Biological Chemistry II/Spring term 2023/24 Mock Exam

Instructor: Prof Giovanni D'Angelo

Question 1

Labeling cholesterol isotopically

Cholesterol is essential for maintaining the structural integrity and fluidity of cell membranes, and it plays a critical role in various biological processes. The precursor of cholesterol is acetate, which is used to produce isoprene units that are then condensed to produce cholesterol.

a. If you use an excess of ^{13}C-labeled acetyl-CoA (as shown in red in **Figure 1**), which carbon atoms of mevalonate are labeled? (Circle them) (1 pt)

b. Which carbon atoms are labeled in cholesterol? (Circle them) (3 pts)

c. If we use statins, what products are formed, and which carbons of these products are labeled? (Draw the molecule(s)) (2 pts)

d. If you use an excess of ^{13}C-labeled acetyl-CoA (as shown in blue in Figure 1), which carbon atoms of mevalonate are labeled (Circle them) (1 pt)

e. Which carbon atoms are labeled in cholesterol (Circle them) 3 pts)

f. With the use of statins what products are formed, and which carbons of these products are labeled ? (Draw the molecule(s)) (1 pt)

g. If we mutate the cholesterol transporter OSBP, rendering it inactive. What happens to the membrane(s) composition ? (2 pts)

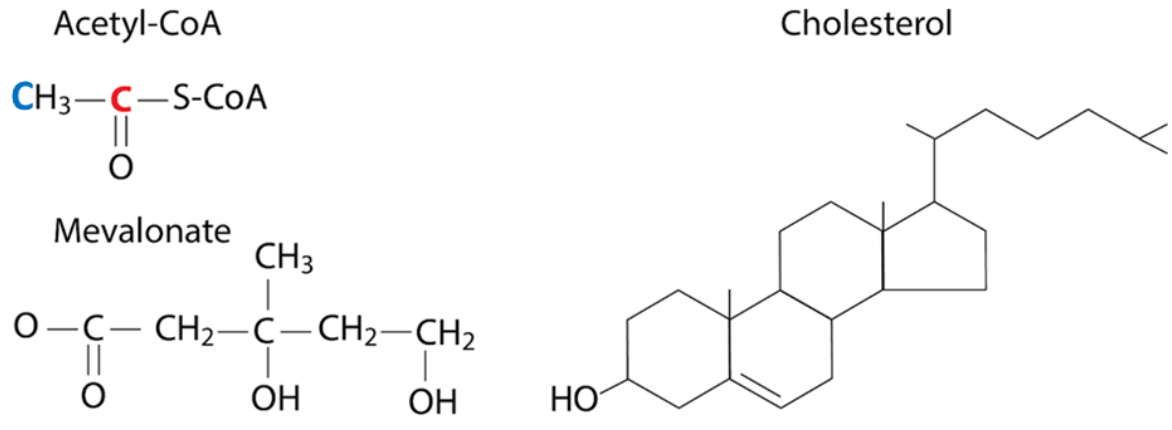

Fig. 1

Question 2

Blood Glucose control

Glucose metabolism is central to animal physiology. Blood glucose control is essential for maintaining metabolic balance and involves a complex interplay between hormones, organs, and biochemical pathways. Insulin and glucagon are the primary hormones regulating blood glucose levels.

a) Upon a sugar-rich meal, beta cells of the pancreas secrete insulin, which in turn induces blood glucose levels to decrease. Specifically, when glucose is available, ATP is generated by β-cells. ATP inhibits an ATP-gated K+ channel, resulting in membrane depolarization and the fusion of secretory granules containing insulin with the plasma membrane, releasing this hormone into the bloodstream. Imagine that the K+-ATP channels are mutated in a way that they cannot close in response to stimuli. How can you stimulate cells to secrete insulin? (2 pts)

b) In an experiment, a researcher subjects hepatic cells to insulin administration by adding it to a medium containing an excess of uniformly ^{13}C-labelled glucose. In which molecule will the labelling most likely be found? (1 pt)

c)) In the same experiment, the researcher now has an experimental point whereby, after having subjected cells to insulin and high ^{13}C-labelled glucose, the medium is washed out and the cells are subjected to a glucose-deprived medium in the presence of glucagon. Where will the labelling be found now? (1 pt)

d) A 12-year-old boy is admitted to the children's hospital. His symptoms include nausea, vomiting, and abdominal pain, and the doctor could smell acetone on his breath. The patient also explains that he has been urinating a lot. The doctor tests for the presence of glucose in the urine, which is positive. The doctor administered sulfonylurea drugs, which did not help the patient. Explain why the drugs did not work and what the doctor should administer instead of sulfonylurea. (2 pts)

e) Lewis's disease is an autosomal recessive metabolic disorder characterized by a deficiency in the enzyme glycogen synthase. The most common clinical history in patients with Lewis's disease involves an infant or child with symptomatic hypoglycemia or seizures that occur before breakfast or after an inadvertent fast. Symptoms include lethargy, apathy, pallor, headache, hypotonia, ataxia, seizures, and coma. How can you rationalize these symptoms? (2 pts)

Question 3

TCA cycle

In diabetes mellitus, the body's ability to use glucose as a primary source of energy is impaired due to insufficient insulin production (Type 1 diabetes) or insulin resistance (Type 2 diabetes). As a result, the body begins to use alternative sources of energy to meet its metabolic needs. When glucose and fat stores are insufficient, the body starts breaking down muscle proteins into amino acids through proteolysis.

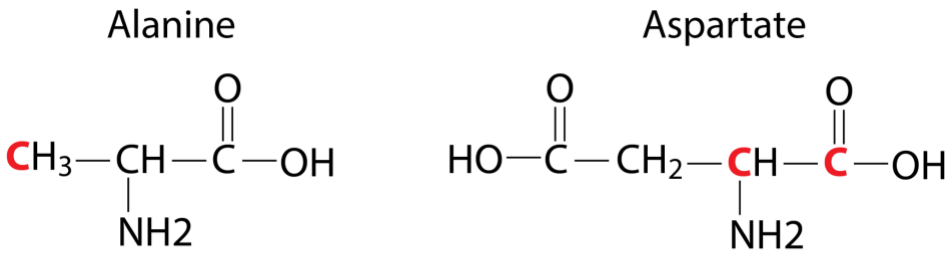

Fig. 2

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.

a) Imagine that the breakdown of endogenous proteins yields labelled amino acids: Alanine labelled at C3 and Aspartate labelled at C1 and C2 **(**red in **Fig. 2**), which then enter the TCA cycle. Where do we find the [^{13}C] label after one TCA cycle? (2 pts)

b) Fluoroacetic acid is a harmful metabolite. Fluoroacetic acid can disrupt the Krebs cycle. The metabolite of fluoroacetic acid is fluorocitric acid, which is very toxic because it cannot be processed by aconitase in the Krebs cycle (where fluorocitrate takes the place of citrate as the substrate). The enzyme is inhibited and the cycle stops working. Where will you find the [^{13}C] label from Aspartate and Alanine (see **Fig. 2**) in the presence of fluoroacetic acid? (3 pts)

c) Now imagine that Aspartate is uniformly labelled at all carbon positions. Where do we find the label after two TCA cycles and where after three? (2 pts)

Question 4

Amino Acid metabolism

A master's student in a biochemistry lab is working with three hepatocyte cell lines that carry different gene mutations. One of them is wildtype (WT), i.e., it has no mutations; one bears an inactivating mutation in the gene encoding aspartate transaminase (AST-KO), and one bears an inactivating mutation in the gene encoding glutamate dehydrogenase (GDH-KO). By mistake, the student erases the label of the cell culture dishes and is now unable to tell one cell line from another. He has some [1,2-^{13}C] glucose (see **Fig. 3**) and has access to a metabolomics technology that allows him to trace ^{13}C. He decides to use this technology to discriminate the three cell lines by looking at which metabolites are produced from the labeled glucose. He also draws a scheme of cellular respiration and some amino acid biosynthesis to help him with the task (**Fig. 4**), where circles represent carbon atoms of the different molecules.

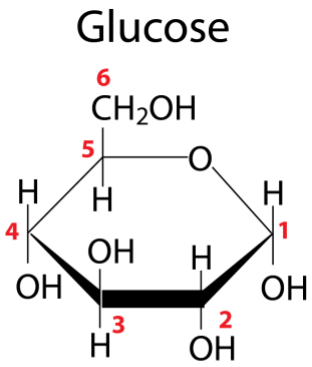

Fig. 3

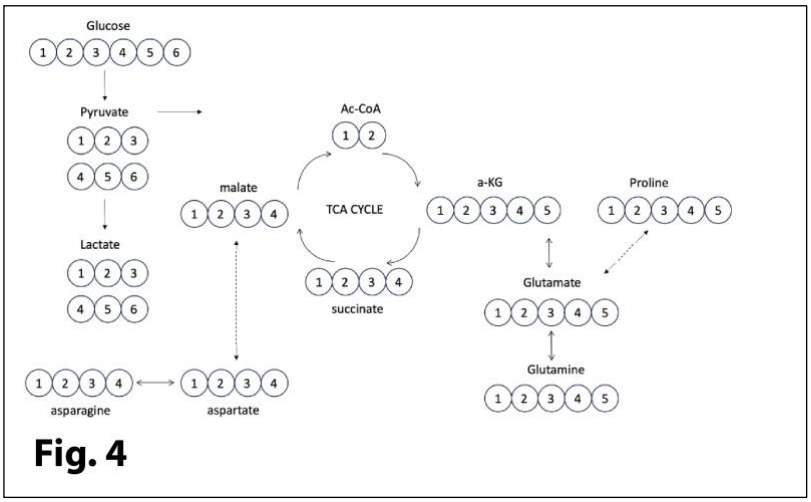

Fig. 4

a) Fill in the circles of the carbon atoms you think would be labeled in each cell line. (3 pts)

b) Our inattentive student gets confused again and instead of using [1,2-^{13}C] glucose, he feeds the cells with [5,6-^{13}C] glucose (see Fig. 3). When he looks at his metabolomics results, he finds a molecule compatible with being Heme, labeled in 8 positions. Can you explain this result? Can you circle the labeled carbon atoms in the Heme structure (Fig. 5)? (4 pts)

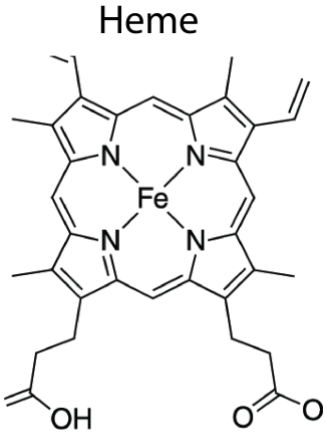

Fig. 5

Question 5

Enzyme Kinetics

Horseradish Peroxidase (HRP) is an enzyme isolated from the horseradish roots (*Amoracia rusticana*). HRP combines with hydrogen peroxide (H_2O_2), and the resultant [HRP–H_2O_2] complex can oxidize a wide variety of chromogenic hydrogen donors making it a useful tool for various immunochemistry applications.

Chromogenic Substrate $_{reduced}$ + H_2O_2 $\rightarrow$ Chromogenic Substrate $_{oxidized}$ + $2H_2O$

a) In the reaction catalyzed by the enzyme HRP, the initial velocity v_0 of the reaction is *⅓ rd* of the maximum velocity V_{max}. If the substrate concentration is 5×10^{-3} mM, determine the value of K_m in µM. (3 pts)

b) k_{cat} (turnover number) is a measure of how many substrate molecules canone enzyme molecule converts per second. For the above reaction, if the total enzyme concentration is 10mM, and V_{max} = 0.04 Ms^{-1}, calculate the K_{cat} of the enzyme. (3 pts)

c) Catalase is also an enzyme similar in function to that of HRP, which uses H_2O_2 as a substrate. Compare the catalytic efficiency of both enzymes. H_2O_2 is a potent cell damaging agent that induces cell death/ apoptosis. In an experiment to protect cells from H_2O_2, which enzyme do you think is ideal? Explain. (3 pts)

Enzyme	K_m (M)	K_{cat} (s^{-1})
Catalase	1.1	4×10^7
HRP	(a)	(b)