Biochemistry II – Exercise 4

Question 1: Stimulation of Oxygen Consumption by Oxaloacetate and Malate

In the early 1930s, Albert Szent-Györgyi reported the interesting observation that the addition of small amounts of oxaloacetate or malate to suspensions of minced pigeon breast muscle stimulated the oxygen consumption of the preparation. Surprisingly, the amount of oxygen consumed was about seven times higher than the amount necessary for complete oxidation (to CO_2 and H_2O) of the added oxaloacetate or malate. Why did the addition of oxaloacetate or malate stimulate oxygen consumption? Why was the amount of oxygen consumed so much greater than the amount necessary to completely oxidize the added oxaloacetate or malate?

Question 2: Labeling studies in isolated Mitochondria

The metabolic pathways of organic compounds have often been delineated by using a radioactively labeled substrate and following the fate of the label.

a) How can you determine whether glucose added to a suspension of isolated mitochondria is metabolized to CO_2 and H_2O?
b) Supposed you add a brief pulse of [3-^{14}C] pyruvate (labeled in the methyl position) to the mitochondria. After one turn of the citric acid cycle, what is the location of the ^{14}C in the oxaloacetate? Explain by tracing the ^{14}C label through the pathway. How many turns of the cycle are required to release all the [3-^{14}C] pyruvate?

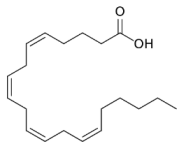
Question 3: Primary Carnitine deficiency

Primary Carnitine deficiency is an autosomal recessive disorder affecting Carnitine transport, with a broad range of phenotypic conditions. Mild symptoms in adults include tiredness, fatigue, more severe symptoms arrhythmias of the heart as well as organ destruction. The disease induced problems become especially prevalent in times of fasting. Based on this given information of the disease and your knowledge on the role of Carnitine, which molecular processes can lead to the manifestation of the described symptoms, start by displaying the role of carnitine in a healthy individual then highlight molecular implications of an impaired carnitine pathway.

Question 4: Fatty acid oxidation.

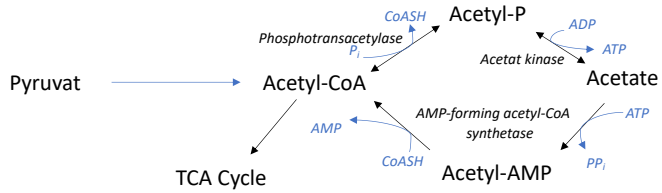
If we follow a hypocaloric diet, our body will start to use the stored fat to produce energy through a process called fatty acid oxidation.

a) How many molecules of acetyl-CoA will be created form a 20-carbon fatty acid? How many ATP can subsequently be produced from that?
b) What changes in the process of fatty acid oxidation if the fatty acid chain has an odd number of carbons?
c) To oxidize Arachidonic acid (shown below), which enzyme(s) are additionally needed to those of the beta-oxidation pathway?



Question 5: *S. aureus* amino acid catabolism.

Staphylococcus aureus (S. aureus) is adapted to a variety of carbon sources. When its primary carbon source glucose is limited, a secondary source can be catabolized. Numerous amino acids can serve as a secondary carbon source.
Besides the TCA cycle, bacteria have an acetate cycle from acetyl-CoA.



Adapted from: De Mets et al. Regulation of acetate metabolism and coordination with the TCA cycle via a processed small RNA. Proc Natl Acad Sci USA 2019.

For experiments of the S. aureus catabolism, it is grown in a medium supplemented with 18 amino acids, but no glucose.

a) The figure below displays the amino acid concentrations in the growth media over the duration of *S.aureus* growth in them.
Which metabolic intermediates feeding into the TCA cycle seem to be primarily made in *S. aureus* from the amino acids supplemented?

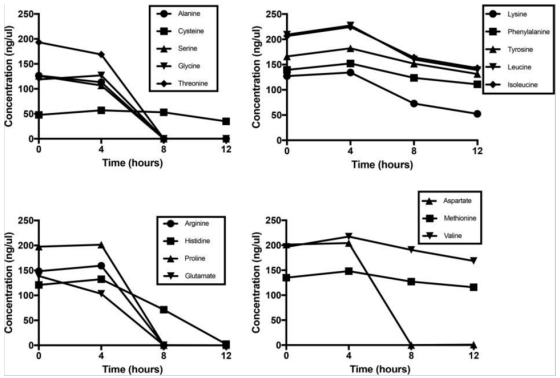


Figure from: Halsey et al. Amino Acid Catabolism in Staphylococcus aureus and the Function of Carbon Catabolite Repression. mBio.2017

b) Knock down (KO) of the gene coding for the glutamate dehydrogenase shows to reduce the growth of *S.aureus* in the amino acid media. Which reaction does the enzyme usually catalyze (also in humans), which conclusions regarding the *S. aureus* catabolism can you derive from the KO experiment finding?

c) A Double KO of the glutamate dehydrogenase gene and acetat kinase gene led to totally abolished growth of *S. aureus* in the medium. Which additional consequence for the *S. aureus* catabolism can be extracted from this finding?

Question 6: Measurement of Alanine Aminotransferase Activity.

The activity (reaction rate) of the alanine aminotransferase, an important measurement for the diagnosis of liver disease, is usually measured by including an excess of pure lactate dehydrogenase and NADH in the reaction system. The rate of alanine disappearance is equal to the rate of NADH disappearance measured spectrophotometrically. Explain how you would imagine this assay to work.