Biological Chemistry II/Spring term 2022/23 Mock Exam

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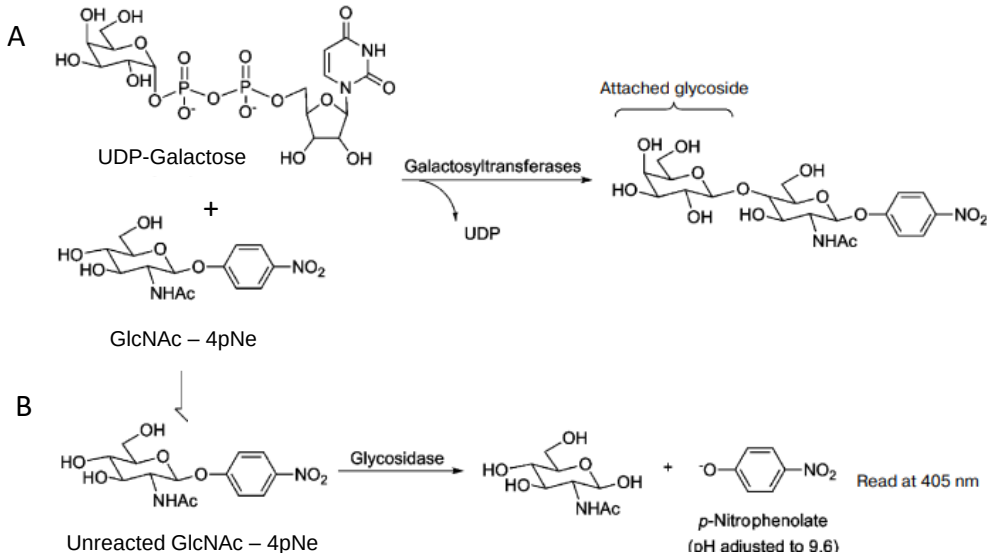
Question 1

Galactosyltransferases are a class of enzyme which adds galactose to a sugar acceptor. You want to measure the activity, K_M, and v_{max} of the galactosyltransferase GalA, which uses UDP-Galactose as a substrate, and adds a galactose onto N-acetyl-glucosamine (GlcNAc). This reaction can be quenched by adding an excess of the enzyme exoglycosidase.



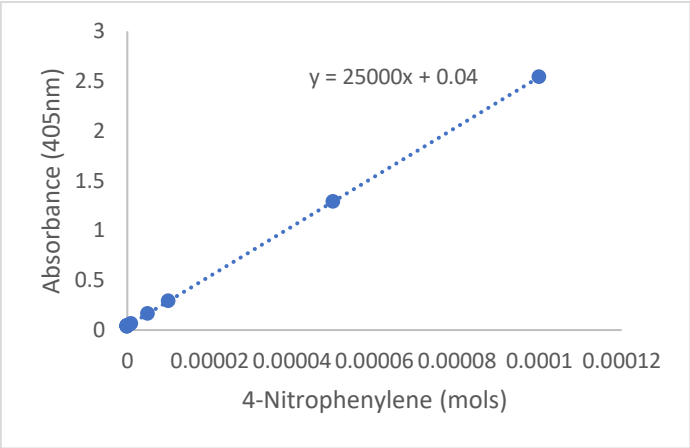
(a) GlcNAc can be conjugated to the chemical group 4-nitrophenylene and 4-nitrophenylene conjugated MONO SUGARS are cleaved glycosidase enzymes, which gives rise to an absorbance at 405 nm. Design an experiment for measuring the K_m and V_{max} of the galactosyltransferase GalA. Assume reaction time of both enzymes to be 30 mins and we initially added 0.1 umol of the substrate GlcNAc-4pNe.

(b) You are currently screening for potential drug candidates which inhibits the activity of GalA as it is an important protein in the virulence of a pathogenic bacteria. You identified a potential candidate JW1996. The kinetics data of GalA with and without JW1996 is shown below. What is the K_d, V_{max} of each respective case? Which type of competitor is JW1996? Assume reaction time of both enzymes to be 30 mins and we initially added 0.1 umol of the substrate GlcNAc-4pNe.

Concentration of UDP-Galactose	Abs 405 (GalA only)	Abs 405 (GalA with JW1996)
0.1 uM	1.48	1.96
0.5 uM	0.57	1.77
1 uM	0.34	1.74

5 uM	0.10	1.71
10 uM	0.07	1.71
15 uM	0.06	1.709
20 uM	0.057	1.708
25 uM	0.053	1.708
30 uM	0.051	1.707
40 uM	0.048	1.707
50 uM	0.047	1.707
60 uM	0.045	1.707
100 uM	0.043	1.707
200 uM	0.042	1.707

Given in the diagram is a standard curve of 4-nitrophenylene absorbance as a function of concentration:



Question 2

Glycolysis and Oxidative phosphorylation are conserved metabolic pathways that allow living organisms to convert reduced carbon sources into energy. Most organisms ranging from bacteria to mammals use glycolysis and/ or oxidative phosphorylation to sustain their energetic needs.

(c) *Escherichia coli* is an example of a facultative anaerobe. Describe what the fate of glucose is when fed to *Escherichia coli* in a CO_2 chamber.

(d) Calculate the energy yield (in terms of ATP molecules) of 5 molecules of glucose fed to *Escherichia coli* in the presence or absence of O_2. Please depict the individual steps of the calculation *(Assume four H^+ ions must flow through ATP synthase to power the synthesis of one ATP molecule)*.

(e) Uncoupling agents negatively affect oxidative phosphorylation. Some of these serve a specific biological purpose. The uncoupling agent thermogenin (a.k.a. UCP1) is a proton ion channel protein present in brown adipose tissue mitochondria. Thermogenin allows the passage of protons from the intermembrane space into the matrix disrupting the flow of protons. What is the effect of the action of Thermogenin and what is its physiological role?

(f) Athletes try various approaches to try to maximize their physical performance potential. Some athletes train at high altitudes to increase their red blood cell levels, which naturally happens in response to the lower oxygen levels at high altitudes. What potential benefit would altitude training have on an athlete's performance, and why? (2pt)

(g) "Carb loading" is a nutritional strategy used by runners, where they eat a lot of carbohydrates for several days before a marathon and rest. Please describe the metabolic process leading to the potential benefits behind this concept? (2pt)

Question 3

Inborn errors of sphingolipid catabolism lead to lysosomal storage diseases which are characterized by the toxic accumulation of metabolic intermediates in the lysosomes. Substrates reduction therapies are based on the idea that reducing sphingolipid production leads to decreased lysosomal accumulation and thus to mitigation of pathology. Gaucher, Fabry and Nieman-Pick Type A/B diseases are characterized by the accumulation of glucosylceramide (GlcCer), globotriaosylceramide (Gb3), and sphingomyelin (SM) respectively.

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(a) You are asked to identify targets for inhibitors to be used in substrate reduction therapies for these diseases. Which are the factors that you might target for each of the cases? Articulate your answer.

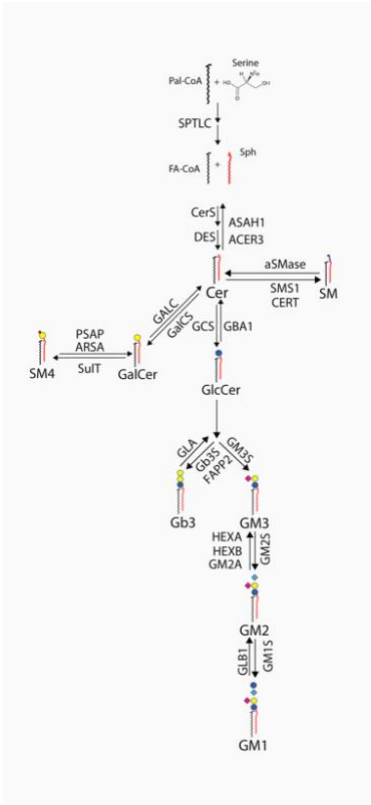


Figure 1: Schematic representation of sphingolipid metabolism

(b) Sphingolipid synthesis occurs in different cellular compartments, however sphingolipid movement through the aqueous cytosol is unfavorable and lipid-transfer proteins are required to facilitate this process which are regulated by phosphoinositides. Explain the role of the lipid-transfer proteins and phosphoinositides regulation in the synthesis of sphingolipids. What is the pleckstrin homology domain, describe its fold, size and function?

(c) Activated isoprene units are synthesized starting from Acetyl-CoA. If 2-[^{14}C]acetyl-CoA is added to a rat liver homogenate, where will the ^{14}C label appear in Δ^3-isopentenyl pyrophosphate (Figure 2)?

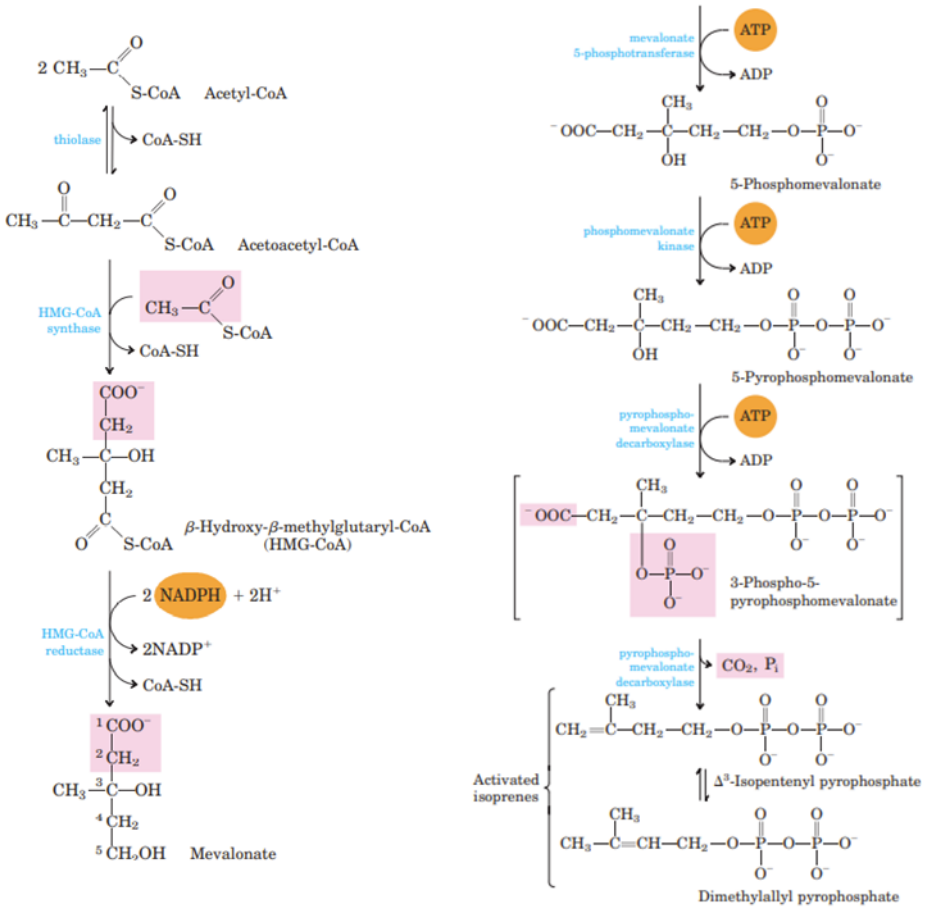


Figure 2: Schematic representation of the biosynthesis of activated isoprene units

(d) HMG-CoA reductase is the rate-controlling enzyme in the mevalonate pathway. It is often targeted by inhibitors – statins. What is one of the most important downstream effects of statin treatment?

(e) PI3K catalyzes the conversion of PtdIns(4,5)P_2 to PtdIns(3,4,5)P_3 while PTEN catalyzes the opposite reaction. Mutations rendering PI3K hyperactive and mutations disrupting PTEN activity are major cancer drivers. Can you explain the mechanism by which disturbed lipid metabolism result in malignant transformation?

Question 4

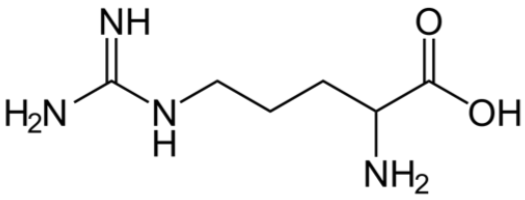
When adapting to their environment, cells (as a consequence of transcriptional changes) change their metabolism.

a) Describe a workflow based on mass spectrometry to compare the metabolomes of a cell line grown under different conditions. Please explain each step.

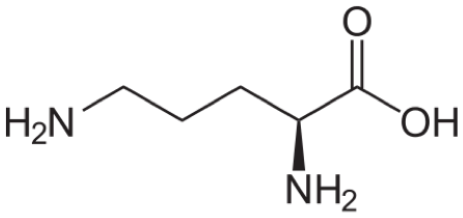
b) According to your metabolomics results intracellular glucose levels are increased when cells are cultured in the presence of *compound A*. In an attempt to evaluate the potency of your compound, you devise an experiment where cells are incubated with increasing amounts of *compound A*. Which types of assay suit better the design of such experiment? Explain why.

c) When administered to mice, *compound A* induces: hypoglycaemia, increased glycogen synthesis in the liver and muscles, increased glycolysis, increased fatty acid and triglyceride synthesis. Based on these effects can you hypothesize a mode of action for *compound A*? Explain your answer.

d) Arginase converts arginine into ornithine and urea. Below, you can find the structure and m/z of arginine and its metabolite ornithine:



Arginine
m/z 174.2



Ornithine
m/z 132.16

How can you measure arginine cellular uptake and its conversion into ornithine by mass spectrometry?

Question 5

Amino acids are important precursors for the production and maintenance of the nucleotide pools in cells.

a) What is the ^{15}N labelling of GMP (guanosine monophosphate) in cells grown in the presence of uniformly ^{15}N labelled glutamine? Explain your answer.

b) How does your answer change if glutamine is labelled as follows?

I. Exclusively on the N atom in the side chain (amide).
II. Exclusively on the N atom in the amine group attached to the α-carbon atom.

c) Name 2 other amino acids that can be labelled with ^{15}N and used to feed cells, so that the GMP synthesized in these cells will be labelled with at least one ^{15}N atom.

d) Another way of synthetizing GMP is through its salvage pathway. HGPRT is a transferase that catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate as part of the salvage pathway. Lesch-Nyhan syndrome is a rare genetic disorder due to a deficiency of HGPRT. Such deficiency causes a build-up of uric acid in all body fluids.

I. Which molecule does HGPRT incorporate into the guanine when it converts it to guanosine?
II. 5-amino 4-imidazolecarboxamide (AICA) is an inhibitor of enzyme guanine deaminase. Allopurinol, used to treat gout, is an inhibitor of xanthine oxidase. Which one of these two drugs would be best suited to alleviate symptoms of a patient suffering from Lesch-Nyhan syndrome and why?