Module Code: CHE00019H

Examination Candidate Number: __________

Desk Number: __________

BSc and MSc Degree Examinations 2018-9

Department:

BIOLOGY

Title of Exam:

Proteins and Disease

Time Allowed:

2 hours

Marking Scheme:

Total marks available for this paper: 100

The marks available for each question are indicated on the paper

Instructions:

Answer all questions in the spaces provided on the examination paper

Materials Supplied:

CALCULATOR

For marker use only:  

Office use only:

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Q1.

a) Structures of three steroid water pollutants 1-3 are shown below.

![Structures of steroid water pollutants](image)

Giving your reasons, identify which of the compounds 1-3 can disrupt the function of Estrogen Receptor (ER). (3 marks)

Only 2 can act as ER disruptor (1 mark). This is as the three main structural features that enable ligand/hormone binding to ER are:
- OH group at the A – first left - ring, and the ring associated with the -OH group must be aromatic, i.e. phenolic and flat, to be pincered between some alkyl amino acid side-chains in the ligand binding cavity (1 mark),
- the length of the steroid must be within the length of the estradiol scaffold, i.e. no longer than estradiol, as the length of the cavity in this direction is limited; 1 and 3 fulfil this requirement, but 1 will not bind as it lacks phenolic A ring (1 mark).

This was “a warm up - easy' question that, surprisingly, delivered many wrong answers. The main issue here was lack of recognition that to achieve any effect the ligand must bind to ER. This is possible only if its contains phenolic ring. However, many answers did not recognize difference between compound 1/3 and ligand 2. ONLY ligand 2 has phenolic ring (aromatic + (-OH) group). Hence only 2 can be a disruptor, and in an agonistic (obviously unwanted - pollutant!) manner.

b) Human cells used in an assay for Nuclear Receptor-induced signalling pathways showed a decreased expression level of heat shock proteins (HSPs). Giving your reasons, state whether expression of HSPs should be addressed to maintain the reliability of this assay. (3 marks)
The unliganded NR is not folded properly hence is not conformationally stable with an empty ligand cavity, and with the terminal C-terminal helix 12 not closing the ligand binding site (1 mark). The aggregation and degradation of ligand-free NR is normally prevented by formation of its complex with some HSPs (1 mark). Therefore, the lower levels of HSPs may lead to the aggregation of NR(s), their proteolytic removal=lower cellular levels, and, ultimately, an unreliable assay (1 mark).

This question delivered many good answers. However, they often claimed that Hsps had direct effect on transcription. The main point here was lack of protection for the unliganded/not well-folded monomeric ER hence it subsequent aggregation.

Q2.

a) The sequence and numbering of the C-terminal part of the insulin B chain is given below:


Sheep insulin differs from human insulin only by Arg instead of Thr at position B27. Outline briefly the main steps of an enzyme-based semi-synthesis of human insulin into its sheep homologue. (6 marks)

Trypsin-like enzymatic digest of pig insulin can be used in the first step to obtain human B-chain cleaved after ArgB22, i.e. without B23-B30 peptide (1 mark). Then the B23-B30 octapeptide containing Arg at B27 (sheep sequence) can be synthesised on the solid phase (1 mark). Both ArgB27 and LysB29 side chains must be protected (1 mark). In the next step the des-octapeptide(B23-B30) insulin and B23-B30 sheep octapeptide can be joined by another application of trypsin but in a water-depleted, e.g. organic, medium. Trypsin will do a trans-amidation reaction (i.e. form a peptide bond) if the nucleophile (water) is not present (2 marks). The full sheep insulin will be obtained after enzymatic deprotection of ArgB27 and LysB29 (1 mark).

This question delivered often calamitous answers as it was not read carefully/till the end. Many answers described semi-synthesis of human insulin although question was about transformation of human insulin INTO sheep one. However, even ‘wrong direction’ answers showed good understanding of such process hence some marks were allocated there.
b) A person acquired a mutation in Insulin Receptor isoform A (IR-A) that abolished functionality of this receptor. Explain briefly whether this person will be more prone to develop cancer. (5 marks)

Cancer cells overexpress frequently IGF-1R and IGF-1/IGF-2 hormones for auto-stimulation and auto-proliferation (one of the major hallmarks of cancer) (1 mark). However, IR-A is also a good binder of IGF-2 and it can also be involved in mitogenic signaling (1 mark). Only activation of IR-B has practically pure ‘metabolic’ outcomes: IR-B is a poor IGFs binder (1 mark). Moreover, IR-A can form heterodimers with IGF-1R providing extra mitogenic signaling (1 mark). Therefore the mutation of IR-A may have an anti-cancer protective effect for this person (1 mark).

This question was answered well although the answers with all above-mentioned effects of IR-A mutation were rare. Either hetero-dimerisation of IR-A:IGF-1R or IR-A as a good binder of IGF-2/1 was missing.

c) Enzyme X is one of the key proteins in the glycolysis metabolic pathway. Should the function of enzyme X be inhibited or enhanced in an effective anti-cancer therapy? Justify your answer. (3 marks)

A switch of metabolic pathways is one of the new emerging cancer hallmarks: Reprogramming Energy Metabolism. Cancer involves not only deregulated control of cell proliferation but also corresponding adjustments of energy metabolism in order to fuel cell growth and division (1 mark). Aerobic glycolysis – although with much poorer efficiency of generating ATP than mitochondrial oxidative phosphorylation - gives cancer cells more chemical ‘building blocks’ variety needed for proliferation (1 mark). Hence glycolytic enzymes should be inhibited in anti-cancer therapies (1 mark).

This question was also answered well with visible impact of some info from cancer module. However, several answers linked wrongly enzyme X with regulation of glucose level and insulin signalling, GLUT4 activation etc. (?).

Q3

a) Briefly describe the human stage of the life-cycle of the malaria parasite *Plasmodium falciparum*. (4 marks)
Infects humans during mosquito blood meal [1]. Sporozoites proceed to liver where parasites multiply and are released as merozoites [1]. These infect red blood cells in a persistent asexual cycle of infection [1]. Some of parasites differentiate into male and female gametocytes which are taken up in an insect bite [1].

This was a straightforward question and most students were familiar with the parasite life cycle and scored well.

b) Briefly explain how the sequence of the *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP-1) relates to its function. (4 marks)

PfEMP-1 is a protein that is localised on the surface of red blood cells infected with the parasite with a transmembrane domain close to the C-terminus [1]. It has a modular structure [1] featuring Duffy-Binding like domains and cysteine-rich interdomain repeats [1] in which the different modules confer the capacity to bind to different cell surface ligands such as chondroitin sulphate and CD36 [1].

Most answers displayed knowledge here. A common oversight was the transmembrane region.

c) Explain how the parasite evades immune responses against PfEMP-1. (2 marks)

PfEMP-1 is encoded by one of the var genes. There are > 50 or so variant copies on the parasite chromosome. These undergo switching from time to time

Again almost all students answered this part correctly.

Q4

a) Identify the following anti-malarial drugs and briefly state what is known of the their mode of action and the principal mechanism of drug resistance. (8 marks)

(i) Pyrimethamine which is an inhibitor of the parasite dihydrofolate reductase an enzyme of the folate pathway. For pyrimethamine, multiple mutations in the
enzyme lower the affinity of the drug without significantly affecting the catalytic activity [2]

(ii) Artemisinin. It is thought to have multiple targets in the parasite. One likely target is phosphatidylinositol-3-kinase. Resistance mutations have been mapped to PfKelch13 which is thought to be involved in ubiquitination pathways. [2]

(iii) Sulphadoxine which is an inhibitor of the parasite dihydropteroate synthase. This is an enzyme of the pathway of folate biosynthesis. [2]

(iv) Chloroquine, which interferes with haem polymerisation in the food vacuole of the parasite. Resistance has been associated with an efflux transporter which pumps the drug away from its site of action. [2]
This question was generally well done. The most common confusion was to mix up chloroquine and pyrimethamine.

Q5

(a) Describe the reaction catalysed by the enzyme N-myristoyltransferase referring to the substrates and products of the reaction.  

Substrates Myr-CoA and Gly-Prot; Products CoASH and Myr-Prot [1]. Compulsory order binding and release. Acyl group transfer to alpha-amino group [2]

Fewer than expected answers scored full marks with omissions of one or more of the substrates or products.

(b) The molecule shown is a multifunctional agent that has been used to define the set of myristoylated proteins in parasite cells. Explaining your reasoning assign the moieties labelled A-C to the functions below

(i) Covalent attachment to the myristoylated proteins
(ii) Purification of tagged myristoylated proteins
(iii) Detection of myristoylated proteins in gels

(i) C this is an azide group which can participate in copper catalysed azide alkyne cycloaddition with an alkyne tagged proteins. [2]
(ii) A this is a biotin group which can be used for affinity purification using streptavidin coated beads [2]
(iii) B this is a fluorophore that allows the protein to be detected in a gel for example [2]

This question proved challenging and there was a tendency to score either full or no marks on this one.

(c) Having pulled-down a mixture of myristoylated proteins tagged with the reagent above, how would you then identify the protein components of the myristome of the organism. (3 marks)

The central technique would be protein fragmentation and mass spectrometry to identify the masses of peptides produced. [1] These would be compared against the genome sequence of the organism of interest. [1] Confidence in the assignment would be provided by the observation of high fragment coverage in candidate proteins. [1]

This question was challenging. Few answers scored full marks here.

Essay

A) Compare the complexities of clinical approaches for Type 1 and Type 2 diabetes. Plenty of material can be exploited here from both lecture and additional papers that have been supplied. Firstly the molecular bases of T1 and T2 Diabetes should be presented. They determine different clinical approaches. While T1 Diabetes therapy must rely on supply of non-endogenously produced insulin the anti-T2 Diabetes approached can vary. The main issue in T1 therapy is the problem with copying physiological effects of insulin, mostly lack of its basal level upon exogenous provision (e.g. injections) of this hormone. Issues of short and long acting insulin analogues should be discussed. Top essay will include different peripheral tissue/liver distribution of Insulin Receptors isoforms (IR-B = metabolic, IR-A = mitogenic) and ‘late’ delivery of insulin to liver/main glycemic response organ upon injection. Therapy of T2 Diabetes can start from diet (slimming) to drug-based tissue re-sensitizing to insulin action (metformin). Both approaches result from obesity-T2 Diabetes correlation. More advanced drug (non-insulin) anti-T2 Diabetes should be discussed, such as stimulation of differentiation of new adipose tissue as a new reservoir of fat storage; this route can result in more insulin-sensitive adipose tissue. Some dangers of diabetes-cancer correlations can be also discussed briefly in the context of insulin-based treatment; e.g. overstimulation of IR-A and its hybrids with IGF-1R.

Despite rather straightforward task this essay was much less popular than essay B. Most of the students addressed the key points, although only minority of essays dug deeper into the overlap of insulin:IGF axes and issues related to different
tissue distribution of IR-A and IR-B isoforms of insulin receptors. A critical approach to T2DM treatments was also rarely observed.

B) Compare and contrast the structure and function of the protective antigen of *Bacillus anthracis* with the haemagglutinin of influenza virus, and explain their roles in disease.

The topic of anthrax is covered in the final lecture with a major focus on PA, meanwhile there are two lectures on flu one of which focuses on HA. There is plenty of material to be covered here. The two proteins are membrane bound and undergo a process of maturation involving proteolysis. PA is a component of a bi-partite toxin that facilitates entry of the toxins, oedema factor and lethal factor into the cytoplasm of target cells. Meanwhile HA recognizes conjugated sialic acids in mediating attachment of the flu virus to the host cell surface. Both proteins have conformations which are sensitive to pH and lowering the pH promotes membrane fusion events. The two proteins have roles in immunity. There are a number of differences too including the oligomeric state and there are specific aspects of the structure and the function of each protein that can be discussed.

This proved a popular essay. The students had no problems identifying relevant material and essays were generally very good. The best essays balanced discussion of HA and PA and structure with function.