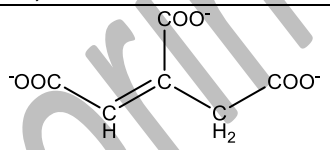
Biochemistry Sample Assignment Solution

Question 1

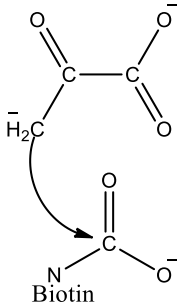
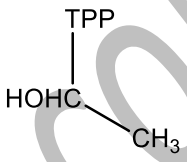
Create a table to compare and contrast the chemical reactions catalyzed by the following enzymes as well as their mechanisms of action. Your answer should include at least 5 of the following where appropriate: the stereochemistry of the product, the stereochemistry of the reaction, the structures of the intermediates, involvement of the amino acid side chains in the mechanism (how are they involved? What chemistry takes place? What intermediates are formed?), special features of the mechanism, involvement of coenzymes and other nonenzyme components, role of induced fit and other enzyme conformational changes, regulation, thermodynamics. You may include other facts as well. Your answer should be 1–2 pages.

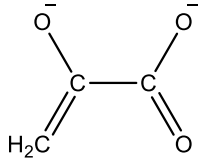
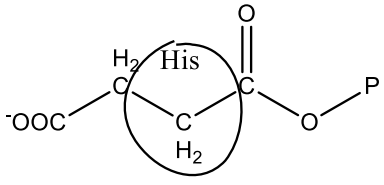
a) aconitase and succinate dehydrogenase
b) pyruvate carboxylase and pyruvate dehydrogenase
c) PEP carboxykinase and succinyl-CoA synthetase
d) fructose bisphosphate aldolase and citrate synthase
e) triose phosphate isomerase and aconitase

Answer:

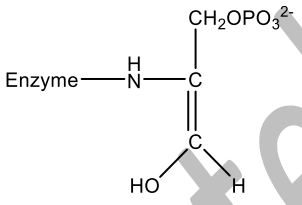
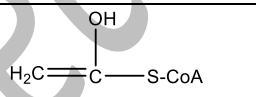
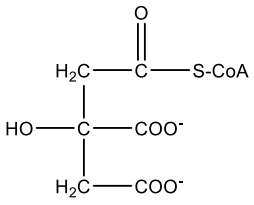
Name of Enzyme	Aconitase	Succinate Dehydrogenase
Reaction Catalysed	Citrate ⇆ Isocitrate Catalyse an aldol condensation reaction	Succinate +FAD^+ ⇆ Fumarate + FADH
Stereochemistry of the reaction/ product	Out of four equivalent hydrogens, only one pro-R H atom of the pro-R arm is removed, 2R, 3S-Isocitrate formed from citrate.	Dehydrogenation is very stereospecific. Pro-R hydrogen removed removed from one carbon atom and pro-S hydrogen removed from the other.
Structure of the intermediates	 cis-Aconotate	
Involvement of coenzymes and other nonenzyme components	Iron sulphur (Fe-S protein) complex	Three different iron-sulfur clusters and one molecule of covalently bound FAD along with enzyme.
Thermodynamics	$\Delta G'^0$ = 13.3 kJ/mol	$\Delta G'^0$ = 0 kJ/mol
Regulation		Regulated by malate, oxaloacetate and malonate, an competitive inhibitor of the succinate dehydrogenase
Involvement of the amino acid side chains in the mechanism (Active site)	Contains an iron sulphur centre, which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H_2O. Three Cystine residues of the enzyme	Threonine-254, Histidine-354, Arginine-399 stabilize the substrate with hydrogen bonding, while FAD removes the electrons and carries them to the first iron-sulfur cluster.

	bind three iron atoms; the fourth iron is bound to one of the carboxyl groups of citrate and also interacts non covalently with a hydroxyl group of citrate	

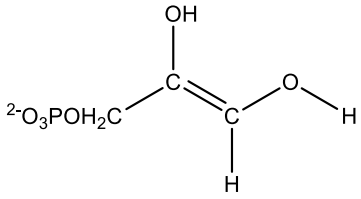
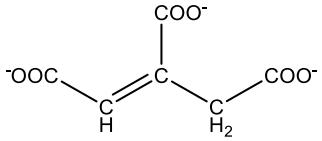
Name of Enzyme	Pyruvate carboxylase	Pyruvate dehydrogenase
Reaction Catalysed	Pyruvate + ATP + CO_2→ Oxaloacetate + ADP + Pi	Pyruvate + NAD^++ CoASH → Acetyl CoA + NADH + CO_2
Structure of the intermediates		
Involvement of coenzymes and other nonenzyme components	Mg^+, Biotin, HCO^{3-}, Mn^+, Acetyl CoA	TPP, FAD, CoA, NAD and lipoate. Four vitamins required in human nutrition are vital components of this system: thiamine (in TPP), riboflavin (in FAD), niacin (in NAD), and pantothenate
Thermodynamics	$\Delta G'^0$ = kJ/mol	$\Delta G'^0$ = -33.4 kJ/mol
Regulation	Acetyl CoA is a positive regulator	Activated by dephosphorylation. Inactivated by the phosphorylation and end products such as acetyl CoA and NADH.
Involvement of the amino acid side chains in the mechanism (Active site)	The cofactor biotin is covalently attached to the enzyme through an amide linkage to the amino group of a Lys residue, forming a biotinyl-enzyme.	PDH complex contains multiple copies of three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Lysine amino acid of E2 actively participate in the reactions.

Name of Enzyme	Phosphoenolpyruvate carboxykinase	Succinyl-coA synthetase
Reaction Catalysed	Oxaloacetate + GTP →Phosphoenopyruvate + CO2 + GDP A lyase catalysed reaction	Succinyl CoA + GDP + Pi ⇆ Succinate + CoASH + GTP Substrate level phosphorylation
Structure of the intermediates	 Pyruvate enolate anion intermediate	
Involvement of coenzymes and	GTP, Mn^{2+} and Mg^{2+}	GDP

other nonenzyme components		
Thermodynamics	$\Delta G'^0$ = kJ/mol	$\Delta G'^0$ = -2.9 kJ/mol
Regulation	Stimulated by glucagon, glucocorticoids, retinoic acid, and cAMP, while it is inhibited by insulin.	Regulated by ATP and NADH level
Involvement of the amino acid side chains in the mechanism (Active site)	Hyper-reactive cysteine-307 is involved in the binding of Mn^{2+} to the active site.	Histidine-246

Name of Enzyme	**Fructose bisphosphate aldolase**	**Citrate synthase**
Reaction Catalysed	β-D-fructose-1,6-bisphosphate ⇆ Dihydroxyacetone phosphate + Glyceraldehyde-3-phosphate A reversible aldol condensation	Oxaloacetate + Acetyl CoA +H2O ⇆ Citrate + CoASH A reversible aldol condensation
Stereochemistry of the reaction/ product	Stereospecific formation of 2R intermediate	only the S-isomer of the product forms
Structure of the intermediates	 Enamine intermediate	 Enol form of Acetyl Co-A  Citroyl-CoA
Thermodynamics	$\Delta G'^0$ = 23.8 kJ/mol	$\Delta G'^0$ = -32.2 kJ/mol
Regulation		Inhibited by high concentrations of ATP, acetyl-CoA, succinyl CoA and NADH
Involvement of the amino acid side chains in the mechanism (Active site)	Amino group of lysine act as a nucleophile on carbonyl carbon and glutamic and aspartic acid acting as a proton donor and acceptor in its mechanism	Catalytic triad formed by Aspartate (375) and Histidine (274 and 320). Negatively charged oxygen atom of carboxylate side chain of Asp-375, deprotonates the acetyl CoA's alpha carbon atom to form an enolate anion which in turn neutralized through protonation by His-274 to form an enol intermediate

Name of Enzyme	**Triose phosphate isomerase**	**Aconitase**

Reaction Catalysed	Dihydroxyacetone-P ⇆ glyceraldehyde-3-P (GAP) Reversible keto-enol isomerization through cis-enediol intermediate	Citrate ⇆ Isocitrate Catalyse an aldol condensation reaction
Stereochemistry of the reaction/ product		Out of four chemically equivalent hydrogens, only one pro-R H atom from pro-R arm is removed.
Structure of the intermediates	 Enediol	 cis-Aconotate
Thermodynamics	$\Delta G'^{0}$ = 7.5 kJ/mol	$\Delta G'^{0}$ = 13.3 kJ/mol
Regulation	Not regulated directly, however it is regulated via the two step previous enzyme catalysed reaction i.e., phosphofructokinase	
Involvement of the amino acid side chains in the mechanism (Active site)	Histidine -95 and glutamic acid -165 plays important role for this isomerization and act as proton donor and acceptor in its mechanism	Contains an iron sulphur centre, which acts both in the binding of the substrate at the active site and in the catalytic addition or removal of H_2O. 3 cystine residues of the enzyme attaches with three iron atoms; the fourth iron is attaches to one of the carboxyl groups of citrate and also interacts non covalently with a –OH group of citrate

Question 2

Part a) In the conversion of pyruvate to acetyl-CoA, a thioester bond is first formed with the lipoamide moiety, and then a transesterification reaction forms a second thioester bonded to acetyl-CoA. Since there is simply a swap of esters in the second step (which is called a transesterification reaction), could this reaction take place without the lipoamide coenzyme? Be sure to use chemical structures to support your reasoning.

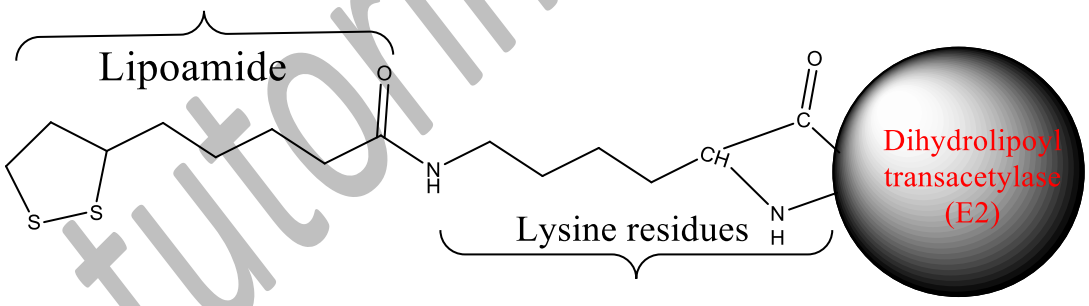
Part b) Would it be possible for the transformation of isocitrate to α-ketoglutarate to occur if the protein was engineered to contain FAD instead of the NAD^+ coenzyme? Ignore any steric effects based on coenzyme size. Explain your reasoning within the context of the course text, notes, and discussions.

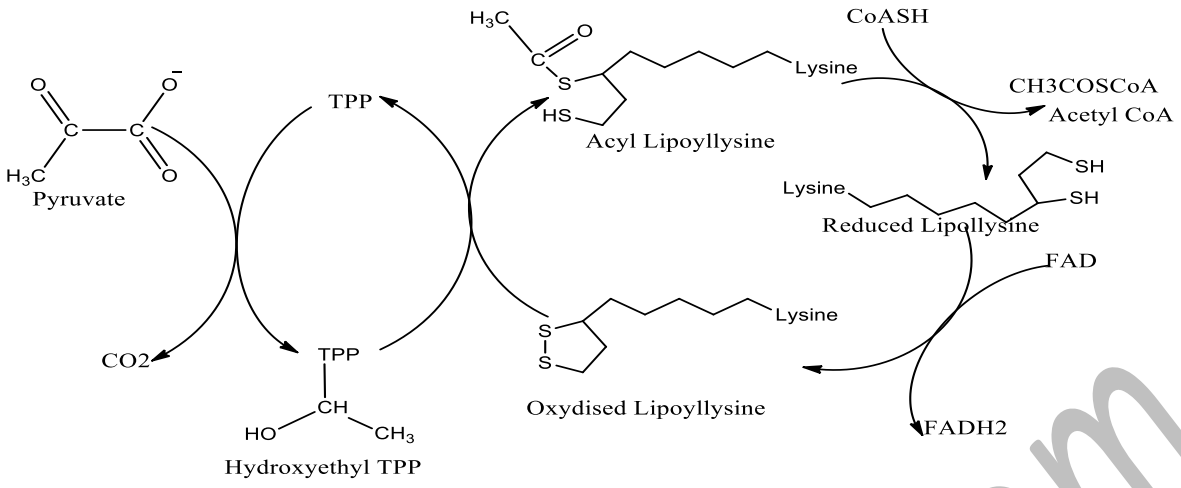
Part c) Do you think that phosphofructokinase could retain catalytic function if the Mg ion were replaced with Zn. Why or why not?

Part d) Ignoring any steric effects, do you think that glyceraldehyde-3-phosphate dehydrogenase could retain catalytic activity if the active site C were mutated to S?

Answer:

(a) Pyruvate dehydrogenase (PDH) complex contains multiple copies of three enzymes—pyruvate dehydrogenase (E1), dihydrolipoyl transacetylase (E2), and dihydrolipoyl dehydrogenase (E3). Lysine amino acid of E2 actively participate in the reactions. The pyruvate dehydrogenase requires five cofactors namely thiamine pyrophosphate (TPP), flavin adenine dinucleotide (FAD), coenzyme A, nicotinamide adenine dinucleotide (NAD), and lipoamide. Lipoamide attached to the dihydrolipoyl transacetylase (E2) via its lysine residue. This attachment helps to form a flexible long arm and acts as tethers which helps in the movement of intermediates from the active site one enzymatic component to the active site of another enzymatic components. In the absence of the lipoamide, intermediates can't move efficiently to the active site and the reaction will be halted.



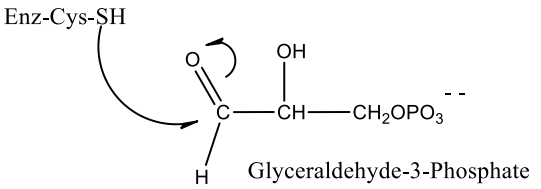


Mechanism of action of Pyruvate Dehydrogenase (PDH) for the formation of Acetyl CoA

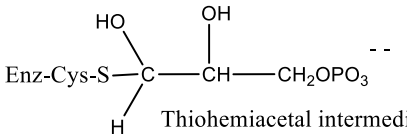
(b) Isocitrate is oxidatively decarboxylated to α-ketoglutarate, along with the reduction of NAD^+ to NADH by the isocitrate dehydrogenase enzyme. The reaction has a net $\Delta G^{o'}$ 8.4 kJ/mol, which is sufficient to reduce the NAD^+ to NADH. The reduction of FAD to FADH requires less energy compared to NAD^+ to NADH reduction. So, engineering of protein or enzyme by FAD instead of NAD^+ will also leads to conversion of isocitrate to α-ketoglutarate (Garrett and Grisham, 2010).

(c) Magnesium ion is required for the stability of ATP. Phosphofructokinase binds both Mg^{2+}ATP and fructose-6-phosphate (F6P) to make fructose-1,6-bisphosphate and Mg^{2+}ADP. In this reaction Mg^{2+} plays dual role as an enzyme modulator and also an activator of substrate. In this reaction ATP helps in the formation of the enzyme-Mg^{2+} complex which modulated the interaction between fructose 6-phosphate and the enzyme. So, replacement of Mg with Zn will not allow the reaction to proceed (Surendranathan, 1992; Evans et al., 1981).

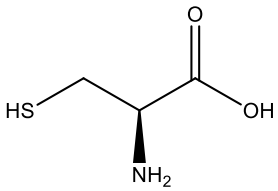
(d) If the active site of glyceraldehydes-3-phospate dehydrogenase enzyme is mutated from cysteine (C) to serine (S) then there would be a difference on beta carbon of amino acid, as cysteine have –SH group whereas serine has –OH group on that carbon. The lone pair of electrons of SH group of cysteine attacks on the carbonyl carbon of glyceraldehydes-3-phospate during the reaction catalyzed by this enzyme. This forms a thiohemiacetal intermediate as shown in following structure. As the oxygen belongs to same class in periodic table where S exist, so similar chemical behavior is expected. But oxygen is one of the most electronegative elements of periodic table, so it is possible that the –OH of serine and carbonyl oxygen will form a hydrogen bond. In this case the catalytic reaction would not take place.



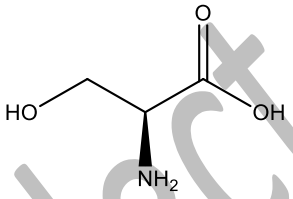
Glyceraldehyde-3-Phosphate



Thiohemiacetal intermediate



Cystein



Serine

References:

1. Nelson, D.L. and Cox, M.M., Lehninger Principles of Biochemistry 5th Ed, 2008, W.H. Freeman and Company, New York, Chapter 16 (pp. 601-30) and 19 (pp. 690-750).
2. Garrett, R.H. and Grishm, C.M., Biochemistry 4th Ed, 2010, Brooks/Cole, Cengage Learning, Canada. Chapter 18-20 (pp. 535-629).
3. Victor, R., David, B., Kathleen, M. B., Peter, J. K., Anthony, W, P. (2003). Harpers Illustrated Biochemistry: 26th edition, McGraw-Hill Education Medical; Chapter 12 (pp. 92-101) and Chapter 15-20 (pp. 122-172).
4. Evans PR, Farrants GW, Hudson PJ. Phosphofructokinase: structure and control. Philos Trans R Soc Lond B Biol Sci. 1981; 293(1063):53-62.
5. Surendranathan K.K., Iyer, M.G., Nair P.M. Mechanism of action of a dimeric phosphofructokinase from banana: role of magnesium on its kinetics and regulation. Plant Science. 1992; 81(1): 29-36.

Question 3

(Part A) For each of the following situations, predict the major consequences for the TCA cycle. Explain your logic in a few sentences. Also, predict whether the level of succinyl CoA would be higher or lower than normal (for the purpose of this question, ignore any other anabolic or catabolic pathways succinyl CoA could participate in). Include a few sentences to support your reasoning:

(i) inhibition of ATP synthetase
(ii) lack of oxygen in the tissue
(iii) an abundance of ATP in the cell
(iv) mutation of the active site H → W in succinyl-CoA synthetase

(Part B) For each of the following situations, predict the major consequences for glycogen metabolism. Explain your logic in a few sentences. Consider the effect on both glycogen phosphorylase and glycogen synthase. Will the amount of glycogen be higher or lower than normal?

(i) an increase in epinephrine in the blood
(ii) loss of a gene encoding UDP–glucose pyrophosphorylase
(iii) loss of the gene encoding fructose-2,6-bisphosphatase
(iv) mutation of S-116 → T in phosphoglucomutase

Answer (Part A):

(i) Inhibition of ATP Synthase:

ATP synthase is an enzyme complex responsible for ATP generation through oxidative phosphorylation via electron transport chain (ETC). TCA cycle and ETC are coordinately regulated. If ATP synthase get inhibited then ETC will also ceases due to high potential gradient generation which ultimately blocks the proton pumping in intermembrane space from matrix of mitochondria. If ETC blocks, TCA cycle will also stop working because there is no consumption of NADH and FADH2 through ETC for ATP synthesis and ultimately non availability of NAD^+ and FAD for running TCA cycle. Succinyl Co A is an intermediate of TCA cycle, which is synthesized by α-ketogluterate and generate NADH. So, the level of succinyl Co A would be lower than normal because succinyl Co A synthesis will also lower with TCA cycle.

(ii) Lack of oxygen in tissue

Oxygen is an ultimate acceptor of electrons in ETC which accept electrons from NADH and $FADH_2$, coming from TCA cycle and generate ATP to fulfil energy requirement of cells in tissue. If there is lack of oxygen in tissue ETC could not work properly because electron transfer from NADH and FADH2 to oxygen is not possible, then NAD^+ and FAD cannot be regenerated and in lack of NAD^+ and FAD, TCA cycle would not work. In this case succinyl Co A would be lower than normal as TCA cycle is not working or less working.

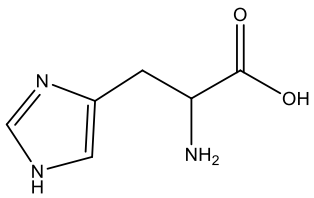
(iii) An abundance of ATP in the cells

If there is an abundance of ATP in cell that means energy requirement of cell is less as well as the concentration of available ADP which is a substrate for phosphorylation would also less. Thus, oxidative phosphorylation through ETC would not work or less work in lack of ADP. If ETC stops or slow down, TCA would also stop or slow down as these cycles are tightly coordinated (because NAD^+ and FAD would not

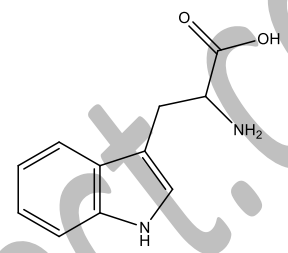
be available to run TCA). In this case succinyl Co A would be lower than normal as TCA cycle is not working or less working.

(iv) Mutation of active site H → W in succinyl-Co A synthetase

Succinyl–Co A synthetase have histidine (H) on its active site but if this amino acid is mutated by tryptophan (W) then nitrogen with free lone pair of histidine would not be available with tryptophan that is very important for its catalytic activity. Histidine's imidazole ring has two nitrogen in which one nitrogen have free lone pair (not involved in ring resonance) which attacks on the phosphate group of succinyl phosphate during reaction. This will not be possible if active site has tryptophan (W) because its indole ring nitrogen not has free lone pair of electrons to attack on phosphorus of succinyl phosphate. Thus succinyl-Co A could not be converted into succinate and GTP would not form. As this conversion does not takes place the level of succinyl Co A would be higher in cell compared to the normal.



Histidine (H)

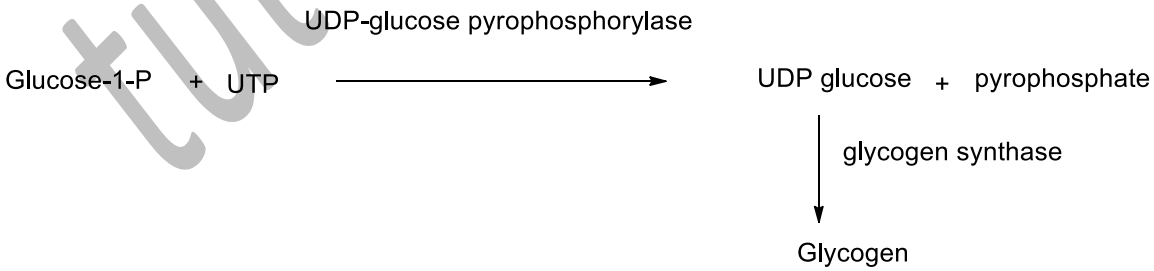


Tryptophan (W)

Answer (part B):

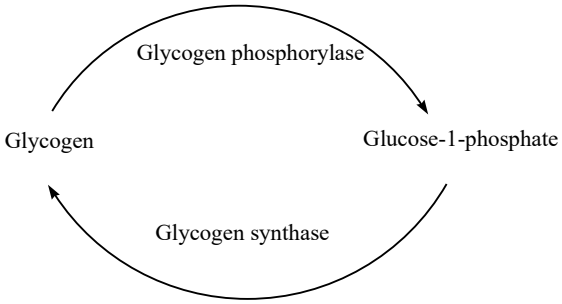
(i) Epinephrine exerts glycogenolytic effect, which increases the glycogen breakdown for production of the energy. So, an increase in epinephrine in the blood activates the glycogen phosphorylase to break the glycogen into glucose-1P and inhibit the glycogen synthase to stop the formation of glycogen from glucose. Increasing the epinephrine concentration in blood will cause to decrease in glycogen concentration.

(ii) The biosynthesis of glycogen is catalyzed by UDP-glucose pyrophosphorylase through transformation of glucose-1-phosphate and uridine 5-triphosphate (UTP) to UDP glucose. Subsequently UDP glucose is converted to glycogen-by-glycogen synthase enzyme.



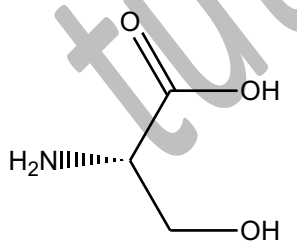
Loss of a gene encoding for UDP-glucose pyrophosphorylase will result in inhibition of transformation of glucose-1-P into UDP glucose. Which lead to down-regulation of glycogen synthase and up-regulation of glycogen phosphorylase enzyme. It also leads to decreased level of glycogen.

(iii) Glycogen is synthesized from glucose and the reaction is catalysed by glycogen synthase whereas it is degraded by glycogen phosphorylase and gives glucose-1-phosphate.

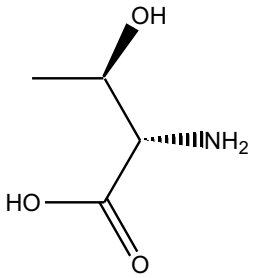


Fructose 2,6 BPase (FBPase-2) and phosphofructokinase-2 (PFK-2) are part of the same polypeptide chain, and both are regulated, in a reciprocal fashion by various factors. If there is a loss of the gene encoding Fructose 2,6 BPase, then there is no dephosphorylation of Fructose-2,6-bisphosphate as a result its concentration will increase. This fructose 2,6-bisphosphate, an allosteric activator for the enzyme's phosphofructokinase 1(PFK-1) and suppressor for the enzyme fructose 1,6-bisphosphatase (FBPase-1). Fructose-6-phosphate is converted to Fructose-1,6-bisphosphate by the enzyme PFK-1, which works as a substrate for glycolysis. Whereas the reverse reaction is catalysed by FBPase-1. Fructose 2,6-bisphosphate levels in the liver include increased glycogen storage through indirectly activating glycogen synthase and increase synthesis of glycogen down.

(iv) Phosphoglucomutase is usually converts glucose-1P to glucose-6P, which is the substrate for glycolysis to produce the energy. Mutation of S-116 (serine 116) to T (threonine 116) in phosphoglucomutase will cause slowdown of its activity because both serine and threonine are having hydroxyl group on side chain which is responsible at catalytic activity by transfering of phophate group through formation of phosphoserine in normal or phasphothreonine in mutated phasphoglucomutase but threonine have one methyl group extra on the carbon where –OH group attached compared to serine which sterically interfere with normal mechanism. So, there are chances that after mutation either phosphoglucomutase will not catalyse its reaction or catalyze but not that efficiently. As phosphoglucomutase will be inactivated or become less efficient, which convert glucose-1-phosphate to glucose-6-phosphate, glucose-1-phosphate will accumulate and as a result glycogen synthesis and their degradation will decrease.



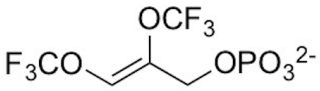
Serine



Threonine

Question 4

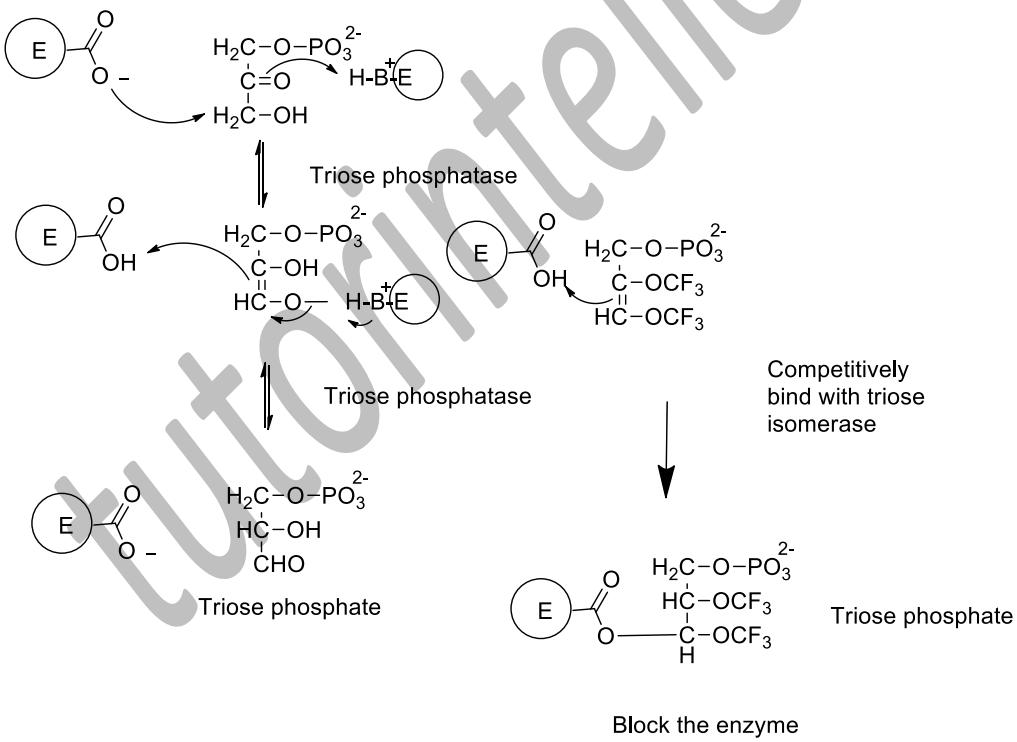
(Part A) Why might the compound shown below be a potent competitive inhibitor of triose phosphate isomerase?



(Part B) A rare inherited metabolic disorder that is characterized by neurological disorders is associated with low activity of aconitase and complex III of the electron transport chain. (It is not clear whether this is directly or only indirectly involved in the pathogenesis of this disease.)

(i) What do aconitase and complex III have in common that a single inherited disease might similarly affect them?
(ii) What would be the effect of this deficiency on aerobic respiration as well as the cellular concentration of ATP? Explain.

(Part A) Ans: Triose phosphate isomerase enzyme is isomerizes triose phosphate to keto or enol isomers through hydrogen shift by the help of proton donor and proton acceptor moieties present on the enzyme as depicted below. The hydrogen shift is promoted by the hydrogen bonding of enzyme with proton donor part of enzyme, due to structural similarity of the given compound, it competitively inhibits the triose isomerase enzyme and block it through covalent binding.



Q.4 Part B

(i)

Both aconitase and Complex III (Coenzyme Q–Cytochrome *c* Reductase) contains the iron-sulfur proteins cluster (Fe-S protein). In aconitase this Fe-S protein (Reiske protein) bind to cysteine group which activate the enzyme and causes the isomerisation of citrate to isocitrate. Similarly, Fe-S cluster is essential for complex III for electron transfer.
The assembly and disassembly of Fe-S is regulated by mammalian iron regulatory protein-1 (IRP-1) and defect in this protein due to inherited neurodegenerative disorder causes in unstable GAA triplet and results in loss of activity of Fe-S protein.

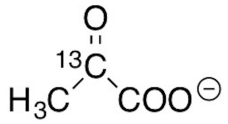
(ii)

The deficiency of iron regulatory protein -1 (IRP-1) causes the loss of activity of Fe-S protein hence TCA cycle and ETC get affected. In aerobic respiration there are 3 stages, glycolysis, TCA cycle and ETC. Glucose enters in glycolysis cycle and gives net 2 ATP, 2 NADH and 2 pyruvate molecules. Later oxidation of pyruvate to acetyl Co-A take place and then it enters to TCA cycle, where the aconitase which is dependent on Fe-S protein, is involved in transformation of citrate to isocitrate. Loss of activity of Fe-S protein will causes loss of activity of aconitase and stops the TCA cycle. Similar effects will also be observed in ETS cycle, because complex I, II and III in ETS are dependent on Fe-S protein. Thus, cellular concentration of ATP will decreases dramatically. Only 2 ATP obtained whereas in normal condition net 36 ATP can be produced by one glucose molecule.

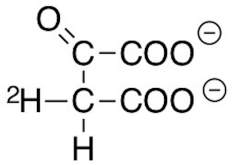
Question 5

(Part a) In a short paragraph (maximum 6 sentences) discuss in your own words the importance of labeling studies in understanding metabolism. Cite a specific example from the course text, discussions, or notes to help you explain the importance.

(Part b) If a liver extract capable of carrying out normal metabolic reactions (including gluconeogenesis) is incubated with labeled pyruvate labeled with carbon-14 at the carbonyl carbon atom (see below), where would the label be found in glucose? Explain, including schemes of the reactions to support your answer.



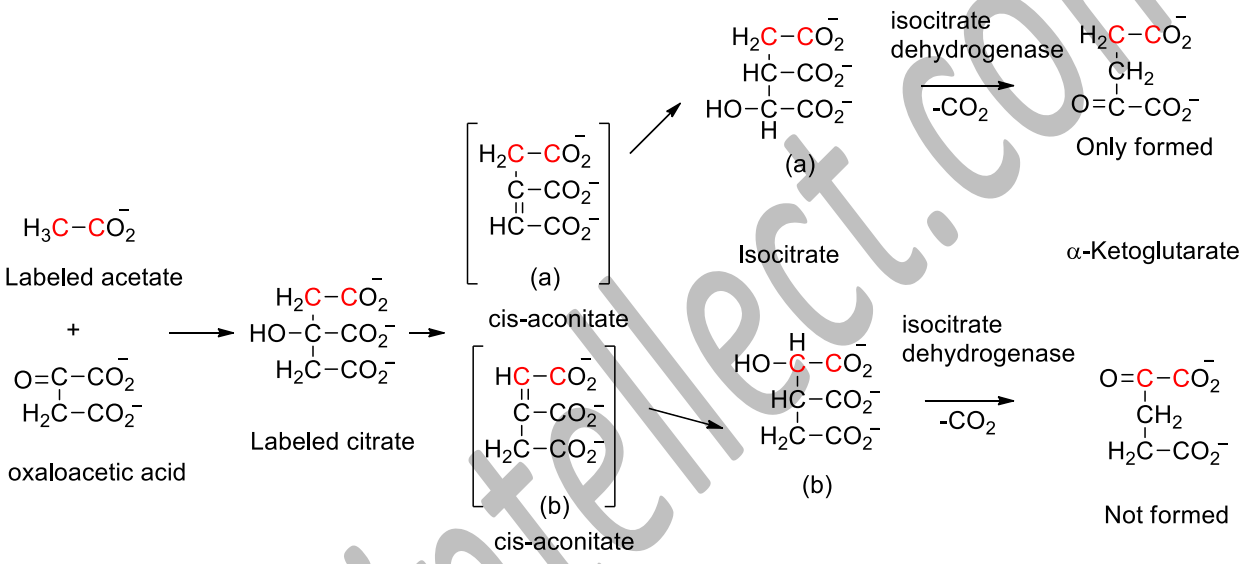
(Part c) If oxaloacetate were labeled with deuterium as shown, where would the label end up after one turn of the cycle? Let's say that one turn of the cycle completes with the reformation of oxaloacetate. What would a labeling study of this nature be able to tell us? Explain, using schemes of relevant reactions to support your answer.



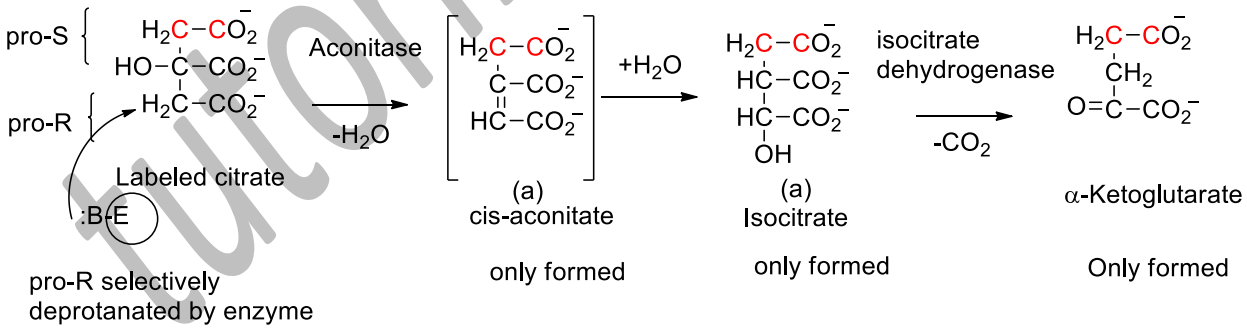
Answer:

(part a)

Isotopic labeling technique is very useful to track the metabolite, along with its formation pathway. One can also come to know the position of incorporation of labeled atom in the metabolites hence it enabled mechanistic understanding of metabolic networks. The example given below which shows the reaction of unlabeled oxaloacetate with labeled acetate (Red color 14-C) which supposed to give a mixture of α-ketoglutarate through symmetrical labeled citrate but only one α-ketoglutarate formed as revealed by labeled product. This contrary result explained that citrate is not only the intermediate during this transformation, there is an asymmetric tricarboxylic acid known as *cis*-aconitate and isocitrate, must be formed which later give the α-ketoglutarate.



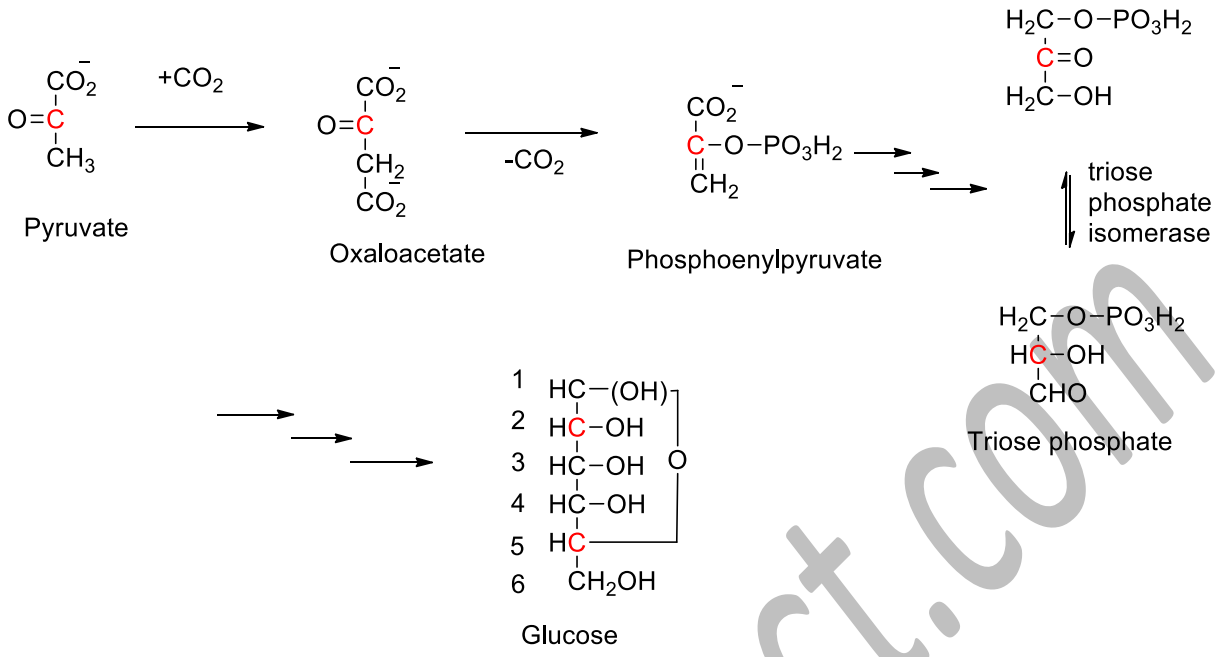
Mechanism: Aconitase enzyme selectively remove proton from pro-R arm of the citrate to convert it into cis-aconitate which later converted to α-ketoglutarate by isocitrate dehydrogenase.



(part b):

If pyruvate carbonyl is labeled with 14-C (Red color) than it will lead to the formation of glucose with labeled 2nd and 5th carbon with 14-C (Red color). As shown in the scheme below pyruvate initially carboxylated with carbon dioxide and later transformed to oxaloacetate with labeled carbonyl. Oxaloacetate then transformed to PEP (Phsphoenylpyruvate) and subsequently through few step

reactions to triose phosphate as keto and enol tautomers and through few steps reaction lead to give glucose with 2nd and 5th carbon labeled with 14-C (Red color).



(part c):

If oxaloacetate labeled with deuterium (D) then at the end of one turn of cycle 50% of oxaloacetic acid will be having the labeled deuterium (D) as shown in scheme below. Initially the aconitase enzyme selectively abstract the H (unlabeled pro-R hydrogen H, leaving labeled D) as E-B-H which gives 100% labeled cis aconitate. Subsequently after few steps it converted to succinate. Later succinate transformed to fumarate with 50% labeled D due to dehydrogenation lead by succinate dehydrogenase with equal probability of removal of D vs H. This further transformed to 50 % labeled malate and hence subsequently produces 50% labeled oxaloacetate. This type of labeling study will tell us the mechanistic understanding of the pathway.