Experimental report

12/02/2017

Proposal: 8-04-790 **Council:** 10/2016

Title: To understand the antimicrobial activity of the salivary protein Histatin 5.

Research area: Chemistry

This proposal is a resubmission of 8-04-777

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Samples: Histatin 5

Instrument	Requested days	Allocated days	From	To
IN16B	4	3	03/02/2017	06/02/2017
IN5	3	1	06/02/2017	07/02/2017

Abstract:

The goal of this project is to understand the antimicrobial activity of the intrinsically disordered salivary protein Histatin 5 (Hst5), which acts in defence against oral candidiasis caused by Candida albicans. Experiments and computer simulations have indicated the importance of the dynamic properties of the protein, and it is hypothesized the binding of ions, for example, Zn(+2) and Fe(+3), might be of importance. We aim to combine IN16B and IN5 to record the quasi-elastic neutron scattering spectra to cover a very complete dynamic range. The use of IN16B and IN15 in combination with novel data analysis frameworks to separate the different hierarchical levels of dynamics, it will allow for new insights that have not previously been accessible. The main goal will be to access the (sub) nanosecond internal diffusive motions of the proteins in solution, with emphasize on the parameters salt (zinc), protein concentration, and temperature. The model system, Hst5, is well established on both the experimental and simulation side, and by combining neutron scattering with simulations, we will obtain a molecular understanding of system that is both of medical and academic relevance.

Experimental report project 8-04-790

To understand the antimicrobial activity of the salivary protein Histatin 5

Main proposer: Marie Skepö, Theoretical Chemistry, Lund University, Lund, Sweden

The system of interest is Histatin 5, a 24 amino acid long cationic antimicrobial saliva protein. Histatin 5 belongs to the family of intrinsically disordered proteins.

The overall aim with the project is to use IN16B in combination with IN5 to record quasi-elastic neutron scattering (QENS) spectra, to cover a very complete dynamic range, using the same samples for the two experiments. The latter is crucial for the experiment due to sample cost. The main goal is to access the (sub) nanosecond internal diffusive motions of the proteins in solution, depending on the parameters salt (zinc), protein concentration, as well as temperature. By doing so, we want to study how these parameters influence the internal mobility of the proteins. In the data analysis, the superimposed center-of-mass diffusion and solvent diffusion will have to be separated. Therefore, in the application, we emphasized that achieving a sufficiently strong scattering signal from sufficiently concentrated samples and performing the rather involved data analysis are among the main challenges of this proposal. The theoretical modelling conducted here will aid in the evaluation of the data and provide complementary molecular information.

The aims with the present study are:

- To reveal dynamic properties of the salivary protein Hst5 to provide a deeper understanding the mechanism of action, e.g. as a defence against oral candidiasis.
- To further develop models for how the dynamics of intrinsically disordered proteins (IDPS) are affect by protein-ligand interactions.
- To establish a quantitative link with MD/MC simulations

We were approved 48 hours at IN16B and 24 hours IN5, and during these experiments we were able to measure on two samples for IN16B and one at IN5 i.e. 200 mg/ml Histatin 5 at high and low salt concentration for the former, and 200 mg/ml Histatin 5 at low salt concentration for the latter.

The preliminary results show indeed that the experimental techniques are feasible for this kind of systems, and shows promising results, see Figure 1 for the preliminary results from IN16B and Figure 2 for IN5, respectively. Hence, we would like to continue by exploring the full parameter space with respect to protein and salt concentration.

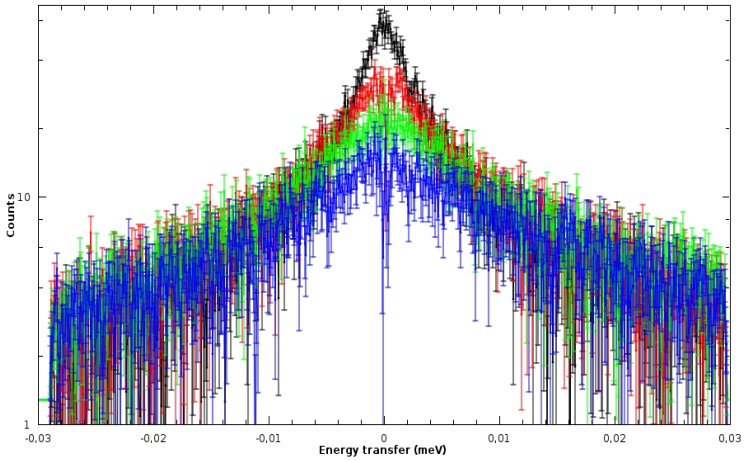

Figure 1. Example of single-detector QENS spectra on a histatin (185mg/ml) aqueous (D2O) solution at T=285K, subsequent to the subtraction of the corresponding pure buffer spectra, recorded on IN16B during February 2017 (exp.8-04-790). The Q-values for the displayed spectra are 0.43, 0.82, 1.18, and 1.39/A (in the order of increasing width and decreasing elastic intensity). No self-shielding correction has been performed yet, explaining the small dip at omega=0 for the highest q-shown. Further analysis is in progress.

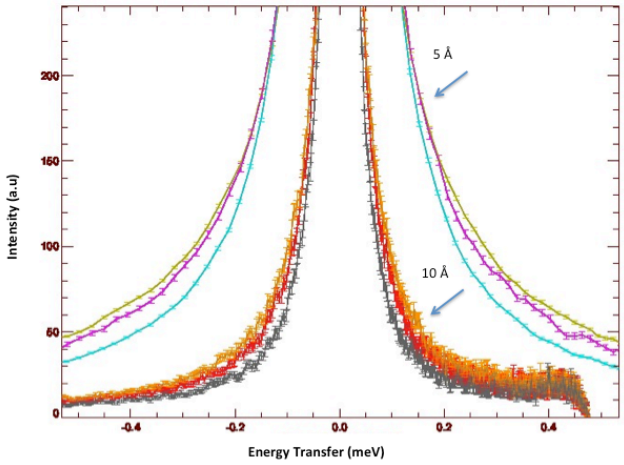

Figure 2. Scattering intensity as a function of energy transfer in meV obtained from IN5 for two wave lengths, 5 Å and 10 Å, respectively. The measurements were performed at three temperatures: 280 K, 298 K, and 310 K. The expected temperature response is captured i.e. the protein moves faster when the temperature is increased, hence, the FWHM is increasing.