Report on SAXS measurements of Histatin 5 and lipid vesicles at BM29 (MX-2141)

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Introduction

Histatin 5 (Hst5) is a cationic intrinsically disordered protein that normally is found in the saliva.[1] It consists of 24 amino acids, of which the majority is either charged of histidine residues. Because of its chain composition, Hst5 has polyampholytic properties at physiological pH, and thus tend to assume extended, flexible conformations in aqueous solution. Additionally, Hst5 has important biological functions in the oral environment. Apart from being part of the dental pellicle,[2] Hst5 also has bactericidal and fungicidal properties.[3] For example, Hst5 plays and important part in the inhibition of growth and germination of *Candida albicans*,[4] which is known for causing candidiasis (thrush). Several mechanisms have been proposed to explain the protective actions of Hst5, and although Hst5 has been shown to be able to use polyamine transporters to enter fungal cells,[5] there are other evidence suggesting that Hst5 can interact with the cell membrane by itself. To investigate the latter statement, we have started to perform several different experiments, including small-angle X-ray scattering (SAXS), dynamic light scattering (DLS), quartz-crystal microbalance with dissipation (QCM-D), neutron reflectivity (NR), and transmission electron cryomicroscopy

(cryo-TEM). If we can obtain a greater understanding of how Hst5 interacts with the bacterial cell membrane, it could potentially open up for new alternatives for treating bacterial infections.

Results and discussion

SAXS measurements were performed on Hst5 and vesicles at beamline BM29 in November 2018. A MES buffer with a salt concentration of 150 mM was used to maintain the pH at 6.5. The Hst5 concentration was varied between 0.8-6.6 mg/mL. Previous measurements of Hst5 has shown that it is monomeric under these conditions. 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-L-serine (POPS) lipids were used at two different compositions: (i) 70 % POPC and 30 % POPS, and (ii) 91 % POPC and 9 % POPS. The lipids were sonicated and extruded to form vesicles of a size of either 30 or 100 nm, and were kept at a concentration of either 0.1 or 0.5 mg/mL. The optimal vesicle size and lipid concentration for the SAXS measurements were found to be 100 nm and 0.5 mg/mL.

The plan was to perform SAXS measurements on solutions containing (A) Hst5 only, (B) lipids only, and (C) a mixture of both Hst5 and lipids, and then subtract the resulting curves of measurement (B) from the resulting curves from measurement (C), and compare to what was found in (A). Following this protocol, we were hoping to determine if Hst5 undergoes any conformational changes upon interacting with the lipids.

Unfortunately, all results came out fairly noisy. Even Hst5, that we have previously measured on at the same beamline, was of poorer quality than usual. This is most likely because the Hst5 powder used for these measurements came from a different supplier. It should be noted that there were two large spots visible in the capillary during the measurements that could not be cleaned off. This was however stated to not affect the measurements, and that possible effects would be removed with the background subtraction. Even though the results

were slightly more noisy than usual, Hst5 (A) could be sufficiently characterized from the obtained data. The results from the subtraction described previously (C) did however give contradicting results that were problematic to interpret. Thus, no conclusive results about conformational changes of Hst5 in the presence of lipid bilayers were obtained from these measurements. Nevertheless, the study is still ongoing, and preliminary cryo-TEM images have suggested that Hst5 has the ability to cause vesicles to "stick together" and form larger vesicle clusters.

References

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