Experimental report

17/09/2019

Proposal: 9-13-780 **Council:** 4/2018

Title: Interactions between poly(amino amides)and biological systems to gaindeeply understand on the mechanismof protection against virus attach

Research area: Physics

This proposal is a new proposal

Main proposer: Emanuela DI COLA

Experimental team: Emanuela DI COLA
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Samples: DNA
mucin type III
DOPE
poly(amino amides)

Instrument	Requested days	Allocated days	From	To
D11	3	3	28/09/2018	01/10/2018

Abstract:

In the framework of a collaboration with chemistry department of University of Milano, we propose to study a new class of linear polyaminoamides molecules, of extremely promising and already proved medical interest, comprising their strong protection against virus infection (mainly HPV and herpes) and their very low toxicity in complex with DNA. We will primarily focus on AGMA1 and ARGO7, cationic agmatine- and arginine- containing polymers, respectively. Particularly intriguing is the mechanism of action of AGMA1, which is so far not fully understood. The current understanding is that its interaction with cell surfaces by means of glycosaminoglycans (HSPG) has a major role in its protective action. Nevertheless, it is active also against viruses whose attachment to the membrane is not dependent by HSPG. We intend to investigate the above indicated properties, i.e. protection against viruses and gene material complexation. The main goals will be to give insights to the mechanism of interaction of the polymers with mucus, constituting the first barrier to the target tissues of their medical application; to study the structural properties of AGMA1 and ARGO7/23bpDNA complexes.

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Title: Interactions between poly(amino amides) and biological systems to gain deeply understand on the mechanism of protection against virus attach

Principal investigators: E. Di Cola [1], P. Brocca [1], I. Grillo [2]

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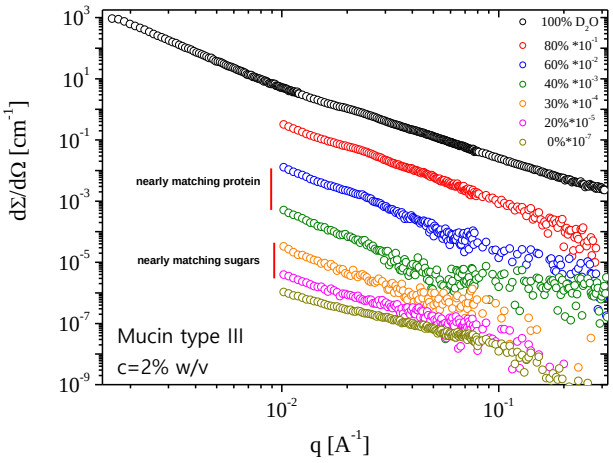
Instrument: **D11**

Summary of Experiment

We investigated by means of SANS the complexation of promising linear polyamidoamines (PAAs) that may be of interest for drug and genetic material delivery. PAAs are intrinsically cationic, containing a tert-amine group in the main chain, however, at physiological pH, most of them bear an overall ionic charge significantly lower than other widely used polycations, such as poly-L-Lysine (PLL) and polyethyleneimine (PEI). Therefore, they are usually less toxic than PLL and PEI. Moreover, unlike chitosan, PAAs are usually water soluble and hydrolytically degradable in aqueous media at pH ≥ 7.0 even without intervention of external factors such as enzymes or strong alkalies. Particularly we focused on two polymers of this family, AGMA1 and ARGO7 (L-, D- and L-D form) and their complexation with biological macromolecules, such as mucin glycoproteins, the key component of mucus. Thanks to their pH tuneable physical-chemical properties, PAAs have been successfully proved to be good candidates to interact with mucin in a model for mucus-covered epithelial cells, to mimick physiological environments for trans-mucosal delivery to tissues [see V. Rondelli, E. Di Cola, A. Koutsioubas, J. Alongi, P. Ferruti, E. Ranucci, P. Brocca *International Journal of Molecular Sciences*, **2019**, 20, 3712]. Being mucin a complex multi-domain protein, with contrast matching SANS (CM-SANS) we intended to benefit from the natural contrast of the protein different domains to study the local change in conformation of mucin once the complexes were formed. To this scope, we firstly performed CM-SANS of pure commercial porcin gastric mucin (PGM, both type III and II, Sigma Aldrich) at different ratios D_2O/H_2O to structurally identify the contributes of the protein backbone (glycosylate sites) from the globular cysteine rich end-domains [example in **Figure 1**].

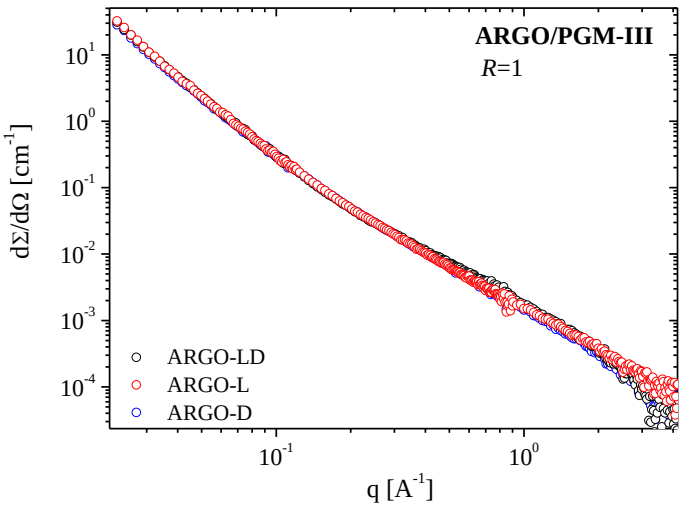
Figure 1: Selected SANS scattering profiles of PGM solutions at *c*=4% w/v, for different D/H ratios. Intensities are rescaled for a factor indicated in the legend.



From the analysis, we found a contrast matching point around 26% D/H ratio. The match point is close to what is reported in literature for polysaccharides, suggesting that we could identify the contribute of the lateral glycosylated chains to the scattering pattern. Previous SAXS investigations have shown that the glycosylate sites (highly negative charged brushes) of PGM may play a major role in stabilizing oppositely charged PAA/PGM interaction, even at high salt conditions [135 mM PBS].

Secondly, we studied the complexation of PGM with both the agmatine- and arginine-based PAAs, at 3 selected ratios R (with $R = \frac{[PAA]}{[PGM]}$), i.e. 2, 1 and 0.5. As an example, we report in **Figure 2** the comparison between ARGO-7 (*L*, *D* and *L*-*D* form) for ratio *R*=1, in 100% PBS-D_2O buffer. These arginine-based PAAs, have the property of being chiral. Notwithstanding, the chiral centers are not a part of the main chain but are part of the side substituents, conferring to the PAA a stable pH-dependent configuration in aqueous solutions. Here the interest to investigate the role of chirality in the formation of the complexes.

Figure 2: SANS scattering profiles for ARGO-7/PGM complexes, at *R*=1.



At first glance, the data seems to suggest that the polymer chirality does not play a fundamental role in the formation of the complexes. The scattering patterns overlap in the whole *q*-range explored in the experiment.

Due to long acquisition time for the CM-SANS experiment, we could not perform in this beamtime the study of the complexes at different ratios H/D, to study the conformational changes of PGM upon complexation, in order to complement our previous NR investigations. This will be the aim of further planned SANS studies.