Report SAXS measurements of the flexible protein Histatin 5 at BM29.

Carolina Cragnell,* Dominique Durand,# Bernard Cabane,** Marie Skepö*
*Chemical department, Theoretical Chemistry, Lund University, Lund, Sweden
#I2BC, Université Paris-Sud, ORSAY Cédex, France
**PMMH, CNRS UMR 7636, ESPCI, F-75231 Paris cedex 05, France

The goal of this project is to understand intrinsically disordered proteins (IDPs) and relate their structure and function in solution with the adsorbed state. For this purpose a combined theoretical and experimental approach will be used and the aim of the project is twofold: (i) to develop a coarse-grained model (for Monte Carlos simulations) for IDPs based on experimental results which can be used for modeling complex mixtures as saliva, and (ii) achieve an understanding of the behavior of salivary proteins and to connect the structure and function in solution with adsorbed state.

This study is directed into Histatin 5 (His5), which is a short multifunctional cationic saliva peptide with a molecular weight of approximately 3 kDa (24 amino acids). His5 acts as the first line of defence against oral candidiasis caused by Candida albicans.[3, 5] The net charge of His5 varies dependent on pH as shown in Figure 1. Hence, it is reasonable to believe that this variance in net charge in combination with varying salivary solution conditions as monovalent and divalent salts have large effects on the electrostatic interactions in the system. Moreover, His5 binds various transition metals such

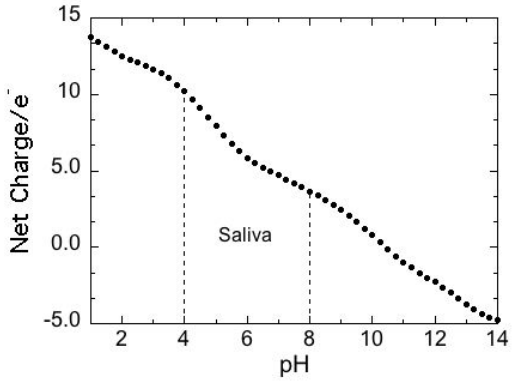
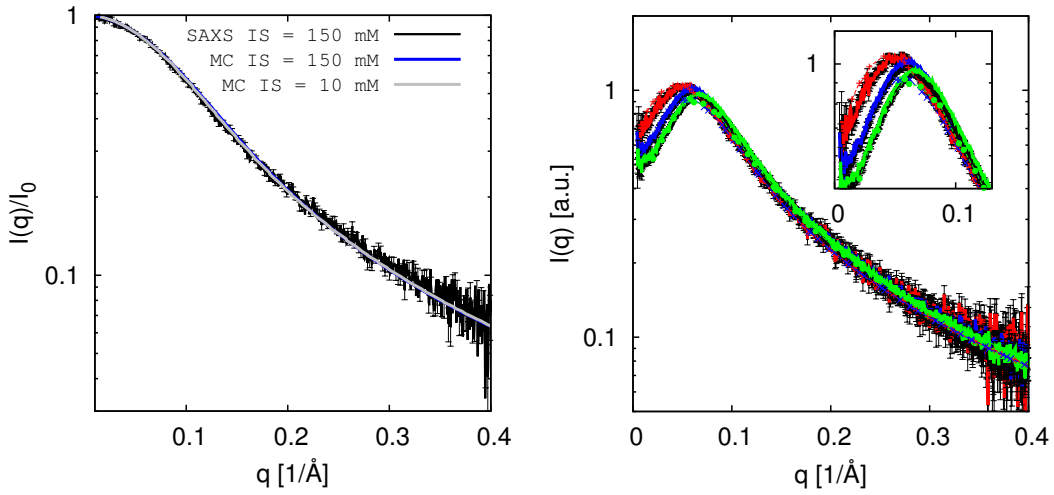


Figure 1: Titration curve for Histatin 5 obtained from Monte Carlo simulations using the inhouse simulation package Faunus.[1]

as Fe and Zn. This ability may significantly modulate its antifungal function. It is suggested in the literature that His5 binds up to ten equivalents of Fe^{3+} and four of Zn^{2+},[4] leading to a total net charge increase of 30 and 8 e, respectively. In addition, there is a strong correlation between peptide cationicity and antimicrobial activity.

In Januari and May 2015 we performed SAXS-measurements at BM29 for Histatin 5 for different solution conditions as varying protein concentration, pH, salt concentration and valency. The results for monovalent salt and pH were very promising and the SAXS-experiments clearly show that electrostatic interactions are of importance. In order to achieve a molecular understanding and a physico-chemical insight of the obtained SAXS results, a model for Monte Carlos simulations has been developed and great correspondence is obtained with experimental results, see Figure 2.



(a) (b)

Figure 2: (a) Form factor determined from SAXS, 1 mg/mL His5, IS =150 mM. The simulated curves were obtained from a single chain, IS = 10 mM and 150 mM, respectively. (b) SAXS curves from different His5 concentrations at low IS (10 mM Tris, pH 7), red 2.4 mg/mL, blue 4.7 mg/mL, and green 6.8 mg/mL. The symbols/dotted lines refer to simulated profiles from similar systems.

However, our main interest was to determine the effect on the intrachain as well as the aggregation state, of ionic strength and divalent ions. The protein concentration range was set to 0.44 – 4.22 mg/mL and the investigated divalent ions were Mg, Ca and Zn. Those were compared with Na. Figure 3 shows the scattering of 1 mg/mL His5 samples at constant ionic strenght (80 mM) set by NaCl solely or by 4 mM $ZnCl_2$, $MgCl_2$, $CaCl_2$ together with NaCl. There are no signinficant effect of Ca, Mg, nor Na on the average of all His5 conformaions. Zn in the buffer on the other hand will make the conformations more compact and the molecular mass higher. Lower ionic strength gives rise to the smallest R_g and the higher the ionic strength the larger the R_g. M_w

is also increasing as ionic strength is increased. It is however unclear if this effect is due to increased contrast with Zn bound to the peptide or due to aggregation in the background. It is observed that there are aggregates in the buffer already in the first frame which becomes worse after approximately five frames, see Figure 4. In the low q region the background scattering is higher than the sample, causing a decrease of the subtracted intensity.

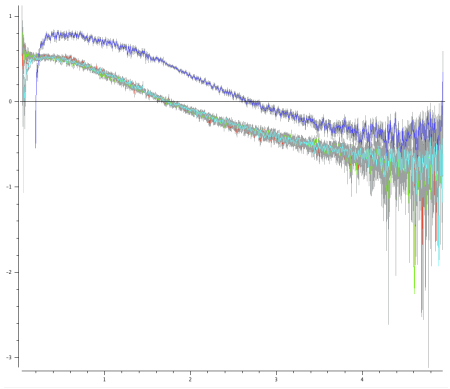


Figure 3: Scattering curve obtained by SAXS 1 mg/mL His5 at pH = 6 with ionic strength of 80 mM set by NaCl (red), 4 mM divalent ions and 68 mM NaCl, Zn (blue), Mg (turquoise), and Ca (green).

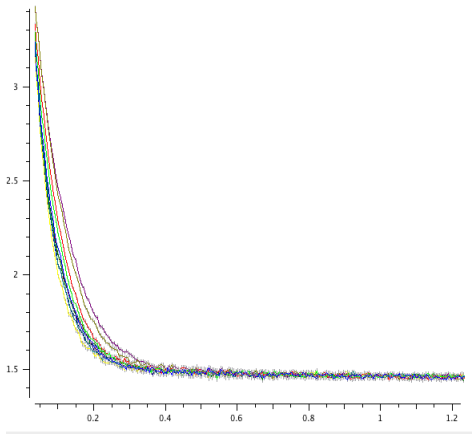


Figure 4: Successive frames of the background containing 4 mM $ZnCl_2$.

Due to biological relevancy as well as from the development of simulation program point of view, the Zn effects on the conformations of His5 still need further investigations. For that purpose we need to extend our experimental studies by performing more SAXS measurements trying to solve the aggregation issue.

References

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