Exercises 3 - Solutions

Glycolysis

March 11th 2024

BIO-213 Biological Chemistry II

Question 1

Escherichia coli, a widely used organism in laboratories, it is a facultative anaerobic bacterium.

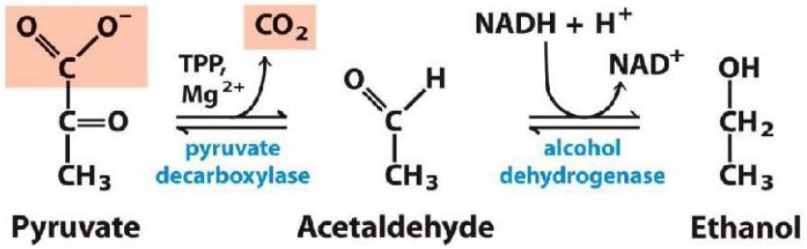
1) Define what a facultative anaerobic organism is.

A facultative anaerobic bacterium is able to produce ATP by aerobic respiration if oxygen is present, but is also capable of switching to fermentation if oxygen is absent.

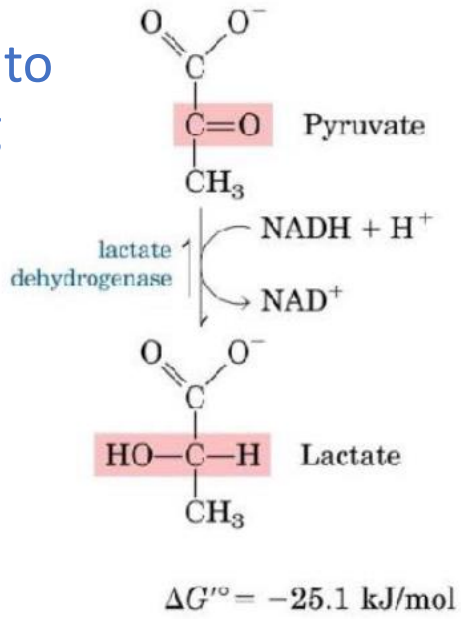
Question 1

2) E. coli fermentation is considered to be mixed, meaning that it is able to produce both lactate and ethanol. Could you describe the two types of fermentations?

In **ethanol fermentation**, pyruvate is first converted to acetaldehyde by pyruvate decarboxylase, releasing a CO2 molecule, then to ethanol by alcohol dehydrogenase, re-oxidizing the NADH to NAD+.



In **lactic fermentation**, pyruvate is directly converted to lactic acid by lactate dehydrogenase, re-oxidizing the NADH to NAD+.

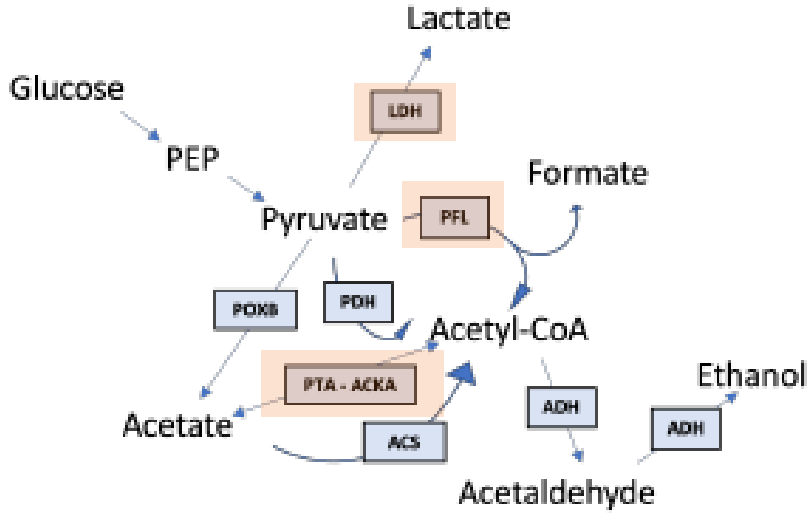


Question 1

3) E. coli is capable of producing ethanol through an endogenous process in which one mole of glucose is metabolized into two moles of formate, two moles of acetate, and one mole of ethanol. Considering the vast knowledge we have on its genome and metabolism (see below), E. coli is the primary choice for the production of biofuels. If you wanted to enhance the production of ethanol using genetic engineering, which gene(s) would you inhibit?

We have to inhibit the production of the other products, in order to enhance ethanol production. Therefore, we could inhibit PFL (formate production), LDH (lactate production) and/or PTA-ACKA (acetate production).

It is not necessary to inhibit the others, as the other compounds they produce can always be reconverted into Ethanol (displacement of the equilibrium if ethanol is collected).

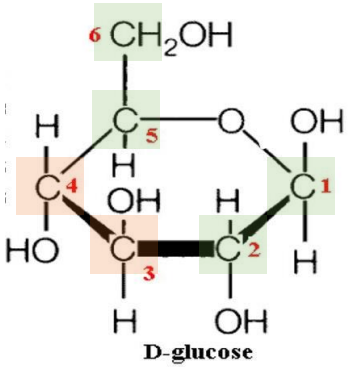


Adapted from
Bernal, Vicente, Sara Castaño-Cerezo, and Manuel Cánovas. "Acetate metabolism regulation in Escherichia coli: carbon overflow, pathogenicity, and beyond." *Applied microbiology and biotechnology* 100 (2016): 8985-9001.

Question 1

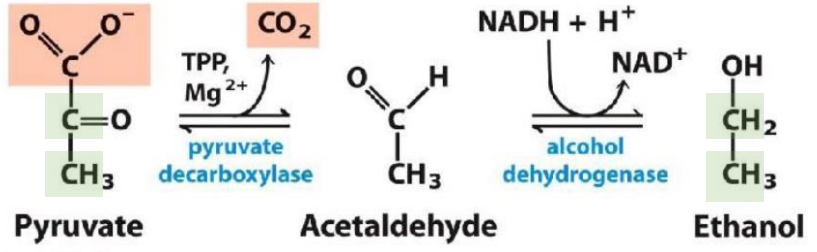
4) In order to study the efficiency of the conversion of glucose into ethanol, you decide to label the glucose with 14C. If you could label the glucose in just one position, which carbon would you choose to obtain labelled ethanol as a product?

We could choose either C1, C2, C5, C6, because they end up forming the carbon atoms of the two ethanol molecules (C1 and C6 end up on position 2 of ethanol, and C2 & C5 are the carbon in position 1, those who carry the –OH group).



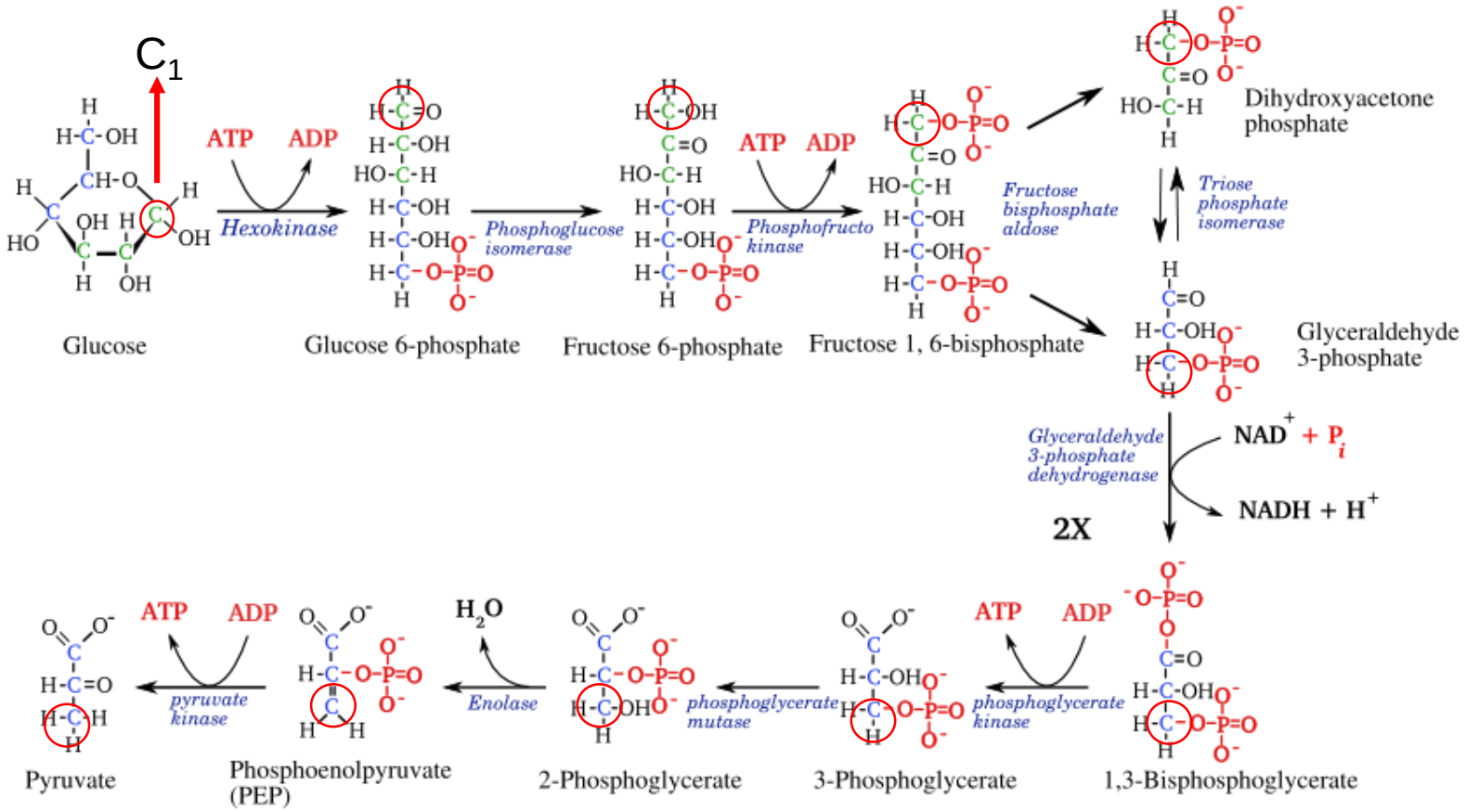
5) Why wouldn't you get any labelled ethanol if you chose position 3?

On the other hand, C3 and C4 carbon atoms of glucose correspond to the carbon atoms of the CO2 molecules that are released, so labelling them would label the CO2



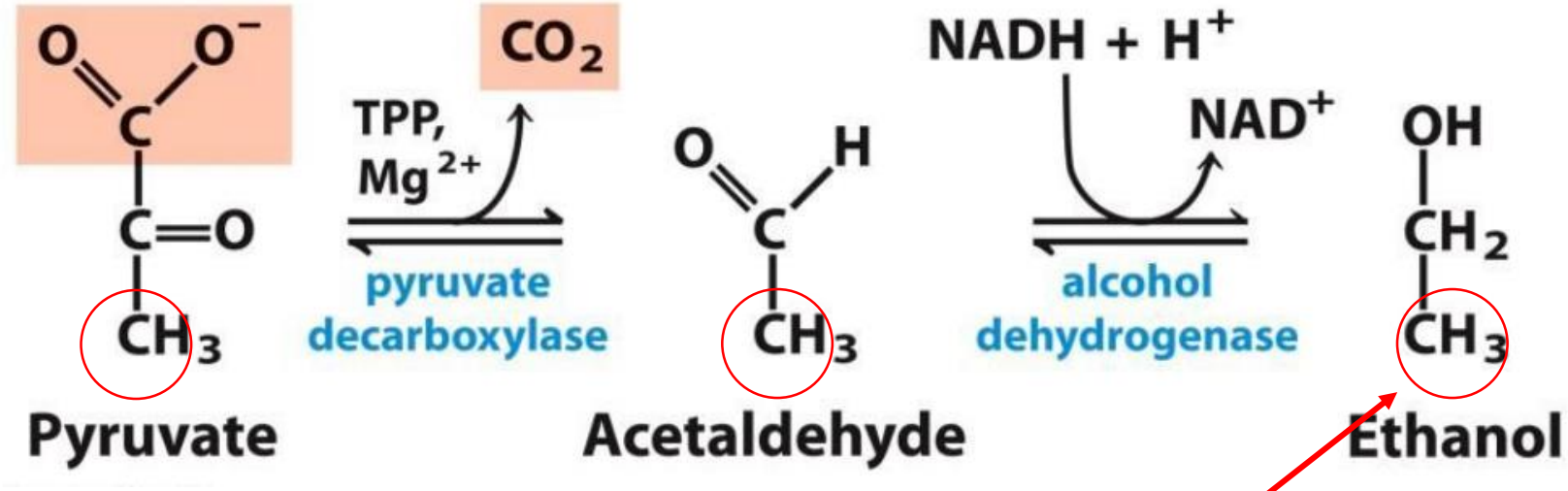
Question 1a

- Recall glycolysis, following carbon 1:



Question 1a

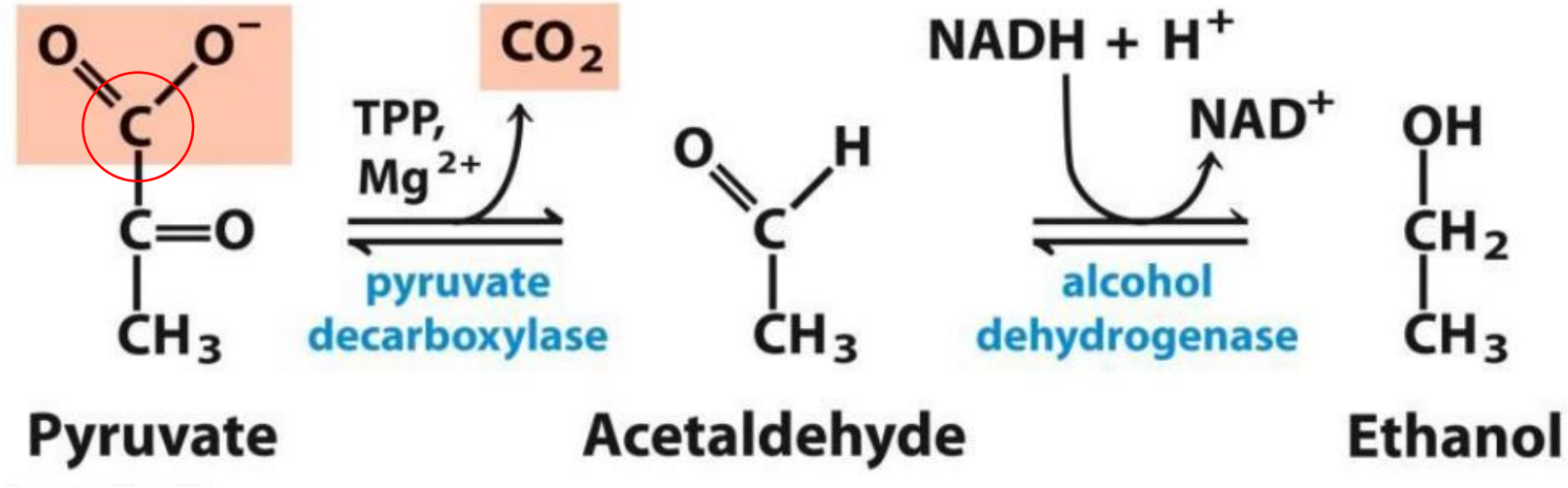
- Continuing onto fermentation:



This carbon should be labelled with ^{14}C

Question 1b

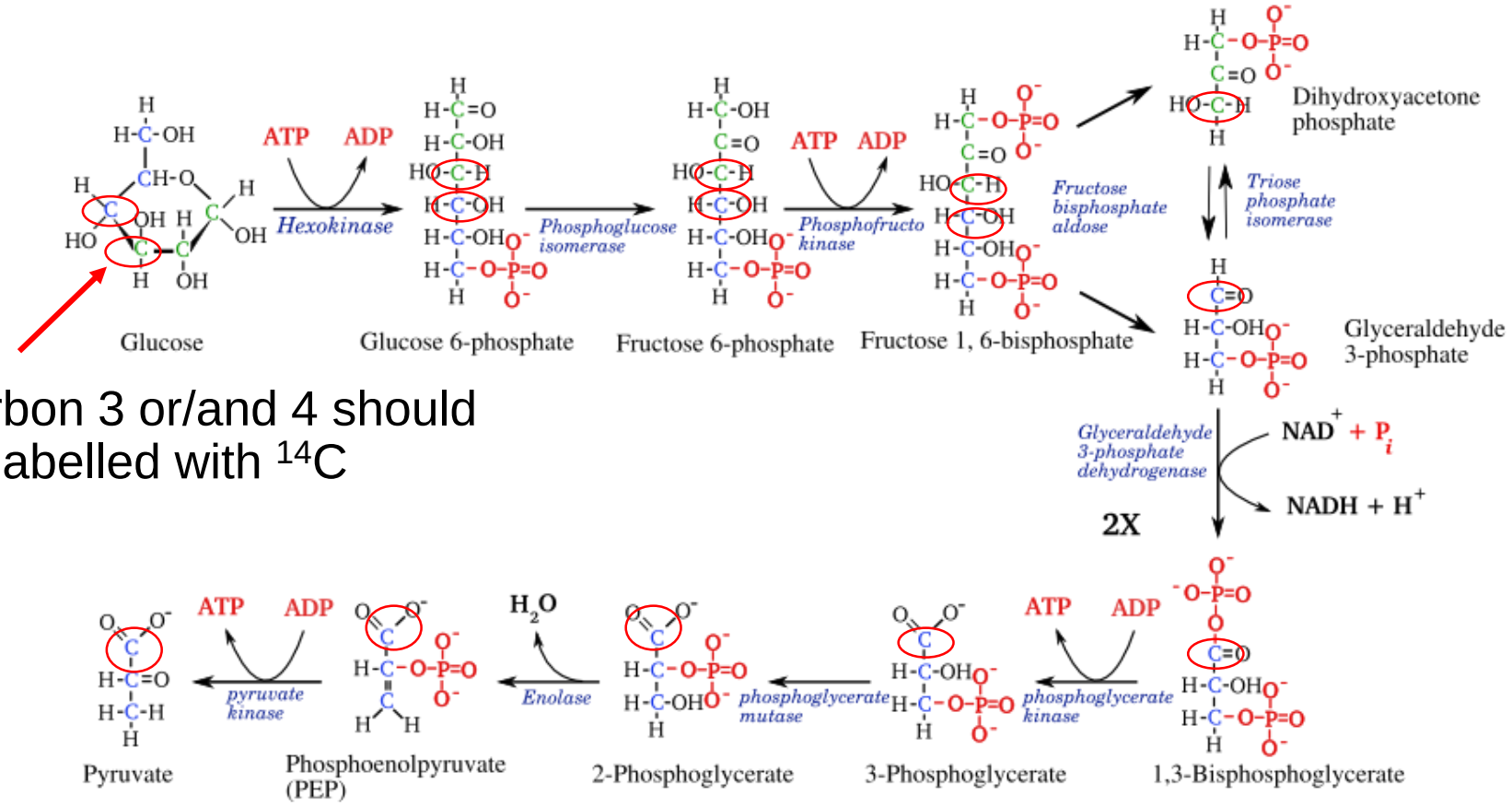
- Lets work backwards by simply circling the carbon to be liberated as CO_2 from fermentation.



- Now work backwards in glycolysis

Question 1b

- Let us work backwards from pyruvate all the way to glucose:



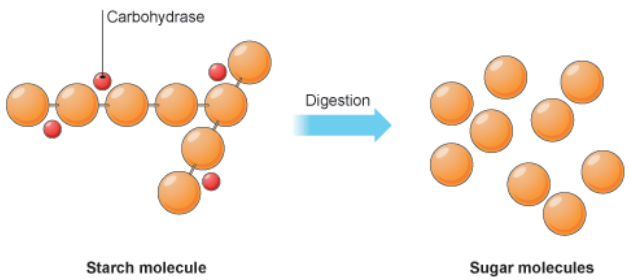
Carbon 3 or/and 4 should be labelled with ^{14}C

- If ^{14}C is liberated as CO_2, the glucose is labelled at the C_3 and or C_4 positions.

Question 2

Fermentation to Produce Soy Sauce Soy sauce is prepared by fermenting a salted mixture of soybeans and wheat with several microorganisms, including yeast, over a period of 8 to 12 months. The resulting sauce (after solids are removed) is rich in lactate and ethanol. How are these two compounds produced? To prevent the soy sauce from having a strong vinegar taste (vinegar is dilute acetic acid), oxygen must be kept out of the fermentation tank. Why?

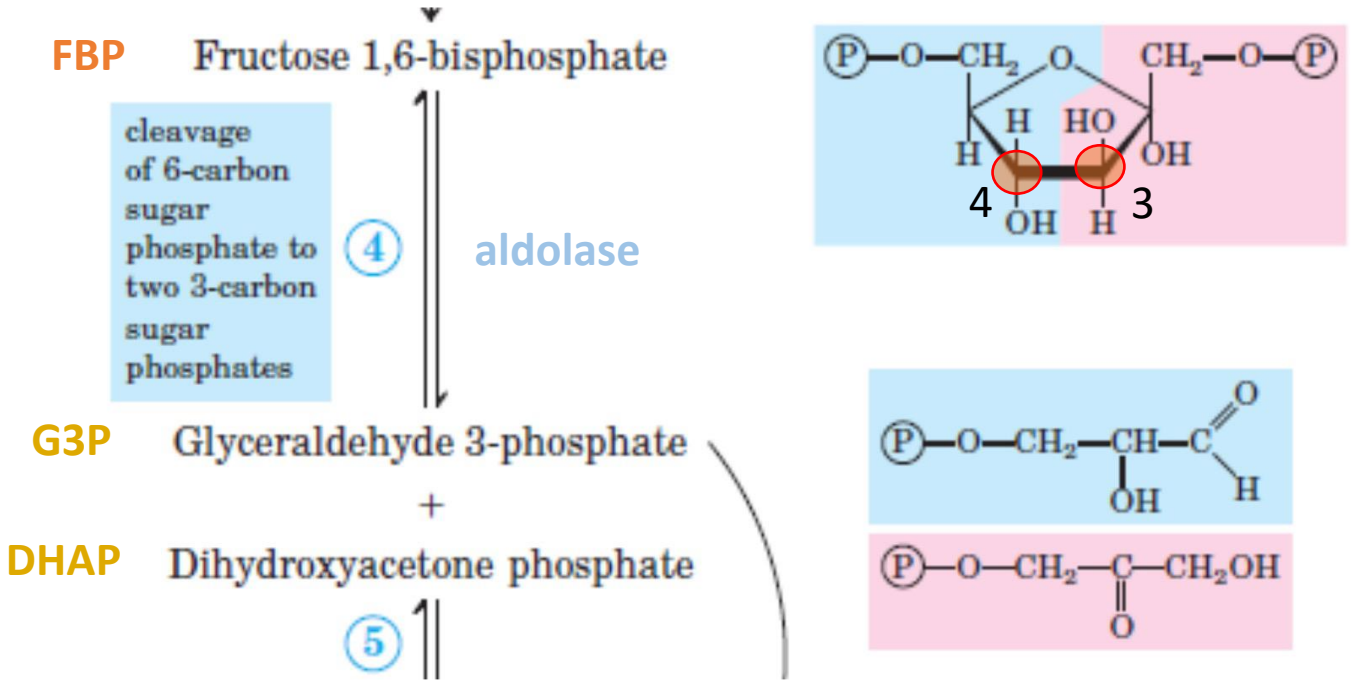
Answer Soybeans and wheat contain starch, a polymer of glucose, which is broken down to glucose by the microorganisms. The glucose is then degraded to pyruvate via glycolysis. Because the process is carried out in the absence of oxygen (i.e., it is a fermentation), pyruvate is reduced to lactic acid and ethanol. If oxygen were present, pyruvate would be oxidized to acetyl-CoA and then to CO_2 and H_2O. Some of the acetyl-CoA, however, would also be hydrolyzed to acetic acid (vinegar) in the presence of oxygen.



Question 3

FBP G3P

Equivalence of Triose Phosphates ^{14}C-Labeled glyceraldehyde 3-phosphate was added to a yeast extract. After a short time, fructose 1,6-bisphosphate labeled with ^{14}C at C-3 and C-4 was isolated. What was the location of the ^{14}C label in the starting glyceraldehyde 3-phosphate? Where did the second ^{14}C label in fructose 1,6-bisphosphate come from? Explain.



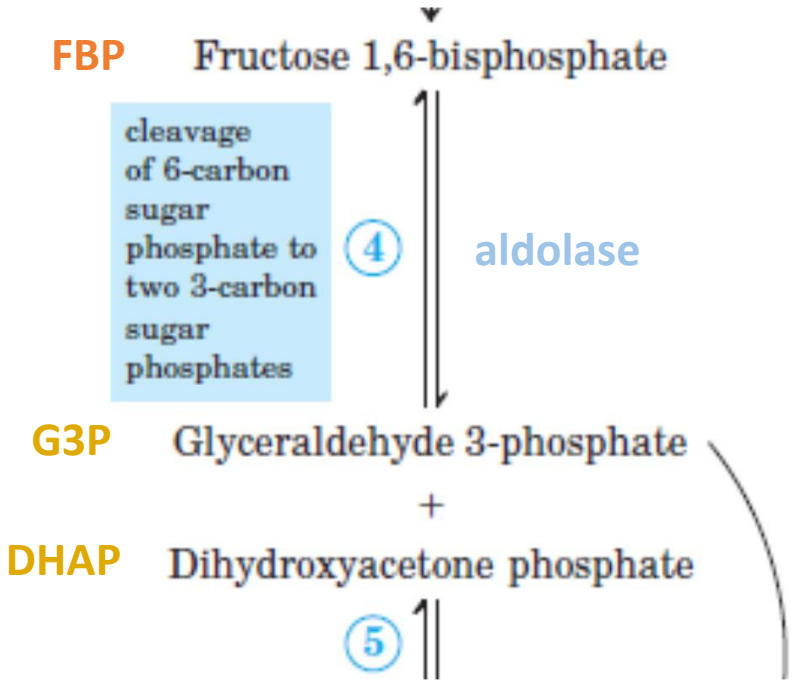
Question 3

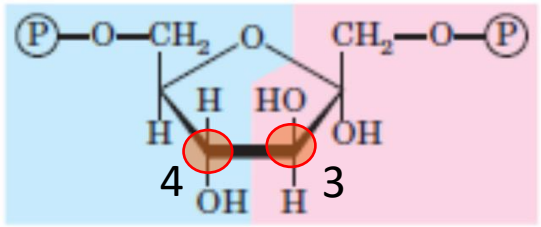
FBP **aldolase** catalysis for **DHAP** and **G3P** formation is reversible. This makes the formation of FBP possible.

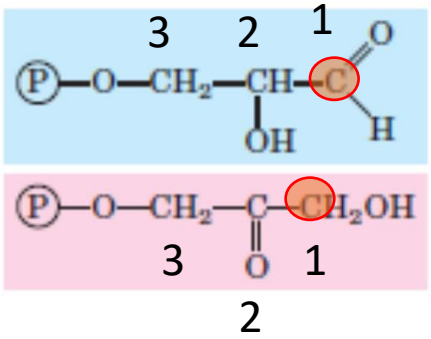
On the other side : **DHAP** and **G3P** are simply isomers (same chemical formula, different bond connections) and can interchange due to the enzyme **Triose phosphate isomerase**

Therefore , labelling the **G3P** at carbon 1, then the labelled product can isomerize and be found in corresponding **DHAP**.

Labelled **G3P** and **DHAP** may then be acted upon by aldolase to form a double labelled **FBP**.

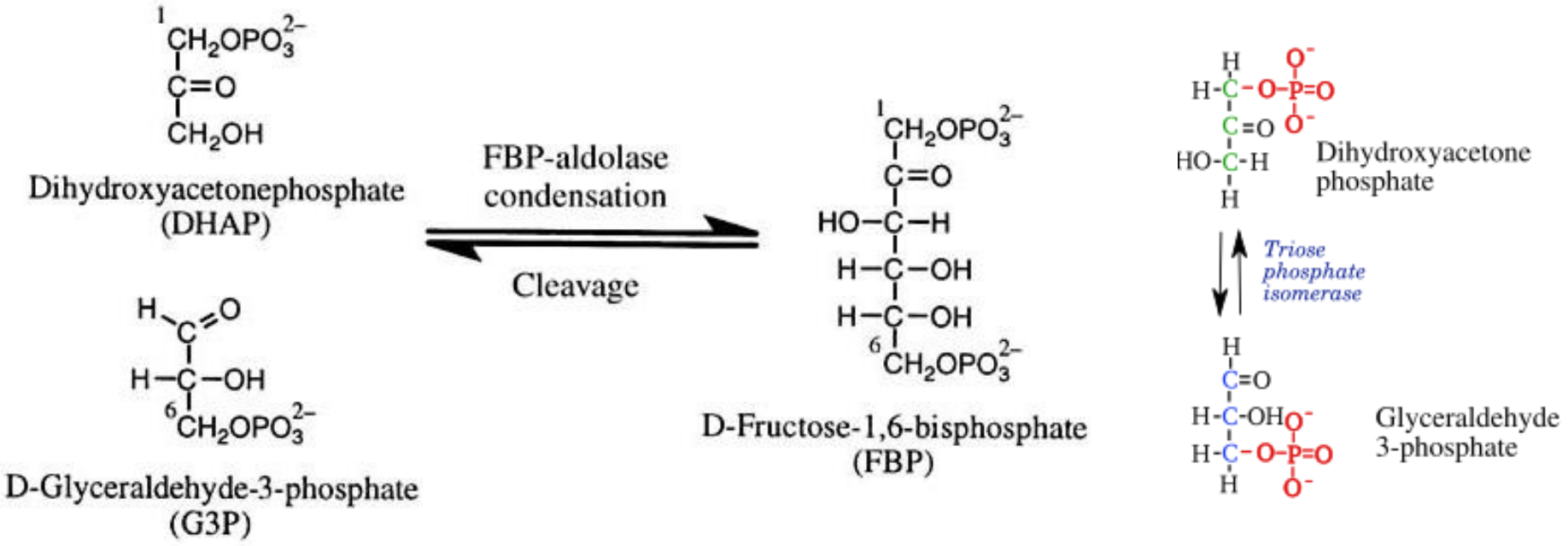






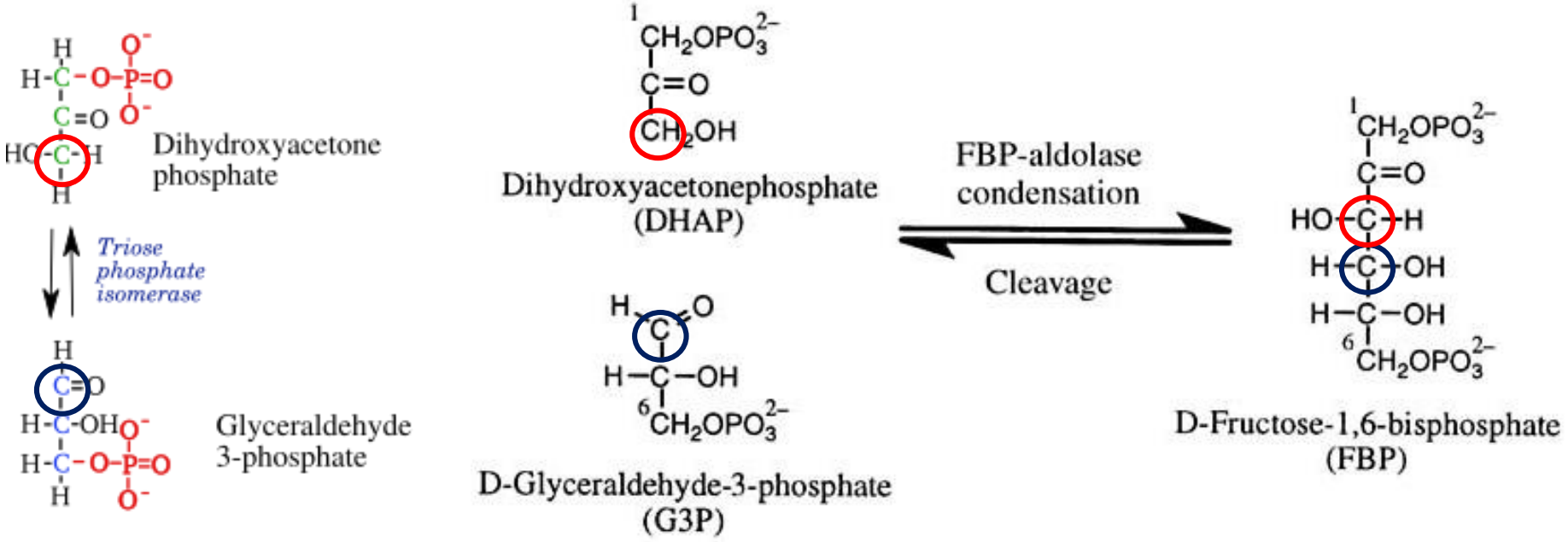
Question 3

- FBP aldolase catalysis for DHAP and G3P formation is reversible.
- This makes the formation of FBP possible.
- On the side: DHAP and G3P are simply isomers (Same chemical formula, different bond connections) and can interchange due to the enzyme Triose phosphate isomerase.



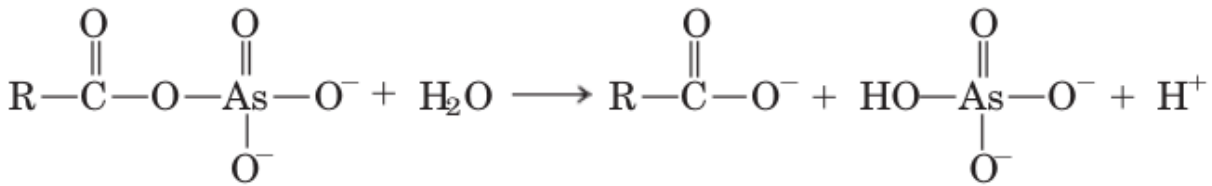
Question 3

- Therefore, labelling the G3P at any carbon with ^{14}C, then the labelled product can isomerize and be found in corresponding DHAP.
- Labelled G3P and DHAP may then be acted upon by aldolase to form a double labelled FBP.



Question 4

Arsenate Poisoning Arsenate is structurally and chemically similar to inorganic phosphate (P_i), and many enzymes that require phosphate will also use arsenate. Organic compounds of arsenate are less stable than analogous phosphate compounds, however. For example, acyl *arsenates* decompose rapidly by hydrolysis:



On the other hand, acyl *phosphates,* such as 1,3-bisphosphoglycerate, are more stable and undergo further enzyme-catalyzed transformation in cells.

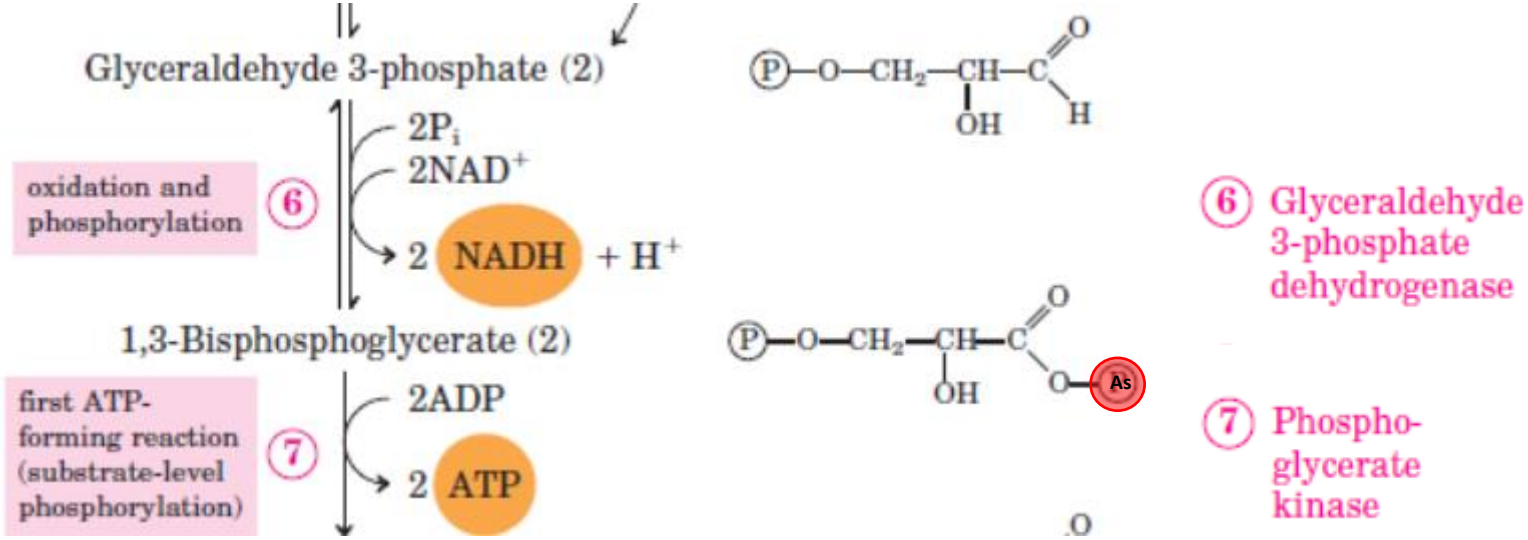
(a) Predict the effect on the net reaction catalyzed by glyceraldehyde 3-phosphate dehydrogenase if phosphate were replaced by arsenate.

(b) What would be the consequence to an organism if arsenate were substituted for phosphate? Arsenate is very toxic to most organisms. Explain why.

Question 4

Answer

(a) In the presence of arsenate, the product of the glyceraldehyde 3-phosphate dehydrogenase reaction is 1-arseno-3-phosphoglycerate, which nonenzymatically decomposes to 3-phosphoglycerate and arsenate; the substrate for the phosphoglycerate kinase is therefore bypassed.



Question 4

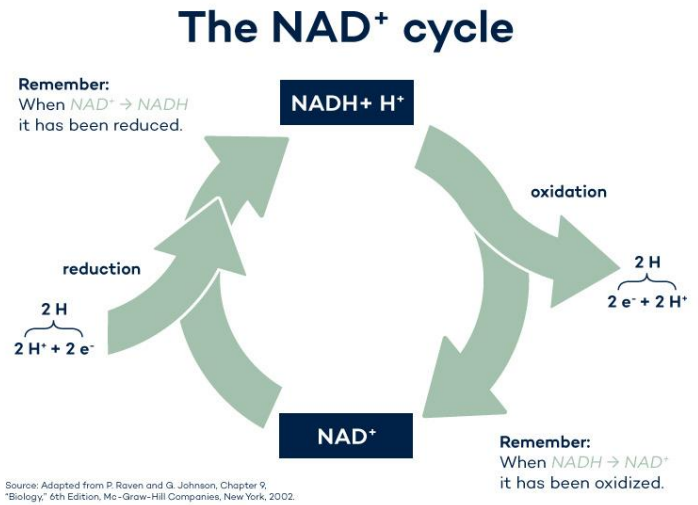
(b) No ATP can be formed in the presence of arsenate because 1,3-bisphosphoglycerate is not formed. Under anaerobic conditions, this would result in no net glycolytic synthesis of ATP. Arsenate poisoning can be used as a test for the presence of an acyl phosphate intermediate in a reaction pathway.

Question 5

Role of the Vitamin Niacin Adults engaged in strenuous physical activity require an intake of about 160 g of carbohydrate daily but only about 20 mg of niacin for optimal nutrition. Given the role of niacin in glycolysis, how do you explain the observation?

Answer Dietary niacin is used to synthesize NAD^+. Oxidations carried out by NAD^+ are part of cyclic oxidation-reduction processes, with NAD^+/NADH as an electron carrier. Because of this cycling, one molecule of NAD^+ can oxidize many thousands of molecules of glucose, and thus the dietary requirement for the precursor vitamin (niacin) is relatively small.

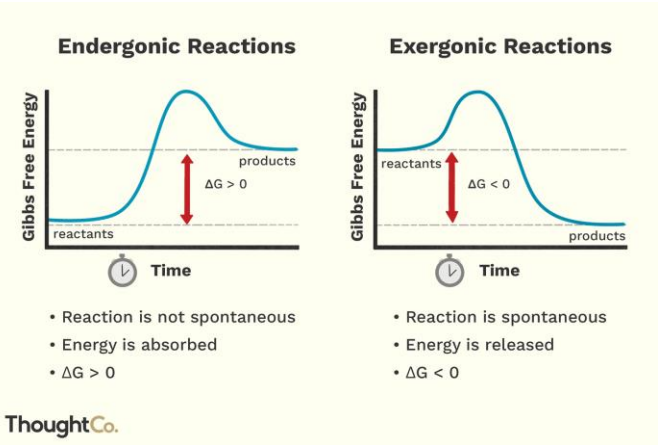
Niacin, aka Vitamin B3, a precursor of NAD+



Question 6

Free-Energy Change for Triose Phosphate Oxidation The oxidation of glyceraldehyde 3-phosphate to 1,3-bisphosphoglycerate, catalyzed by glyceraldehyde 3-phosphate dehydrogenase, proceeds with an unfavorable equilibrium constant ($K'_{eq} = 0.08$; $\Delta G'° = 6.3$ kJ/mol), yet the flow through this point in the glycolytic pathway proceeds smoothly. How does the cell overcome the unfavorable equilibrium?

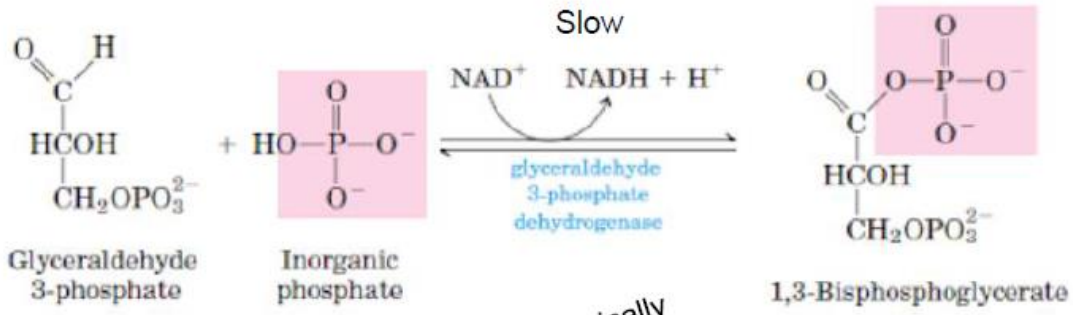
Answer In organisms, where directional flow in a pathway is required, exergonic reactions are coupled to endergonic reactions to overcome unfavorable free-energy changes. The endergonic glyceraldehyde 3-phosphate dehydrogenase reaction is followed by the phosphoglycerate kinase reaction, which rapidly removes the product of the former reaction. Consequently, the dehydrogenase reaction does not reach equilibrium and its unfavorable free-energy change is thus circumvented. The net $\Delta G'°$ of the two reactions, when coupled, is -18.5 kJ/mol + 6.3 kJ/mol = -12.2 kJ/mol.



Question 6

Fast utilization of 1,3 biphosphoglycerate lead to equilibrium shift towards the unfavourable formation of 1,3 biphosphoglycerate.

6

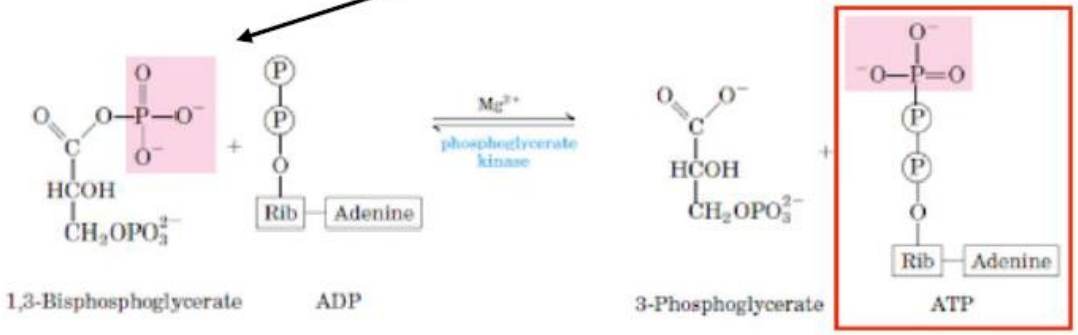


$\Delta G'^{\circ}$ = +6.3 kJ/mol

Fast, thermaldynamically favourable

Product is quickly cleaved by PGK, driving equilibrium towards the 1,3-Bisphosphoglycerate

7



$\Delta G'^{\circ}$ = -18.5 kJ/mol

Exam question

Studying the mechanism of catalysis of the glycolytic enzyme triose phosphate isomerase (TPI) that mediates the conversion between DHAP and G3AP, in 1972, Barbara Plaut and her colleagues discovered that the activity of the enzyme decreased both when incubated in a high or a low pH solution before acting on its substrates (see Fig. 1).

a) Looking at the known molecular mechanism of catalysis of TPI how would you interpret the effect observed by Plaut and colleagues?

The pH profile obtained suggests that maximum catalytic activity occurs around a neutral pH (around 7). This could be due to the following : the amino acids present at the active site of the enzyme need to be in a **specific ionization state** in order to make the enzyme catalytically active. A drastic change in pH could lead to a **change in ionization** state of aa and therefore in a **decreased enzymatic activity**.

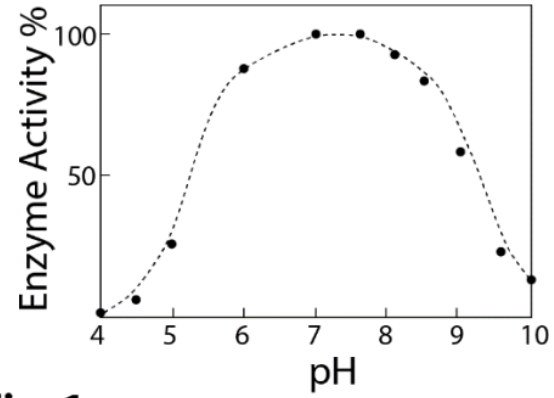


Fig. 1

Exam Question

b) In a second experiment, Plaut and colleagues provided TIP with a 3H (tritium)-labelled version of DHAP at position [C1] (Fig .2A**). After the treatment with TIP, to what carbon atom(s) would you expect the 3H to be bound?**

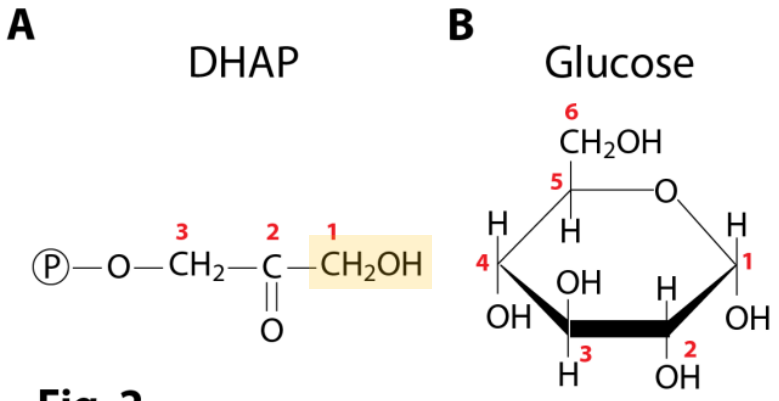
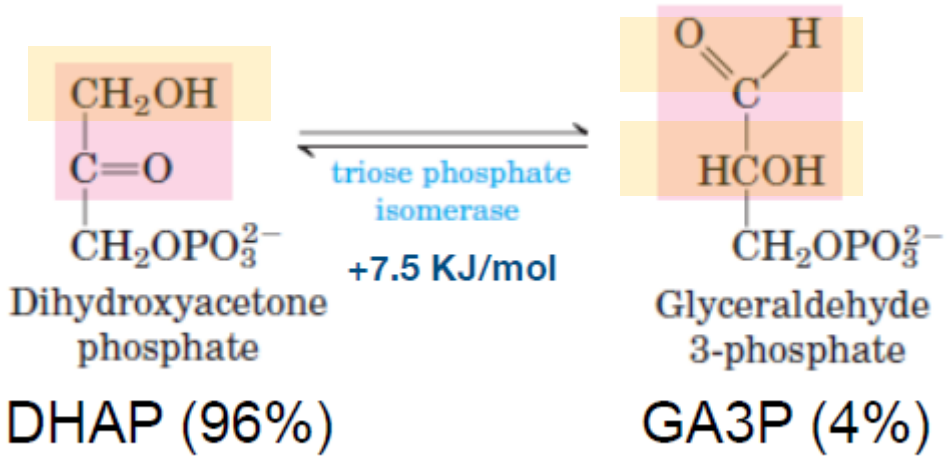


Fig. 2

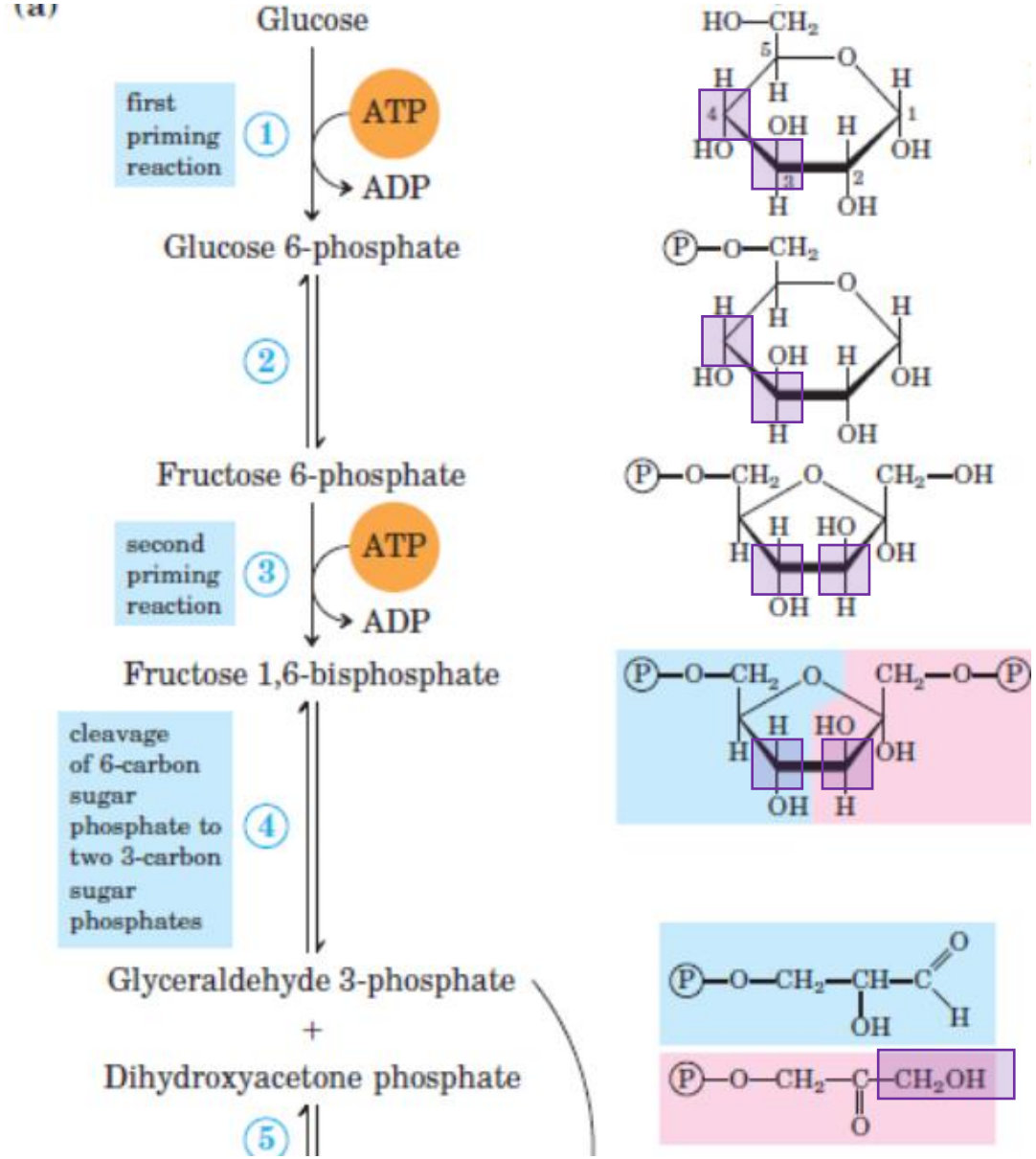
"TPI catalyses the conversion of the ketone DHAP to the aldehyde (GA3P) via an intramolecular redox reaction in which a hydrogen is transferred from C1 to C2"



Exam Question

If you were to label Glucose (Fig. 2B) to replicate this experiment in live cells, what carbon atom(s) of Glucose would you need to label? Articulate the reason for your answers.

We would need to label carbons 3 and 4 of glucose. Not only carbon 3 because DHAP and G3P can interchange due to TIP; so a G3P labelled C1 could change into a DHAP also labelled at C1.



Exam Question

c) When performed in laboratory conditions in a tube, the metabolic reaction in which TPI is involved is reversible, with the equilibrium significantly shifted to what we could consider the “undesired” compound. How come our cells don’t accumulate this “useless” form then? Why do cells treated with arsenate accumulate this “useless” product?

In live cells, there is no accumulation because fast utilization G3P leads to an equilibrium shift (similar to what is explained in question 6). In laboratory conditions in a tube, this doesn’t occur as only this specific reaction is being reproduced, not the whole glycolysis.

However, in cells poisoned with arsenate, this accumulation does occur. In this case, arsenate replaces the Phosphate added in step 6, and the substrate for the following step is no longer produced, blocking the glycolysis flow.