SPR BINDING STUDY BETWEEN MULTIVALENT *MANNO*-GOLD NANOPARTICLES AND THE HIV-1 BROADLY NEUTRALIZING ANTIBODY 2G12

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Multivalency is an important issue in biological interactions. The 2G12 is one of the few monoclonal antibodies (mAb) able to neutralize a broad range of HIV-1 primary isolates [1]. The 2G12 recognizes a conserved dense cluster of oligomannose residues on the "silent face" of gp120, the envelope protein of HIV-1. The crystal structure of Fab 2G12 free and complexed with Manα1-2Man and with Man9GlcNAc2 shows that this antibody provides an extended surface consisting of two classical binding sites and one previously uncharacterized interface region for multivalent interaction with a cluster of oligomannose. The cluster presentation may explain why 2G12 can recognize a self-antigen and binds this kind of glycans with unusually high affinity [2].

To better understand the molecular mechanism of this interaction, we have prepared glyconanoparticles that present oligomannosides in a multivalent way in order to mimic the oligomannose clusters on gp120. We used a strategy developed in our laboratory [3, 4] to prepare new mannose gold glyconanoparticles (GNPs). We have designed, synthesized and characterisized mannose oligosaccharides (mono-, di-, tri-, tetra-, penta- and heptasaccharides) and the corresponding thiol-functionalised neoglycoconjugates. With these structures we have prepared GNPs with different density of oligomannose (10 %, 50% and 100%) on the gold surface.

The binding of the GNPs to the 2G12 was studied by using Surface Plasmon Resonance (SPR) technology. Binding experiments were carried out in Tris buffer (pH 7.4) with a ProteON instrument (Bio-Rad). Two channels of chip surface were activated by injection of different concentrations of sulfo-NHS/EDAC, followed by injection of 2G12 solution in an acetate buffer until 4300 and 2500 response units (RU). The channels were then saturated with a pulse of ethanol amine HCl. The reference channel was prepared in the same way but without the antibody. The free oligosaccharides and the corresponding neoglycoconjugates and GNPs were injected at six different concentrations to reference and 2G12 activated channels to evaluate the binding affinity and the multivalence effect. Competitive experiments using gp120 were also performed.

References:

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