Experimental report

17/02/2020

Proposal: **9-13-820**

Council: 10/2018

Title: Elucidating electrostatic and hydrophobic interactions in protein-surfactant systems using contrast variation SANS

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: $C_{12}H_{25}SO_4Na$
Bovine Serum Albumin
Human growth hormone
$C_{15}H_{34}Cl$

Instrument	Requested days	Allocated days	From	To
D11	3	2	26/07/2019	28/07/2019

Abstract:

Liquid pharmaceutical formulations are commonly comprised of proteins and surfactants, which interact in solution in order to provide an increase in the system stability. Although these systems have been significantly investigated in the last few years, there is a lack of consensus concerning the interaction between proteins and surfactants. We believe that small-angle neutron scattering and contrast variation can provide specific information about these systems and contribute to resolve the puzzle. In this experiment we aim to elucidate the interactions between human growth hormone and different prototypical ionic amphiphiles at room temperature. The use of isotope labelling and contrast matching will provide detailed information of the different parts of the system, which will be co-refined in order to provide structural information of the protein-surfactant complexes.

Introduction

The interactions between proteins and amphiphilic compounds in solution play an essential role on the development of liquid pharmaceutical formulations. Commercial products usually contain surfactants that increase the stability of proteins and enzymes in solution by hindering the protein unfolding promoted at hydrophobic-hydrophilic interfaces.[1] Detailed structural models of protein-surfactant complexes and interactions are rather scarce, and a general theory is yet missing. For this purpose, small-angle scattering has been proposed as a key technique to shed some light on these systems. Human growth hormone (hGH) in particular is relatively unexplored in this context in spite of the considerable industrial interest. It is well known that such a protein readily aggregates in solution under certain conditions. The aggregation process is partially hindered in the presence of some surfactants, although the mechanism is still not well understood and variations in composition promotes protein denaturation. Thus, the development of a solid understanding of the interactions of between proteins and surfactant using isotope-labelling SANS is expected to have a significant impact on the field.[1]

Experiment

The SANS experiment performed on D11 (ILL) was focused on the structure determination of the complexed formed by hGH (0.135 mM) and sodium dodecyl sulfate (SDS) at different surfactant concentrations. A contrast variation approach was followed, where four different contrasts were measured at equivalent surfactant/protein ratios:

1. Surfactant-solvent contrast matched (only protein visible).
2. Surfactant-protein contrast matched (complex visible).
3. Solvent-protein contrast matched (only surfactant visible).
4. Zero-average contrast condition.

Furthermore, an initial characterization of the system containing a cationic surfactant (C_{12}TAC) was performed using the same approach. It was shown that significant structural differences appear between the two surfactants. Unfolding at early stages appears in both systems. Whilst the SDS-hGH systems retains the colloidal stability, the system containing the cationic surfactant visits several regions in the phase diagram, from a colloidally stable suspension to the formation of a surfactant-protein gel.

Results

Figure 1 shows data and best fits obtained through the simultaneous analysis of the four contrasts. Data were obtained on D11 and SANS2d (ISIS, UK), and analyzed using the Indirect Fourier Transform (IFT) and model-based fitting.

The results show structural variations in the system, that evolve in size and charge with the addition of surfactant. At low SDS concentrations, it is shown that the protein-surfactant system forms a globular complex with sparsely adsorbed SDS molecules onto a partially unfolded protein. With increasing surfactant concentrations, a decorated micelle morphology is observed, where the protein sits at the surface of a surfactant (pseudo-) micelle. Similarly, structural transitions on the surfactant phase are observed, where the segregation of surfactant in the complex is observed above the critical association concentration, and protein-free surfactant micelles appear above the CMC.

Thus, the addition of surfactant was shown to affect the structure of the protein, resulting in a rich conformational landscape that depends on the surfactant/protein ratio. The use of the contrast variation approach and the zero-average contrast condition allowed to develop a detailed structural model of interaction for this system.

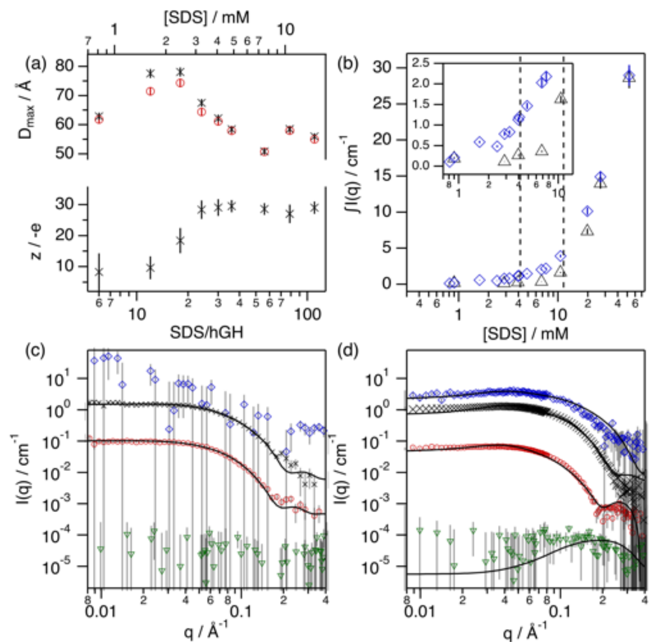

Figure 1 Results from the analysis of SANS data. (a) Dmax and particle charge values for the protein (red circles) and complex (black crosses) upon addition of SDS. (b) Integrated scattered intensity at different surfactant concentrations in the absence (black triangles) and presence (blue diamonds) of 0.135 mM hGH. The inset shows the expanded region where the CAC and CMCeff are located, as indicated by the dashed lines. SANS data and best fits of (c) SDS/hGH=18 and (d) SDS/hGH=56 at 0.135 mM hGH in different contrasts: Contrast 1 (red circles), Contrast 2 (black crosses), Contrast 3 (blue diamonds) and Contrast 4 (green triangles). Solid lines show best fits to the data. Data and fits were offset for clarity. Data collected on SANS2d (c) and D11 (d).

Conclusion

The outcome of this experiment has been highly positive, and the involvement of Sylvain Prevost resulted in very fruitful discussions. The system has shown an interesting and unexpected behaviour with increasing surfactant concentration. Furthermore, the data obtained is high quality with a good signal-to-noise ratio, and the instrument functioned well during most of the experimental time.

This data is the central part of the advanced study of protein surfactant interactions. A manuscript was submitted (February 2020) to an international, peer-reviewed journal and we expect that this publication will settle a solid approach to investigate this type of systems. Furthermore, this investigation also provides a valuable point of view to the industrial partners of the project, who have been witness of the possibilities of SANS characterisation of complex systems.

This project will continue with parallel investigations that aim to shed some light on protein refolding in the presence of mixed surfactant systems, dynamic equilibrium in protein surfactant systems, and conformation retrieval upon dialysis.

References

[1] Otzen, D., Protein–surfactant interactions: A tale of many states. *Biochim. Biophys. Acta* **2011,** *1814* (5), 562-591.