Report on MX-1923: SAXS measurements of Statherin at BM29

E. Rieloff and M. Skepö*

Division of Theoretical Chemistry, Lund University, P.O. Box 124, S-221 00 Lund, Sweden

Email: marie.skepo@teokem.lu.se

1 Introduction

Statherin is an intrinsically disordered protein (IDP) present in saliva, where the main function is to inhibit precipitation of calcium phosphate and crystal growth in the supersaturated environment. We have seen that the protein self-assembles to oligomers with increased protein concentration. The aim of this project is twofold: (i) to understand the self-assembly of Statherin; what intermolecular interactions govern the process and characterise the oligomers with respect to size, shape and polydispersity, and (ii) to further develop coarse-grained and atomistic models for IDPs. The project is also part of a larger project with the aim to study the structure-function relationship of IDPs, especially the ones in saliva.

2 Results and Discussion

SAXS measurements on Statherin were performed at BM29 in September 2017. The experiments were performed at 20 °C and pH 8.1, with 10-500 mM NaCl, and also with an addition of 0.4-1.6 mM $CaCl_2$, since Statherin is known to be calcium binding. The protein concentration was varied between 0.22 and 12.4 mg/mL. Also, two runs of SAXS connected to size exlusion chromatography (SEC) was performed.

2.1 Monomeric Statherin

Despite the tendency to self-associate we obtained a form factor at the protein concentration 0.24 mg/mL, measured at 150 mM NaCl (Figure 1a). The molecular weight was determined to 5.29 kDa, based on I(0) obtained from the pair distance distribution function, P(r), shown in Figure 1c. This is in good agreement with the theoretical molecular

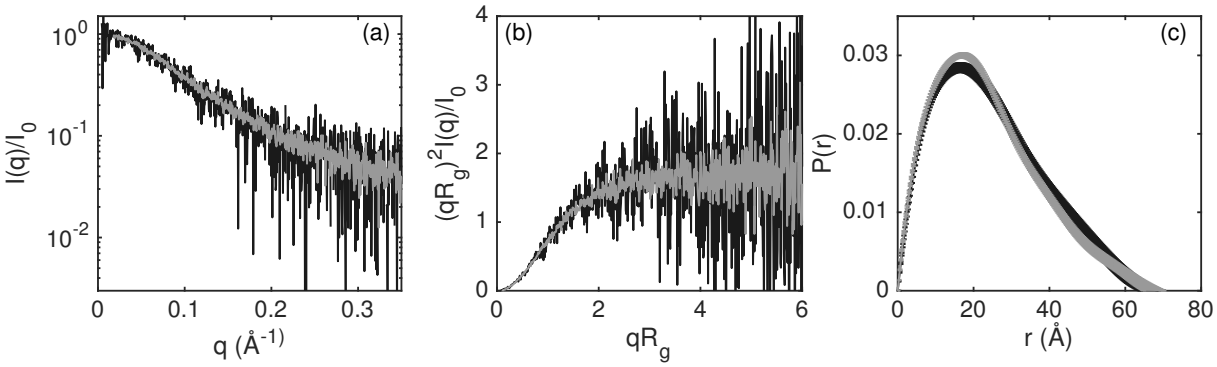

Figure 1: SAXS data for Statherin obtained by measurement at 0.24 mg/mL (black) and by SEC-SAXS (grey), at 150 mM NaCl. (a) Form factor, (b) dimensionless Kratky plot, and (c) Pair distance distribution function.

weight of 5.38 kDa, confirming monomeric Statherin at this protein concentration. The dimensionless Kratky plot (Figure 1b) shows that Statherin indeed behaves as an intrinsically disordered protein.

The SEC-SAXS intensity trace showed one peak with a small shoulder. The data extracted from the peak is also presented in Figure 1, and shows excellent agreement with the data measured at 0.24 mg/mL. The radius of gyration, R_{g}, determined from P(r) are also consistent between the measurements, 19.8 ± 0.6 Å at 0.24 mg/mL and 19.3 ± 0.2 Å from SEC-SAXS. Hence, we have drawn the conclusion that the peak corresponds to monomeric Statherin. The signal from the shoulder was too small to give good data. With a better column it would be possible to increase the protein concentration loaded onto the column, and therefore receive better data. Regarding the effect of calcium, no structural changes were observed upon addition of Ca^{2+}.

The form factor and R_{g} has been compared with simulations of Statherin utilising a course-grained model developed in our group for the protein Histatin 5 [1]. As stated above, the experimental R_{g} is 19.3 ± 0.2 Å, while the model gives a slightly smaller value, 18.0 ± 0.02 Å. We also see deviations between model and experiment in the form factor and Kratky plot. This suggests that the model, based on excluded volume and electrostatics, cannot accurately account for Statherin, which is both more hydrophobic and has a higher proline content than Histatin 5, and also contains phosphorylated serines. Therefore, we will continue with model development.

2.2 Oligomer formation

Oligomer formation was observed at all of the salt concentrations when increasing the protein concentration. Figure 2a shows that at 150 mM NaCl the average size of the monomers grows linearly with concentration in the studied range. At 10 mM NaCl the highest concentration shows repulsion in the system (Figure 2b), suggesting a limit to the

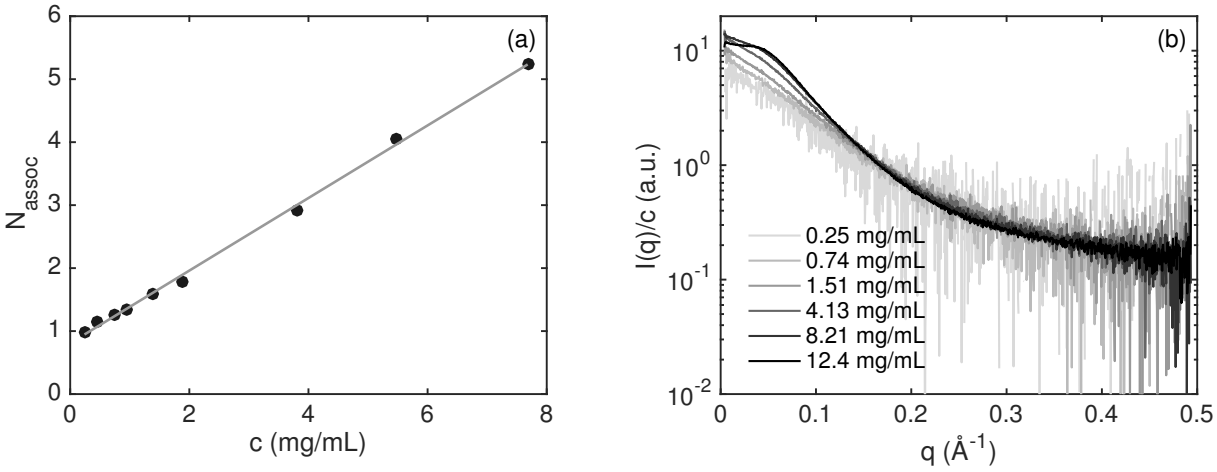

Figure 2: (a) Association number obtained by SAXS measurements versus protein concentration at 150 mM NaCl, and (b) SAXS curves at 10 mM NaCl.

growth. The question is if this also is valid for higher ionic strengths, and to answer that more measurements are required at higher protein concentrations.

To capture self-association in our coarse-grained model an extra hydrophobic interaction is required, in addition to the electrostatics, excluded volume and weak van deer Waals-attraction. Work is carried out to set the strength of this interaction to account for the results seen in experiment.

3 Conclusions

We indeed obtained a form factor crucial for our modelling, and saw that at 150 mM salt the oligomers grow linearly in size with increased protein concentration. To gain a complete understanding of what interactions govern the association process and the polydispersity of the oligomers, further measurements are required.

References

[1] C. Cragnell, D. Durand, B. Cabane, and M. Skepö, "Coarse-grained modeling of the intrinsically disordered protein Histatin 5 in solution: Monte carlo simulations in combination with SAXS," *Proteins: Structure, Function, and Bioinformatics*, vol. 84, no. 6, pp. 777–791, 2016.