The carbohydrate moiety of serum IgM from Atlantic cod (*Gadus morhua* L.)

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Ig glycoproteins I

• All the immunoglobulin classes are glycosylated

• Several functions have been attributed to the carbohydrate moiety of immunoglobulins

• There are basically 2 types of carbohydrate linkages associated with immunoglobulins:
  N-linked: always NAcGlc to Asp-X-Ser/Thr
  O-linked: commonly NAcGal to Ser/Thr
Ig glycoproteins II

- There are 3 types of N-linked oligosaccharides all with a common trimannosyl-chitobiose core:
  - Complex
  - High mannose
  - Hybrid

- Not all the available Asp-X-Ser/Thr sequons are occupied by a glycan (60 - 70%)

- Those occupied usually carry several but characteristic types of glycans (1 - 16), the protein therefore exists as many glycoforms
The possible glycosylation of cod IgM
Aims of the project

• To analyse the oligosaccharides associated with cod IgM

• To assign a possible functional role to the carbohydrate moiety
Material: Cod serum IgM

- Isolated from pooled sera
- Isolated from 6 individual sera
Analytical methods

• SDS-PAGE analysis before and after PNGase and O-glycanase digestion

• Oligosaccharide sequencing analysis:
  - Glycan release and labelling
  - Normal phase (NP)-HPLC analysis and enzyme arrays

• Other complementary methods used:
  - WAX-HPLC: Weak anion exchange
  - LC/ESMS: Liquid chromatographic/electrospray mass spectrometry
  - MAL-DI-MS: Matrix-assisted laser desorption ionization mass spectrometry
SDS-PAGE of PNGase digested IgM
Oligosaccharide sequencing

Hydrazinolysis or PNGase

N-X-S/T
2AB (2-aminobenzamide)

A3G3FS3(6)(6)(3)

NDVS: α 2-3 sialic acid
ABS: α 2-3 and α 2-6 sialic acid
BTG: galactose
BKF: fucose

N-acetyl glucosamine
Mannose
Galactose
Fucose
Sialic acid
NP-HPLC of 2-AB labelled glycan pool and enzyme arrays

<table>
<thead>
<tr>
<th>aliquote:</th>
<th>Fluorescence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>a b&lt;sup&gt;1+2&lt;/sup&gt; c d e f g h&lt;sup&gt;1+2&lt;/sup&gt; i j k l m n</td>
</tr>
<tr>
<td>2</td>
<td>+ NDV (ii)</td>
</tr>
<tr>
<td>3</td>
<td>A2G2F A2G2 A3G3 A3G3F</td>
</tr>
<tr>
<td>4</td>
<td>A2G0 A2G0F A3G0 A3GOF + ABS (iv) + BTG</td>
</tr>
<tr>
<td>5</td>
<td>A2G0 A3G0 + ABS (v) + BTG + BKF</td>
</tr>
</tbody>
</table>

(i) GU

(ii) + NDV

(iii) + ABS

(iv) + ABS + BTG

(v) + ABS + BTG + BKF

(iii) + ABS

(iv) + ABS + BTG

(v) + ABS + BTG + BKF
A2G2

A2G2S(6)

A3G3

A3G3F

A2G2S2(6)(6)

A2G2FS(6)

A3G3S(6)

A3G3FS(6)

A3G3S2(6)(6)

A3G3FS2(6)(6)

A3G3S3(6)(6)(3)

A3G3FS3(6)(6)(3)

Man 5

Man 6

A2G2F

A3G3S2(6)(6)
The % of different glycans

- 60% contain sialic acid
- 40% contain inner fucose
- 50% are tri-antennary (A3)
- < 3% are oligomannose
Fluorescence

(i) A2G0F + ABS

(ii) A3GOF + NDV

(iii) A2G2F + A3G3 + ABS

(iv) A2G0 + A3G0F + ABS + BTG

(v) A2G0 + A3GOF + ABS + BKF
### The A2G0 / A3G0 ratio of individual cod IgM glycans

<table>
<thead>
<tr>
<th>IgM</th>
<th>cod size</th>
<th>temperature</th>
<th>origin/feed</th>
<th>A2G0 / A3G0</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM-1</td>
<td>1.3 kg</td>
<td>1°C</td>
<td>wild/herring-shrimps</td>
<td>1.0</td>
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<tr>
<td>SM-7</td>
<td>2.2 kg</td>
<td>7°C</td>
<td>wild/herring-shrimps</td>
<td>1.45</td>
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<td>SM-14</td>
<td>3.8 kg</td>
<td>14°C</td>
<td>wild/herring-shrimps</td>
<td>1.77</td>
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<tr>
<td>SM-S</td>
<td>0.7 kg</td>
<td>7°C</td>
<td>cultured/pellets</td>
<td>0.44</td>
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<tr>
<td>SM-919</td>
<td>0.7 kg</td>
<td>9°C</td>
<td>cultured/pellets</td>
<td>0.59</td>
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<tr>
<td>SM-921</td>
<td>0.7 kg</td>
<td>9°C</td>
<td>cultured/pellets</td>
<td>0.30</td>
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</table>
Analysis of the functional role of the carbohydrate moiety

- Protection against protease digestion
- The effects on the anti-TNP-BSA activity of cod IgM
The carbohydrate moiety and trypsin sensitivity of cod IgM H-chain
The carbohydrate moiety and anti-TNP-BSA activity of cod IgM
Summary

• The carbohydrate moiety of cod IgM is about 10% of the total molecular weight
• Associated with the H-chain, N-linked, complex type
• Some of the five available sites may not be occupied
• Considerable heterogeneity - 16 glycan forms identified
• High % of terminal sialic acid
• Individual variation in the proportion of core extensions (A2/A3)
• Protects against protease digestion
• Partial deglycosylation abolishes anti-TNP-BSA activity
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