Biochemistry II Exam questions 4

The TCA cycle. Fatty acid oxidation. Amino acids oxidation.

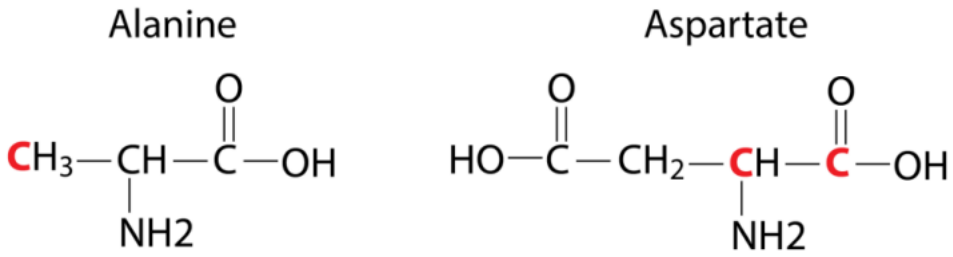
Solutions

March 17, 2025

Question 1

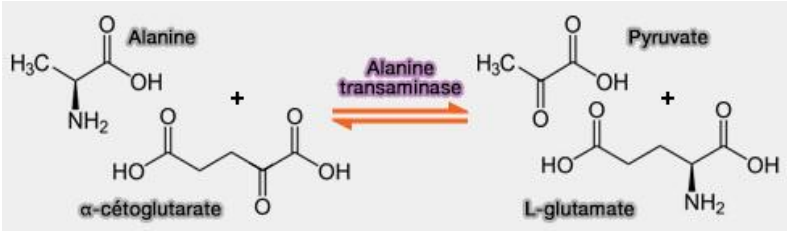
In diabetes mellitus, the body's ability to use glucose as a primary source of energy is impaired due to insufficient insulin production (Type 1 diabetes) or insulin resistance (Type 2 diabetes). As a result, the body begins to use alternative sources of energy to meet its metabolic needs. When glucose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

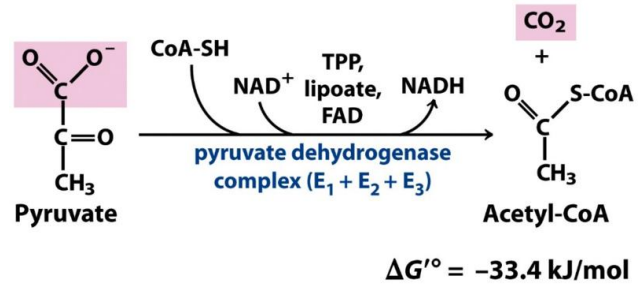
a) Imagine that the breakdown of endogenous proteins yields labelled amino acids: Alanine labelled at C3 and Aspartate labelled at C1 and C2 (Fig. 2), which then enter the TCA cycle. Where do we find the [13C] label after one TCA cycle? (2 pts)



Alanine is fed into the TCA cycle through Acetyl-CoA via being concerted to pyruvate first, aspartate is fed into the TCA cycle through oxaloacetate.

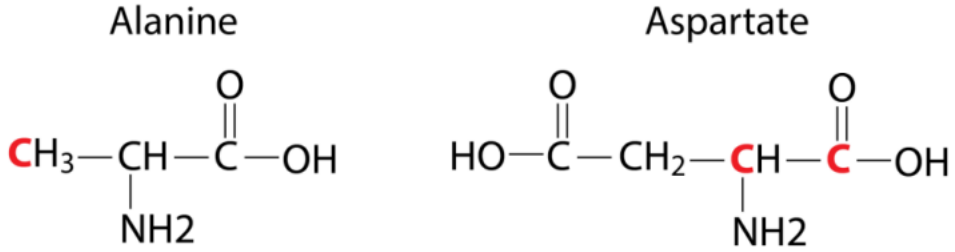
The C3 label of alanine corresponds to C3 of pyruvate and C2 of acetyl-CoA. The carbon atoms from acetyl CoA end up being released as carbon dioxide (CO2) during the Krebs cycle, and the energy stored in acetyl CoA is transferred to energy-carrier molecules such as ATP, NADH, and FADH2. But first they need to be incorporated into the oxaloacetate after the first cycle. Then in subsequent cycles the acetyl CoA carbon atoms get released as CO2.





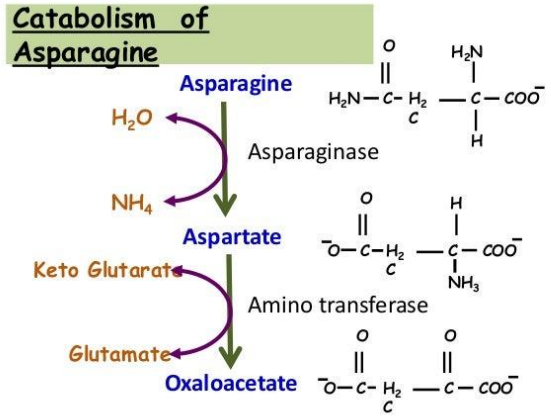
Question 1

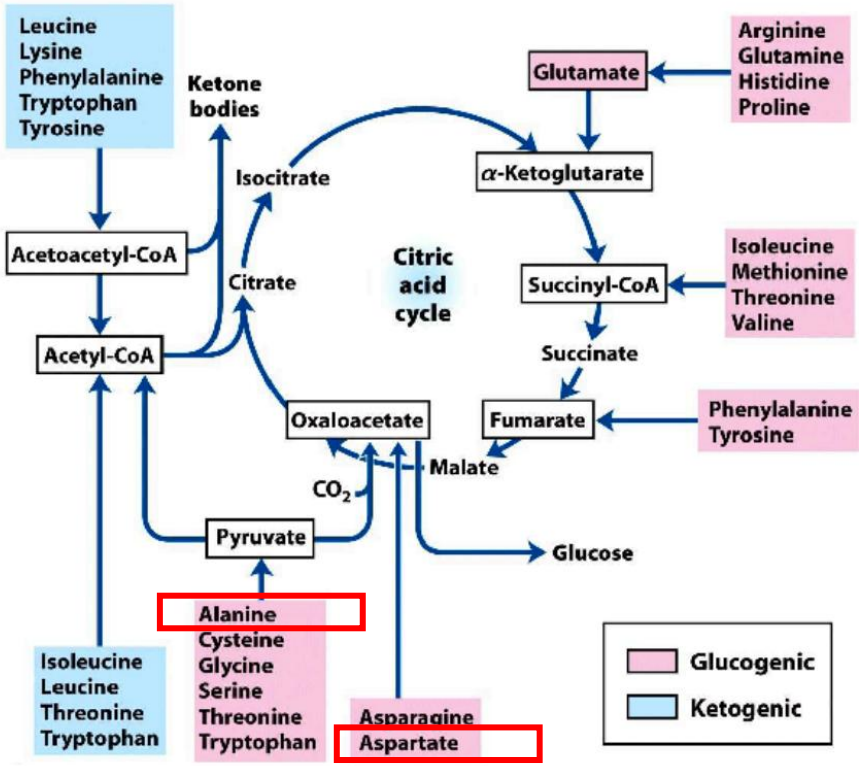
a) Imagine that the breakdown of endogenous proteins yields labelled amino acids: Alanine labelled at C3 and Aspartate labelled at C1 and C2 (Fig. 2), which then enter the TCA cycle. Where do we find the [13C] label after one TCA cycle? (2 pts)



Alanine is fed into the TCA cycle through Acetyl-CoA via being concerted to pyruvate first, aspartate is fed into the TCA cycle through oxaloacetate.

The C1 and C2 labels of aspartate correspond to C1 and C2 of oxaloacetate. After one TCA cycle the first carbon gets lost as CO2 and the second carbon of the oxaloacetate gets regenerated as oxaloacetate.

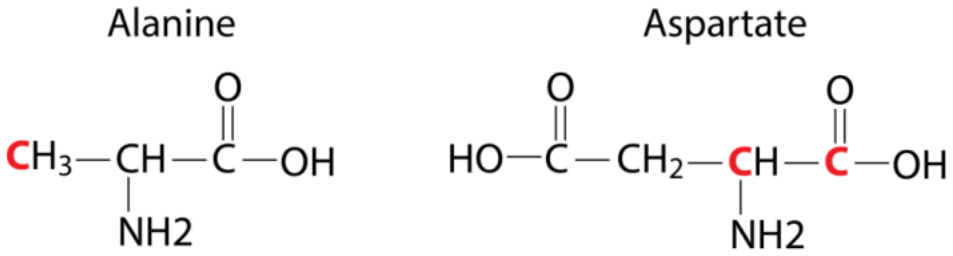


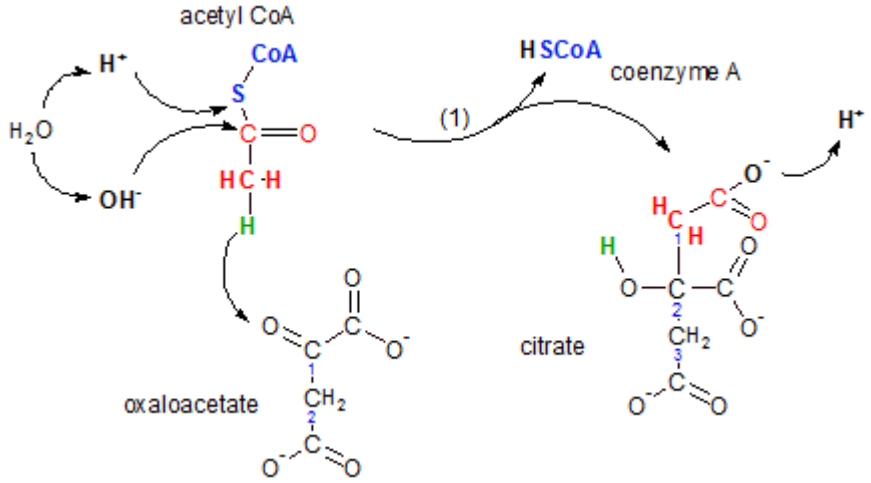


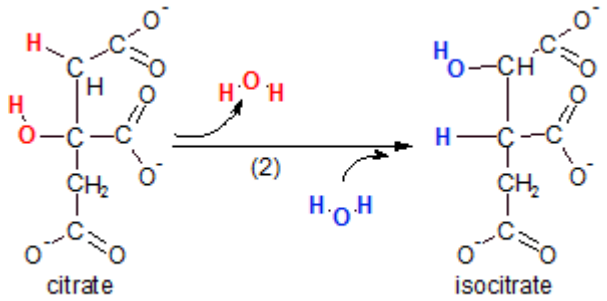
Question 1

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 [13C] label from Aspartate and Alanine (see Fig. 2) in the presence of fluoroacetic acid? (3 pts)

Fluoroacetate (FA; CH2FCOOR) is highly toxic towards humans and other mammals through inhibition of the enzyme aconitase in the tricarboxylic acid cycle, caused by 'lethal synthesis' of an isomer of fluorocitrate (FC). Aconitase in TCA cycle converts citrate to isocitrate. The labeled carbons from Aspartate (C1, C2) and Alanine (C3) remain in citrate and do not get redistributed into later intermediates.



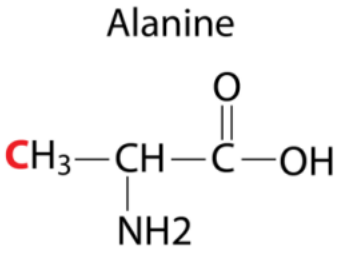


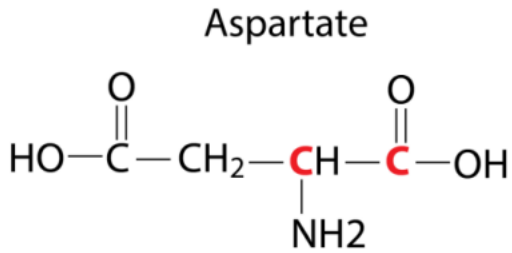


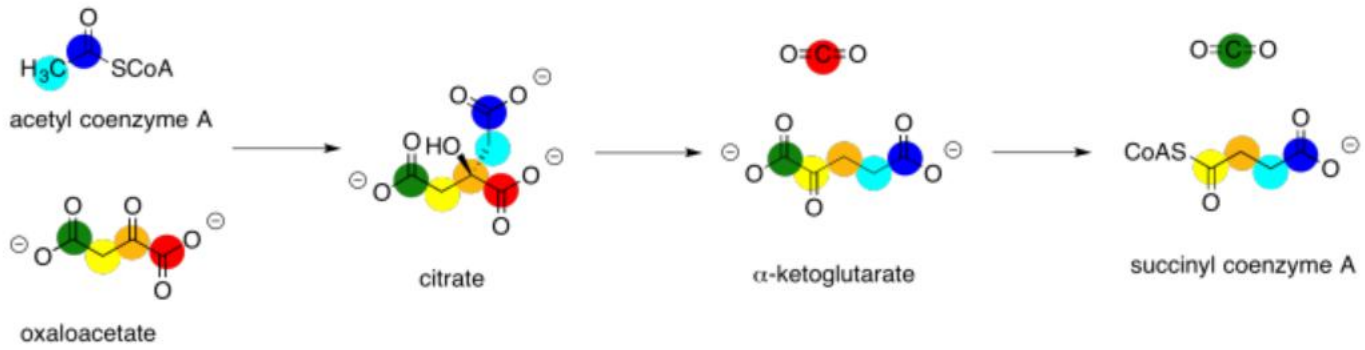
Question 1

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)

The C1, C2, C3, and C4 labels of aspartate correspond to C1, C2, C3, and C4 of oxaloacetate. After the first round of TCA cycle, carbons C1 and C4 get released as CO2. C2 and C3 end up as C3 and C4 of the regenerated oxaloacetate. After the second cycle C4 (initial C3) gets released as CO2. After the third cycle C4 (initial C2) gets released as CO2.







Question 2

Myocytes (skeletal muscle cells) are specialized in generating ATP as a source of energy for contraction. Slow-twitch muscles (red muscles) provide low tension but are resistant to fatigue. They produce ATP through a relatively slow but steady process of oxidative phosphorylation. Fast-twitch muscles, on the other hand, use glycolysis, can develop greater tension, and do so more quickly. In contrast to slow-twitch muscles, they fatigue more quickly.

a) A group of students decides to perform an experiment where they would use myocytes belonging to both muscle types in separate cell culture dishes. They will stimulate myocytes to contract to 100 % (maximum capacity). The culture medium in which myocytes grow contain the following -[13C] labelled molecule: Fatty acid (17 C atoms) labelled at C2, C10 and C17 (Figure 5). Which myocytes will contain the label and in which molecules (assume only one TCA cycle is allowed) (5 pts)

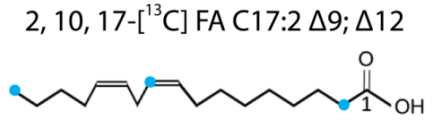


Fig. 5

In slow-twitch muscle cells fatty acids will undergo fatty acid oxidation. Once in the mitochondrial matrix, fatty acyl-CoAs are progressively oxidised by an iterative sequence of four reactions that produce acetic units in the form of acetyl-CoA (Stage1). The newly formed acetyl-CoA can thus feed in the TCA cycle (Stage 2) and then the electrons subtracted in these oxidative reactions are used to produce ATP in the oxidative phosphorylation chain (Stage 3). The even carbons (C2 and C10) will end up in acetyl-CoA and at the end of one TCA cycle be incorporated into oxaloacetate. The odd carbon C17 will end up in propionyl-CoA then feed into TCA cycle as succinyl-CoA and at the end of the TCA cycle be incorporated into the oxaloacetate.

The fast-twitch muscle cells will not incorporate the carbon labels as they favour glycolysis over fatty acid oxidation and will derive their ATP via glycolysis.