Effects of N-glycans on structure and dynamics of IgA2 and their implications on receptor binding via SAXS

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What is N-Glycosylation?

Attachment of a sugar moiety to a protein in the Asn-X-Ser/Thr sequon. The first sugar is attached co-translational and post-translational is modified.

Why is important to study N-glycosylation in therapeutic antibodies?

The structure and function of IgG1 Fc is mediated by interactions between the Fc polypeptide and N-glycan residues.

Modification on IgG glycosilation affects IgG Fc structure and function.

Exemples:
→ no fucose → more binding → more antibody dependent citotoxicity
→ more sialic acid → more anti-inflammatory activity
What do we know about IgA glycosylation and its impact on receptor binding?

In contrast to IgG, little is known how N-glycans impact the protein structure and function of IgAs. While the N-glycan in IgG is in the interstitial space between two heavy chains, in IgAs glycans are located at the external surface of the protein, possibly interacting with Fc receptors.

Features of IgA antibodies

• First line of defense
• Most abundant antibody at mucosal surface
• Neutralization activity of bacterial toxins and virus
• Monomeric, dimeric and secretory forms
• Short half life

Research Questions

• Do the IgA N-glycans have an impact on the protein conformation in solution?
• Do FcRL4- receptor N-glycans play a role for IgA binding?
Production of IgA2

Production of different glycoforms of IgAs

1. $\Delta$XF (no xylose, no 1-3 fucose)
2. $\text{Man}_3\text{GlcNAc}_2$ (MM) and $\text{GlcNAc}_1\text{Man}_3\text{GlcNAc}_2$
3. Terminally galactosylated glycans
4. Terminally sialylated N-glycans

How?

2. $\beta$-hexosaminidases
3. coexpression of N-acetylglucosaminyltransferase $\text{GnTII}$ and 1,4-galactosyltransferase
4. coexpressing the entire CMP-N-acetylneuraminic acid (CMP-Neu5Ac) biosynthesis pathway

Production of IgA2 receptors

FCRL4

Recently discovered binding to systemic IgA
The binding mode and a putative role of N-glycans are currently unknown

FCαRI

Binds all types of IgA, very well studied

Binding mode depending on N-glycosilation

Production of different glycoforms of IgAs

1. WT
2. Man9
3. GlcNAc
4. Deglycosylated in one or more points

How?

2. class I–mannosidase inhibitor kifunensine added to the cell culture
3. Kif + EndoH digestion
4. Site directed mutgenesis N$\rightarrow$ Q
Biophysical Characterization

- LC-ESI-MS analysis → site-specific N-glycan composition
- Thermal stability → differential scanning calorimetry
- In vitro binding studies SPR spectroscopy and isothermal titration calorimetry (ITC)

Characterization via SAXS

1. Coarse grained model IgA2 (from PDB)
2. Application of glycans library modeled from GROMOS
3. Comparison with experimental SAXS data
4. Determine lowest energy model
5. Hypothesize influences on structures by glycans

- Free Antibody (different glycosylation)
- Antibody and Receptor FCRL4
References


