seems to show MrPd only according to electron density. To analyze this result, we used mass spectrometry to measure the molecular weight of MrPd-WW in solution, and the result showed that the degradation of WW domain has already happened~~.~~. As for GST-WW crystals, we built the model of GST; however, there are still exist additional electron density at the C terminus of GST on the electron density map. This result reveal that the C terminus of GST might be followed by thrombin cutting site and/or the WW domain. My future work is to verify the existence of additional residues at the C terminus of GST, and continue searching crystallization conditions for other fusion proteins.

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