UNIVERSITY OF YORK

BSc Stage 3 Degree Examinations 2017-18

Department:
BIOLOGY

Title of Exam:
Glycobiology

Time allowed: 2 hours
Total marks available for this paper: 100

This paper has two parts:

Section A: Short Answer / Problem / Experimental Design questions (50 marks)
- Answer all questions in the spaces provided on the examination paper

Section B: Essay question (marked out of 100, weighted 50 marks)
- Answer either question A or question B
- Write your answer on the separate paper provided and attach it to the back of the question paper using the treasury tag provided
- The marks available for each question are indicated on the paper
- A calculator will be provided
1. The Glut4 glucose transporter responds to insulin signalling by translocating to the plasma membrane. Once the insulin stimulus stops, Glut4 is endocytosed to be stored in intracellular vesicles awaiting the next insulin stimulus. Glut4 is glycosylated, and the following experiments look at the effects of this glycan on the insulin responsiveness of Glut4.

**Figure 1.** The amount of cell surface Glut4 was determined and normalized to the first bar in each of the two graphs.

(A) Cells were untreated (-KIF) or treated with the mannosidase I inhibitor kifunensine (+KIF). Both sets of cells were serum starved for 3 hours before treatment with insulin (black bars) for 30 min. White bars show controls not treated with insulin.

(B) Cells were serum starved and then insulin treated as in ‘A’. Cells used for the left pair of bars express wild type (WT) Glut4, cells used for the other bars use the N57Q mutant.

a) Explain the significance of the N57Q mutation for this experiment.

(4 marks)
b) The level of galectin-3 has been shown to decrease in the serum of diabetes patients. Based on the results of figure 1 formulate a hypothesis to explain what role galectin-3 could be playing in diabetes.  (7 marks)

c) Design an experimental strategy to analyse the monosaccharide composition of Glut4’s glycan.  (4 marks)
2. Fibroblasts from a newly discovered \( N \)-glycosylation CDG patient were analysed by glycan profiling. The profiles were compared to fibroblasts from a healthy volunteer. The table below shows the obtained data for the eight most abundant complex \( N \)-glycans.

<table>
<thead>
<tr>
<th>( N )-glycan</th>
<th>Proportion relative to all complex ( N )-glycans</th>
<th>Patient</th>
<th>Healthy control</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{Hex}_4\text{HexNAc}_4\text{NeuAc}_2\text{Fuc} )</td>
<td>4.7 ± 0.6%</td>
<td>33.6 ± 2.3%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_5\text{HexNAc}_4\text{NeuAcFuc} )</td>
<td>1.3 ± 0.5%</td>
<td>18.8 ± 1.8%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_6\text{HexNAc}_4\text{NeuAc}_2 )</td>
<td>39.0 ± 3.2%</td>
<td>11.7 ± 0.5%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_5\text{HexNAc}_4\text{NeuAc} )</td>
<td>11.2 ± 2.3%</td>
<td>0.8 ± 0.03%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_5\text{HexNAc}_4\text{Fuc} )</td>
<td>1.1 ± 0.3%</td>
<td>5.5 ± 0.2%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_4\text{HexNAc}_4\text{Fuc} )</td>
<td>0.6 ± 0.2%</td>
<td>3.3 ± 0.8%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_6\text{HexNAc}_5\text{NeuAcFuc} )</td>
<td>0.2 ± 0.03%</td>
<td>9.1 ± 1.1%</td>
<td></td>
</tr>
<tr>
<td>( \text{Hex}_6\text{HexNAc}_5\text{NeuAc} )</td>
<td>12.7 ± 1.1%</td>
<td>3.8 ± 0.4%</td>
<td></td>
</tr>
<tr>
<td>Sum</td>
<td>70.8%</td>
<td>86.6%</td>
<td></td>
</tr>
</tbody>
</table>

**Table 1.** Relative amounts of the eight most abundant complex \( N \)-glycans in a healthy control subject’s fibroblasts, and the relative amounts (compared to total complex \( N \)-glycans) of the same glycans in a CDG patient. The shown \( N \)-glycans are Hex: hexose, HexNAc: N-acetyl-hexosamine, NeuAc: sialic acid, Fuc: fucose.

a) Which glycosylation enzyme gene is mutated in the CDG? Explain.

(4 marks)
b) The sum of the glycan amounts in the patient sample is much lower than in the control. Draw one glycan structure that could make up some of this difference. (3 marks)

c) This patient could have some problems with their immune function. Explain why. (4 marks)

3. Sialic acids are important molecules in the interactions between bacterial pathogens and their hosts.

   a) Some gut commensal bacteria secrete enzymes that cleave sialic acid from host glycans but cannot use that sugar themselves. Suggest two possible hypotheses as to why this phenomenon might have evolved. (2 marks)
b) Outline two methods you could use to determine whether the lipopolysaccharide on the surface of a bacterium has been decorated with sialic acid. (4 marks)

c) Some bacterial sialoglycans mimic host structures. Outline the potential pathological consequences of this phenomenon. (4 marks)
4. You identify a new pathogenic bacterium that mimics mucin type O-glycans on its surface to evade detection by the host.

a) Based on the following observations draw the structure of the predominant glycan with as much detail as possible, explaining your logic.

i) Glycan profiling shows that the predominant glycan has a size that is consistent with the presence of two hexoses, two N-acetyl-hexosamines, a sialic acid and a fucose.

ii) Monosaccharide analysis confirms the presence of N-acetyl-glucosamine in the glycan.

iii) Treatment with an endo-galactosidase leaves three disaccharides: one consisting of two hexosamines, one with a hexose and a fucose, and one with a hexose and a sialic acid.

iv) Treatment with an endo-glucosaminidase leaves a trisaccharide containing a hexose, a hexosamine and a sialic acid.  

(6 marks)
b) You find that the bacterium binds very strongly to the nasal epithelium, and you suspect this could be dependent on the presence of fucose or sialic acid. How could you test which monosaccharide is involved?  

(4 marks)

c) Your efforts to bind the isolated glycan to epithelial cells during experiments are unsuccessful. Explain why.  

(4 marks)
SECTION B: Essay question

Answer one question on the separate paper provided

Remember to write your candidate number at the top of the page and indicate whether you have answered question A or B

Mark total for this section: 50

EITHER

A) Explain why it is difficult to control the glycosylation status of biologics, and why do we care?

OR

B) Contrast the functions of cytosolic and cell surface glycans in eukaryotic physiology and disease.