Experimental report

20/09/2022

Proposal: 9-13-1033 **Council:** 4/2021

Title: Contrast variation studies of protein/polyelectrolyte complexes with encapsulated drug

Research area: Soft condensed matter

This proposal is a new proposal

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Samples: Myoglobin
Insulin
glucagon
poly(ethylene oxide)-block-poly(dimethylaminoethyl methacrylate -d15)
Poly(ethylene oxide)-block-poly(dimethylaminoethyl methacrylate)
protoporphyrin-IX

Instrument	Requested days	Allocated days	From	To
D11	2	2	30/08/2021	01/09/2021
D22	2	0		
D33	2	0		

Abstract:

The main goal of our proposal is to use the contrast matching technique for small-angle neutron scattering (SANS) measurements to reveal morphology of complexes formed by the assembly of proteins with diblock copolymers composed of a neutral hydrophilic block and a polycationic block and the effect of encapsulation of an ionic low-molar mass drug into the complexes on their internal structure. Polymer/protein assemblies can be used for targeted delivery and controlled encapsulation/release of both proteins and low-molar mass drugs. The goal of our study is to reveal the effect of protein's nature and drug loading on the morphology of the complexes formed by poly(ethylene oxide)-block-poly(dimethylaminoethyl methacrylate) copolymer and glucagon, insulin or myoglobin with and without encapsulated protoporphyrin-IX by masking various components that is possible thanks to the large differences in their scattering length density (SLD) values for neutrons.

Contrast variation studies of protein/polyelectrolyte complexes with encapsulated drug

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1. Introduction

The internal structure of the polyelectrolyte (PE)/protein complexes and their stability can be tuned by varying nature of a protein, concentration of a protein and a PE, pH and ionic strength, and therefore, such structures can be used for targeted delivery and controlled release of proteins. The internal structure of the complexes affects their ability to encapsulate and release protein, and, in addition, to encapsulate low-molar mass drug into the compact PE/protein layer. The main goal of this proposal was to use the contrast matching technique for small-angle neutron scattering (SANS) measurements to follow morphology transition of complexes formed by proteins and block copolymers composed of a neutral hydrophilic block and a polycationic block by varying polymer charge and concentration, and to reveal the effect of protein nature and encapsulation of a drug into the complexes on their internal structure. Thanks to a significant difference of polymer and protein contrasts for neutrons, the scattering from protonated and deuterated polymer segments, proteins and a drug can be masked, that will allow us to obtain detailed information about the morphology of polymer/protein complexes with or without a drug that is crucial for characterization of their encapsulation and release properties. We planned to follow the co-assembly of insulin and myoglobin with weak PE (poly(ethylene oxide)-block-poly(N,N-dimethylaminoethyl methacrylate), PEO-PDMAEMA and PEO-PDMAEMA-d15) at different protein concentrations and solvent compositions.

2. Experiment

We measured SANS for complexes of 2 copolymers (PEO_{205}-$PDMAEMA_{40}$, PEO_{205}-$PDMAEMA_{40}$-d15) with human insulin and myoglobin with and without a drug (protoporphyrin-IX) at various protein concentrations; in total 37 measurements were performed.

Scattering from empty capillary and buffer were subtracted from each curve, and the scattering intensities were recalculated to absolute values. The obtained scattering curves were used to calculate forward scattering and gyration radius. Based on these calculations and our preliminary data, we will choose the proper models for fitting the curves. However, such data analysis requires more time.

3. Results

Scattering intensity for polymer/insulin complexes increases with increasing protein concentration, indicating formation of larger particles. By matching PE block or insulin, the slope of the curve changes, however fitting of the data is required for understanding complex morphology. Addition of the drug to the complexes decreases forward scattering, due to the changing of the contrast of the complexes. However, no micelle formation was observed for polymer/myoglobin complexes that could be explained by heterogeneous charge distribution in protein or insufficient negative charges on myoglobin surface.

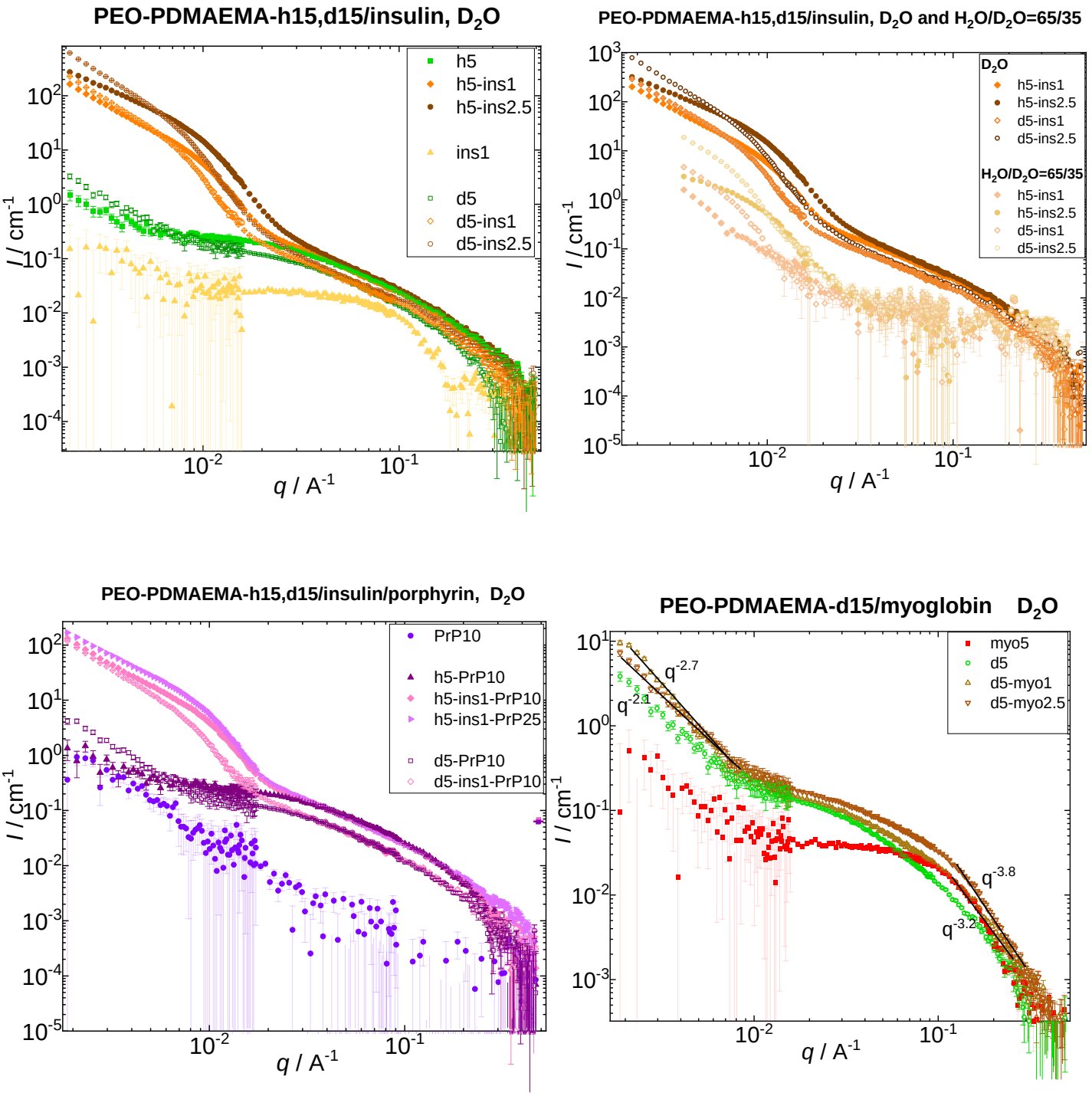

Fig. 1. *SANS curves for PEO-PDMAEMA-h15 (h), PEO-PDMAEMA-d15 (d), insulin (ins), myoglobin (myo) and protoporphyrin-IX (PrP) and their complexes at protein concentrations (indicated in g/l for PE and insulin, and in μM for drug).*

4. Conclusion

Using contrast matching technique by variation of deuterated blocks, we were able to measure scattering from polyelectrolyte/protein complexes with matched PE block. By fitting the curves at different contrasts, we will be able to obtain detailed information about the internal structure of the complexes. Moreover, we

proved drug encapsulation into the complexes. Further, we plan to perform SANS experiment for polypeptide chains that compose insulin to reveal the contribution of electrostatic and hydrophobic interactions.