Indraprastha Institute of Information Technology Delhi (IIITD)

Department of Computational Biotechnology

BIO211 – Cell Biology and Biochemistry

ASSIGNMENT-2 (November 28, 2022)

Instructions:

1. You have to submit a PDF file with your roll no. as its name.
2. You are required to submit the assignments by Sunday, 04 December 2022.

Question 1. Answer the following questions:

A. The complete oxidation of 1 mole of palmitic acid (a 16-carbon fatty acid) to CO_2 and H_2O yields 2355 kcal of free energy. How many ATP molecules could maximally be generated from one molecule of palmitic acid, if the useful chemical energy available in the high energy phosphate bond of 1 mole of ATP is 7.3 kcal? [2 marks]

Answer: Maximum no. of ATP molecules that can be generated from the complete oxidation of 1 palmitic acid molecule = 2355 kcal/7.3 kcal = ~323

B. However, it is known that beta-oxidation of palmitic acid in cells produces only 129 moles of ATP from 1 mole of palmitic acid. How does this compare with your answer in part (A)? Estimate the overall efficiency of ATP production from fatty acids? [2 marks]

Answer: Not all the energy released by beta-oxidation of palmitic acid gets converted into high-chemical bond energy of ATP molecules.

Overall efficiency = (No. of ATP molecules produced/No. of maximum ATP that can be generated) X 100% = (129/323) X 100 = 39.9% = ~40%

Rest of the energy gets dissipated as heat.

C. Assume that the cells of your body are oxidizing fatty acids and no energy is being dissipated as heat to the environment. However, the heat not converted into chemical-bond energy is increasing the temperature of your body. How much would the temperature of your body rise upon oxidation of 1 mole of palmitic acid if your body consists of 75 kg of water. [2 marks]

[*Hint: One kilocalorie (kcal) is defined as that amount of energy that heats 1 kg of water by 1^oC*]

Answer: 60% of the energy is not converted to high chemical-bond energy of ATP molecules.

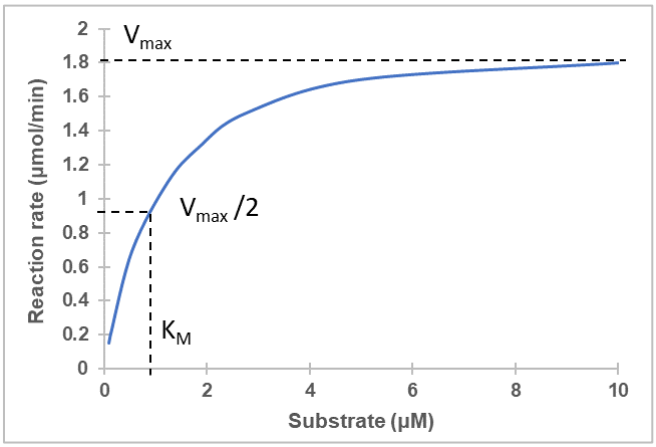
This equals to ~1413 kcal of energy produced from oxidation of 1 mole of palmitic acid.

Rise in temperature of 75 kg of water by 1413 kcal energy = 1413 kcal/75 kg = 18.84^oC

Question 2. Answer the following questions:

A. The following experimental data was collected during a study of the catalytic activity of an intestinal peptidase with the substrate glycylglycine. Plot the data as a graph, and use it to estimate the K_m and the V_{max} for this enzyme. [5 marks]

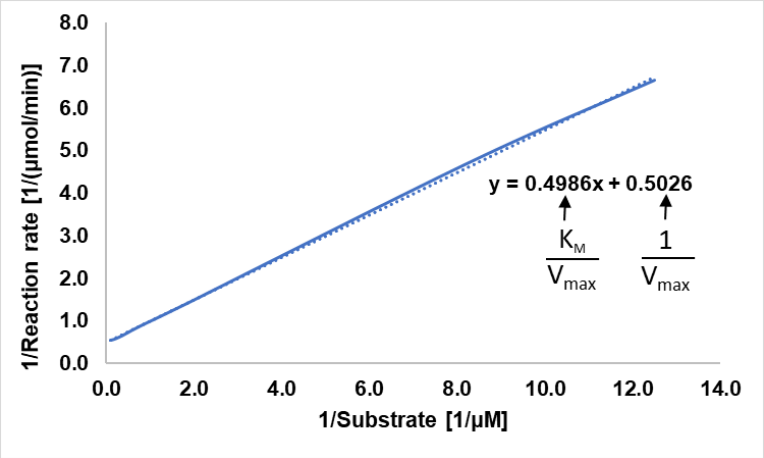
Substrate conc. (µM)	Reaction rate (µmol/min)
0.08	0.15
0.12	0.21
0.54	0.7
1.23	1.1
1.82	1.3
2.72	1.5
4.94	1.7
10.00	1.8



V_{max} = ~ 1.8 µmol/min and K_M = ~1µM

B. Now transform this data to plot it as a straight line (Lineweaver-Burk plot). Determine K_m and the V_{max} for this enzyme using this new plot. Do your results agree with the estimates made from the first graph of the raw data (from 2A)? [5 marks]

Substrate conc. (µM)	Reaction rate (µmol/min)	1/ Substrate conc. [1/µM]	1/Reaction rate [1/(µmol/min)]
0.08	0.15	12.5	6.7
0.12	0.21	8.3	4.8
0.54	0.7	1.9	1.4
1.23	1.1	0.8	0.9
1.82	1.3	0.5	0.8
2.72	1.5	0.4	0.7
4.94	1.7	0.2	0.6
10.00	1.8	0.1	0.6



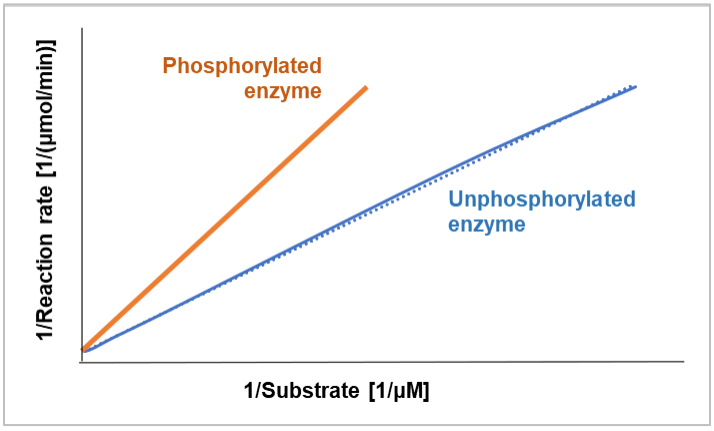
V_{max} = 1/0.5 = 2 µmol/min

K_M = 0.4896 x 2 = 0.9978 µM = ~1µM

C. Now assume that the activity of this intestinal peptidase is regulated by covalent modification of its catalytically active amino acid. Upon phosphorylation, the K_m of the catalyzed reaction has been observed to increase by a factor of 3 without any effect on its V_{max}. Is the enzyme getting activated or inhibited upon phosphorylation? Justify your answer. [3 marks]

If the K_M increases, then the concentration of substrate needed to give a half-maximal rate is increased. As more substrate is needed to produce the same rate, the enzyme-catalyzed reaction has been inhibited by the phosphorylation.

D. How will the Lineweaver-Burk plot of the phosphorylated enzyme differ from the plot of the unmodified enzyme (from 2B)? Give a rough graphical representation of the same. [3 marks]



Question 3. Find out the mechanism of action of the following chemical compounds: [8 marks]

(i) Cyanide poison.

Cyanide mainly inhibits oxidative phosphorylation, a process where oxygen is utilized for the production of essential cellular energy sources in the form of ATP. It does so by binding to the enzyme cytochrome C oxidase and blocks the mitochondrial transport chain. After that, cellular hypoxia and the depletion of ATP occur, leading to metabolic acidosis. The utilization of oxygen by the tissue occurs and is followed by the impairment of vital functions.

(ii) Curare, a drug used for making poison arrows.

Curare is as a neuromuscular blocking agent that produces flaccidity in skeletal muscle by competing with the neurotransmitter acetylcholine at the neuromuscular junction. Acetylcholine normally acts to stimulate muscle contraction; hence, competition at the neuromuscular junction by curare prevents nerve impulses from activating skeletal muscles. The major outcome of that competitive activity is profound relaxation (comparable only to that produced by spinal anesthesia). Relaxation begins in the muscles of the toes, ears, and eyes and progresses to the muscles of the neck and limbs and, finally, to the muscles involved in respiration. In fatal doses, death is caused by respiratory paralysis.