EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF	**Experiment title:** Anti adhesive glycosylated asterisks ligands for inhibiting lectin carbohydrate multivalent interactions Proposal N°23036	**Experiment number**: MX 1019
Beamline: ID14 eh3	**Date of experiment**: from: 2 dec 2009 to: 3 dec 2009 5 feb 2010 to 6 feb 2010	**Date of report**:
Shifts: 6/6	**Local contact(s)**: Adam Round and Louiza Zerrad	*Received at ESRF:*

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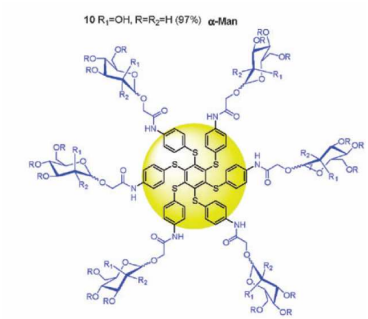
Report:

Objectives of the study: Several biological recognition events involve the generally weak interactions between a carbohydrate epitope and a receptor protein (lectins). These have been observed in diverse biological processes such as cellular recognition, adhesion, cancer cell metastasis, and inflammation. Considering the importance of these interactions, the synthesis of glycodendrimers, monodispersed macromolecules carrying several copies of carbohydrate ligands, was investigated with the aim to increase their binding interactions by a glycocluster effect. A new class of sulfurated, semi-rigid and low-valent glycosylated asterisk ligands has been designed[1] (figure 1) that has some of the highest inhibition potencies of Concanavalin A-induced hemagglutination to near nanomolar concentrations with the a-D-mannose asterisk (Figure 2).



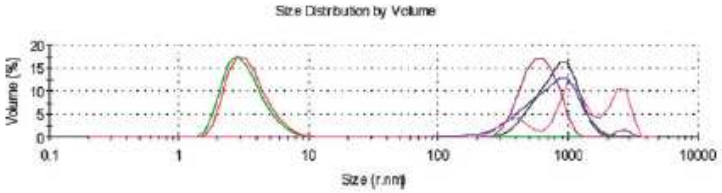


Fig. 1 DLS experiments on α-Man asterisk **10** with Con A. (a) Red: Con A 17 μM in 450 μl of HEPES 20 mM, pH 7.2, 150 mM NaCl, 2 μM $CaCl_2$, 2 μM $MnCl_2$: solution **A**. (b) Green: **A** + 50 nM of **10**; (c) Blue: **A** + 100 nM of **10**. (d) Black: **A** + 150 nM of **10**. (e) Purple: **A** + 200 nM of **10**. (f) Pink: **A** + 400 nM of **10**.

Figure 1 (left): hexa-amino persulfurated benzene asterisk decorated with either α-glucose, β-glucose or α-mannose epitopes.
Figure 2 (right): evidence by DLS of a strong aggregation effect of Concanavalin A with nanomolar concentration of α-mannose asterisk (100nM α mannose asterisk for 17μM ConA monomer).

The main objective of this project was to get structural and kinetic information about the recognition process between bacterial lectins and multivalent glycosylated ligands. Since no structural information could be obtain from light scattering about the glycoasterisk/lectin assembly, we have used Small Angle X ray Scattering to obtain some biostructural data and some preliminary information about the kinetic/thermodynamic aspects of the interaction between our multivalent glycosylated ligands and lectin Con A; the latter being a classic reference before using less known and important bacterial lectins.

Experimental results: In this project, SAXS experiments have been performed on the beamline ID14-eh3 at room temperature using the automatic sample changer. Two aspects were examined: the formation of glycoasterisk with ConA at different concentrations of different asterisks (with mannose, glucose and galactose epitope) and the kinetic of formation of this assembly. But previoulsy to these experiements, the structures in solution of ConA at different pH and glycoasterisks in pure water and in buffer have been cheked.

Form factors of ConA and glycoasterisk : the structure in solution of ConA in Hepes buffer pH 7.2 in a tetrameric form, whereas in acetate pH 4.5 it is a dimeric form, with one carbohydrate site per monomer. The asterisks in solution seems to assemble into oligomers larger in salt solution (Rg 2.63nm) than in pure water (Rg 2.07nm) and with mass which seems to double in salt solution.

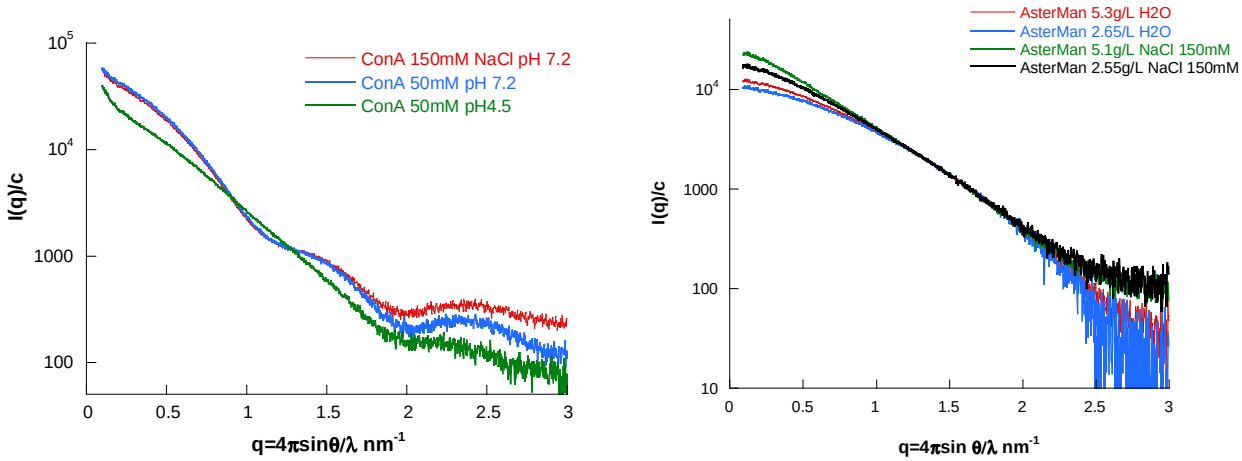


Figure 3 (left) SAXS pattern of ConA at pH 7.2 and 4.5 ; (right) SAXS pattern of a mannose asterisk in pure water and Hepes pH 7.2 150mM NaC

Structure in solution of ConA/glycoasterisk assembly: To get a good signal/noise ratio, we have chosen a concentration of ConA higher than those used for DLS experiments, but keep equivalent asterisk/ConA ratio. On Figure 4, we observe small agregates at 1µM, which is consistent with DLS and larger aggregates at 10µM with a change in the form factor (assembly formation) and saturation at 100µM. There is no affinity of galactose asterisk and lower affinity for glucose asterisk than for mannose astertisk.

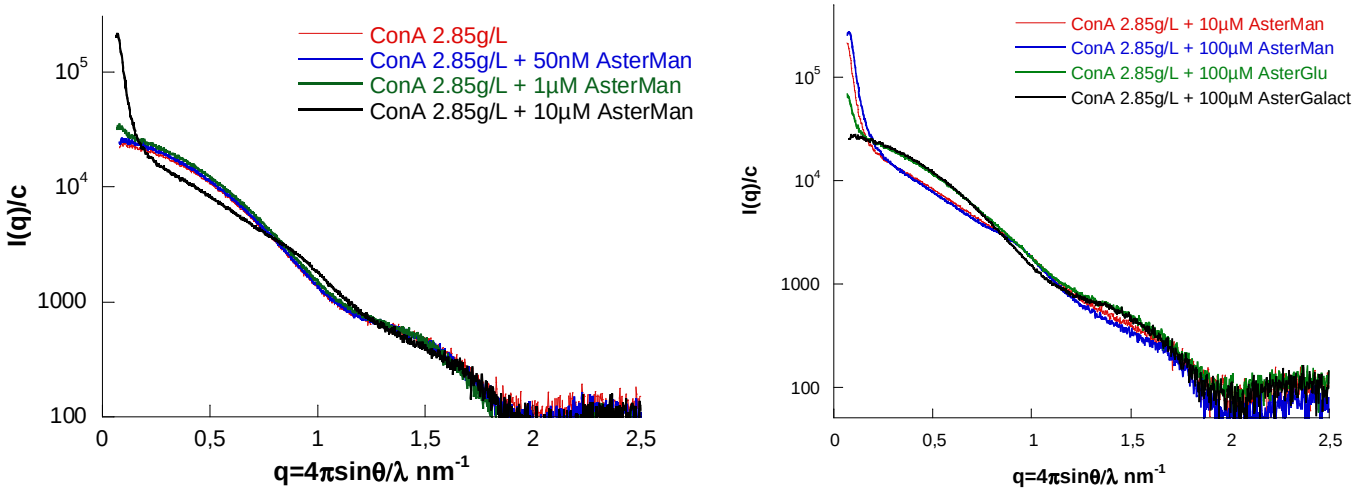


Figure 4 (left) SAXS patterns of ConA with increase amount of AsterriskMannose; (right) with different epitopes on asterisk

It is clear from these first equilibrium SAXS experiments that there is formation of glycoasterisk/Concanavalin A assemblies, which is rather complicated due to the number of carbohydrate fixation sites, the number of carbohydrates on the surface of the asterisk and the problably the oligomer formation of glycoasterisk. These experiments will be deepened, but need also to be complemented by kinetic experiments.

1. M Sleiman, A Varrot, J-M Raimundo, M Gingras and P G. Goekjian
Glycosylated asterisks are among the most potent low valency inducers of Concanavalin A aggregationw
Chem. Commun., 2008, 6507–6509