BIOSC 1820
Metabolic Pathways and Regulation
Spring, 2010
Prof. Jeffrey L. Brodsky
Mid-Term Exam
March 4, 2010

NAME: ___KEY___

Each question is worth 2 points – Good luck!

1. The protein phosphatase-1 enzyme (PP1) is phosphorylated by activated protein kinase A. PP1 is more active once phosphorylated. During glucagon-stimulated glycogen breakdown in the liver, does this mode of regulation make sense? Why?
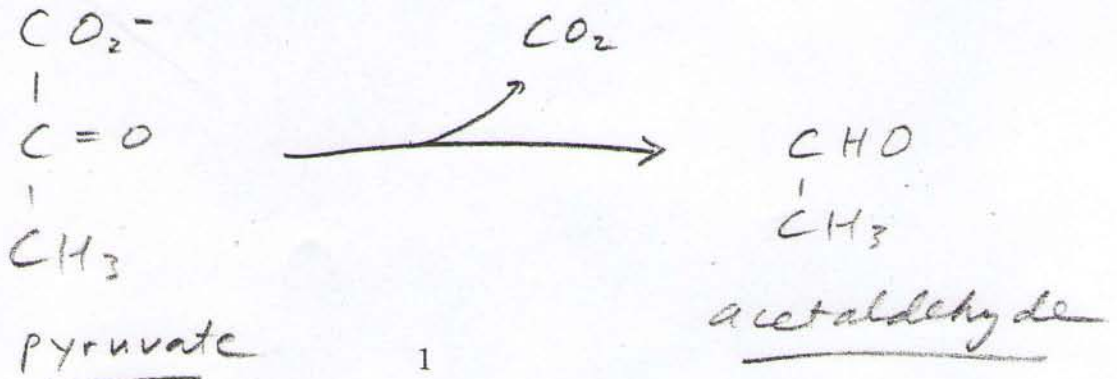
Once activated, PP1 will de-phosphorylate enzymes downstream of PKA, thus inactivating them. This serves as a means of feedback inhibition.

2. Brazil has been growing large batches of yeast for over 30 years in bio-reactors in order to reduce their dependence on foreign oil—they use the generated ethanol as an additive to gasoline, which burns better and cleaner than most grades of gasoline. (The down side is that the production of the sugars for this process has contributed to deforestation in Brazil.)

A. What must be absent from the bioreactors for the production of ethanol to proceed efficiently?

Oxygen

B. Very recently, the DNA of these bio-reactor yeast cultures was sequenced and compared to the genome in lab strains. Strikingly, the yeast had mutated to express extra copies of the genes required to make thiamine pyrophosphate from vitamin B_6. Write out the reaction that requires this cofactor in yeast, which you think was critical for these cultures to thrive. Be sure to include the full names of the reactants and products and the name of the enzyme.

$$
\begin{array}{c}
CO_2^- \\
| \\
C=O \\
| \\
CH_3
\end{array}
\xrightarrow{\quad CO_2 \quad}
\begin{array}{c}
CHO \\
| \\
CH_3
\end{array}
$$

pyruvate   acetaldehyde

3. Draw the α-D-pyranose form of glucose, and indicate with an arrow which carbon is released as CO_2 in the oxidative phase of the pentose phosphate pathway:
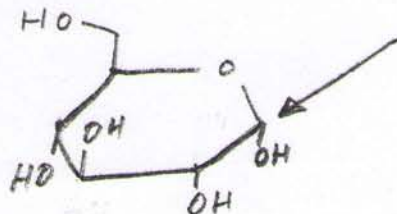
4. In which cellular compartment are you most likely to find the following molecule?

peroxisome

5. Give the name of the metabolite that would most likely immediately build-up in cells treated with:

A. malonate: _____Succinate (inhibits succinate dehydrogenase)._____

B. fluoroacetate: _____Citrate (Fluorocitrate inhibits aconitase)_____

C. caffeine _____Cyclic AMP (inhibits phosphodiesterase)_____

6. A major target of insulin, which we have not discussed, is the transcriptional repression of the gene encoding phosphoenolpyruvate carboxykinase. Draw the structures of the reactants and products for this enzyme (any molecules other than carbohydrates can be abbreviated):
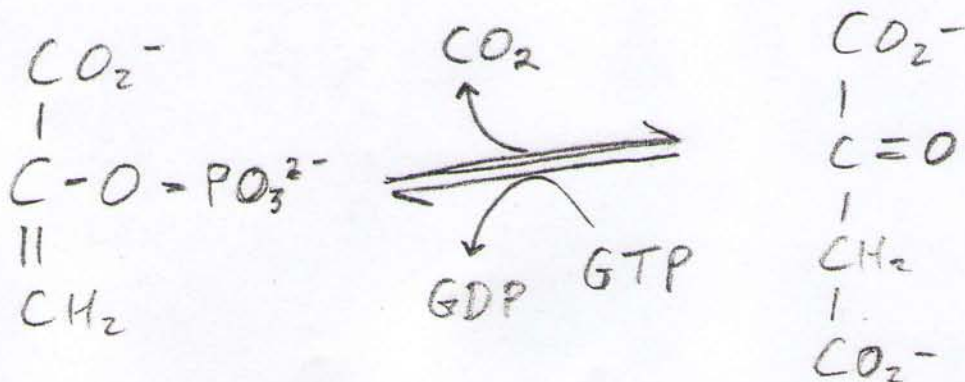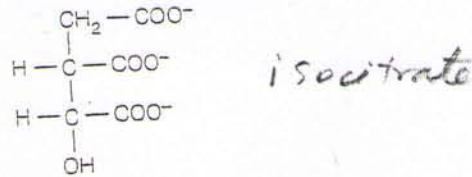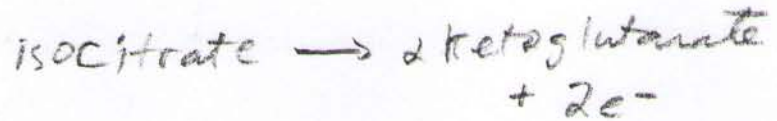
Does it "make sense" that insulin *in*/activates phosphoenolpyruvate carboxykinase?

Yes -- the enzyme is required for gluconeogenesis. Insulin favors glycolysis, which is needed to make AcCoA for fat synthesis

7. Use the following table to calculate the ΔE'° for the delivery of 2 electrons from the following molecule to NAD.

Half-reaction	E'° (V)
$\frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O$	0.816
$Fe^{3+} + e^- \longrightarrow Fe^{2+}$	0.771
$NO_3^- + 2H^+ + 2e^- \longrightarrow NO_2^- + H_2O$	0.421
Cytochrome f $(Fe^{3+}) + e^- \longrightarrow$ cytochrome f (Fe^{2+})	0.365
$Fe(CN)_6^{3-}$ (ferricyanide) $+ e^- \longrightarrow Fe(CN)_6^{4-}$	0.36
Cytochrome a_3 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a_3 (Fe^{2+})	0.35
$O_2 + 2H^+ + 2e^- \longrightarrow H_2O_2$	0.295
Cytochrome a $(Fe^{3+}) + e^- \longrightarrow$ cytochrome a (Fe^{2+})	0.29
Cytochrome c $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c (Fe^{2+})	0.254
Cytochrome c_1 $(Fe^{3+}) + e^- \longrightarrow$ cytochrome c_1 (Fe^{2+})	0.22
Cytochrome b $(Fe^{3+}) + e^- \longrightarrow$ cytochrome b (Fe^{2+})	0.077
Ubiquinone $+ 2H^+ + 2e^- \longrightarrow$ ubiquinol $+ H_2$	0.045
Fumarate$^{2-} + 2H^+ + 2e^- \longrightarrow$ succinate^{2-}	0.031
$2H^+ + 2e^- \longrightarrow H_2$ (at standard conditions, pH 0)	0.000
Crotonyl-CoA $+ 2H^+ + 2e^- \longrightarrow$ butyryl-CoA	−0.015
Oxaloacetate$^{2-} + 2H^+ + 2e^- \longrightarrow$ malate^{2-}	−0.166
Pyruvate$^- + 2H^+ + 2e^- \longrightarrow$ lactate$^-$	−0.185
Acetaldehyde $+ 2H^+ + 2e^- \longrightarrow$ ethanol	−0.197
FAD $+ 2H^+ + 2e^- \longrightarrow FADH_2$	−0.219*
Glutathione $+ 2H^+ + 2e^- \longrightarrow$ 2 reduced glutathione	−0.23
$S + 2H^+ + 2e^- \longrightarrow H_2S$	−0.243
Lipoic acid $+ 2H^+ + 2e^- \longrightarrow$ dihydrolipoic acid	−0.29
$NAD^+ + H^+ + 2e^- \longrightarrow$ NADH	−0.320 ←
$NADP^+ + H^+ + 2e^- \longrightarrow$ NADPH	−0.324
Acetoacetate $+ 2H^+ + 2e^- \longrightarrow$ β-hydroxybutyrate	−0.346
α-Ketoglutarate $+ CO_2 + 2H^+ + 2e^- \longrightarrow$ isocitrate	−0.38 ←
$2H^+ + 2e^- \longrightarrow H_2$ (at pH 7)	−0.414
Ferredoxin $(Fe^{3+}) + e^- \longrightarrow$ ferredoxin (Fe^{2+})	−0.432

$$CH_2-COO^-$$
$$H-C-COO^-$$
$$H-C-COO^-$$
$$OH$$

isocitrate

isocitrate → α ketoglutarate
+ 2e⁻

+ 0.38 V

$NAD^+ + 2e^- \longrightarrow NADH$

− 0.32 V

0.38 − 0.32 =

+ 0.06

8. Assuming that Farraday's constant is 100 kJ/(Volts • mol), how many ATPs—in theory—could be generated from the delivery of these 2 electrons in question #7?
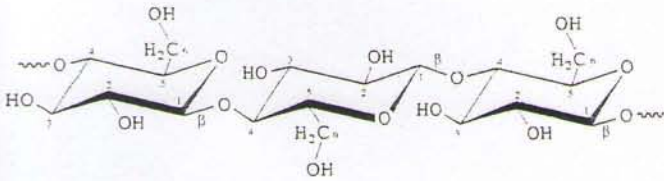
$$\Delta G'° = -n \mathcal{F} \Delta E'°$$

$$= -(2)(100)(0.06) = -12 \ kJ/mol$$

NO ATPs could be made .....

(need ~30 kJ/mol)

9. The following portion of a polymer forms what molecule?

Cellulose.

10. Some evidence suggests that the β and γ subunits associated with G protein coupled receptors may also be important for hormone-dependent signaling.

    A. In one publication, the released β subunit was found to directly activate a chloride channel that is expressed in kidneys. Let's say that the activated α subunit is involved in opening a water channel, which acts in parallel to the chloride channel. What do you think the GAP is for this process?

The water channel

(which will turn the α-GTP "off" — α-GDP will be formed).

    B. Name one way that the α subunit of a G protein might become constitutively activated (not including genetic mutations):
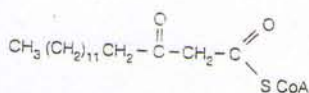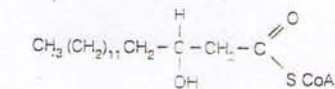
— GTPγS.
— bacterial toxins
— AlF₄

11. Would the following events lead to **(A)** higher or **(B)** lower levels of circulating fats in the blood stream?

    A. removal of the gallbladder *(no digestion)*   *B*

    B. a perilipin mutant protein that cannot be phosphorylated   *B*
         (no breakdown)

    C. a constitutively activated form of PKA in adipocyes   *A*
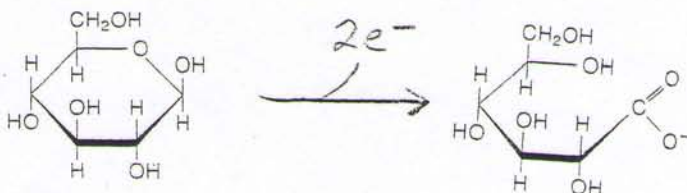   *(lots of breakdown)*

4

12. What is the full name of the electron acceptor for the following reactions (no abbreviations):

A.

$$CH_3(CH_2)_{11}-CH_2-\overset{\overset{H}{|}}{C}-CH_2-C\overset{\diagup O}{\diagdown S\,CoA}$$
$$\overset{|}{OH}$$

$\downarrow 2e^-$

$$CH_3(CH_2)_{11}-CH_2-\overset{\overset{O}{||}}{C}-CH_2-C\overset{\diagup O}{\diagdown S\,CoA}$$

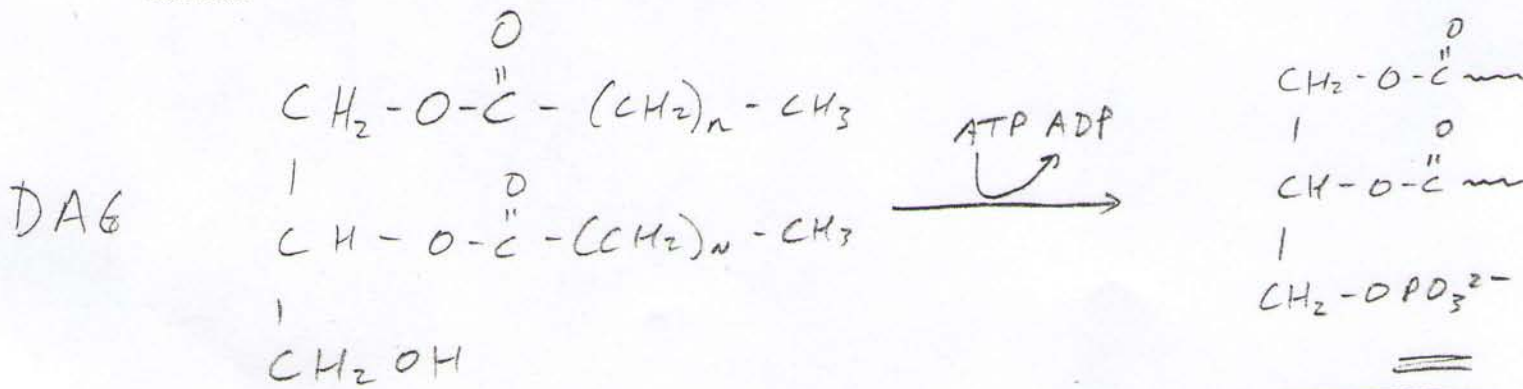nicotinamide adenine dinucleotide

B.

$2e^-$

Copper

(Fehling's soln).

13. After diacyl glycerol is generated from the action of phospholipase C, there is a kinase that acts on this molecule and that is required to generate a precursor for the regeneration of PIP₂. Draw the structure of the product the reaction catalyzed by this kinase:

DAG

$$CH_2-O-\overset{\overset{O}{||}}{C}-(CH_2)_n-CH_3$$
$$|$$
$$CH-O-\overset{\overset{O}{||}}{C}-(CH_2)_N-CH_3$$
$$|$$
$$CH_2OH$$

ATP ADP $\xrightarrow{}$

$$CH_2-O-\overset{\overset{O}{||}}{C}\sim$$
$$|\qquad\quad O$$
$$CH-O-\overset{\overset{||}{}}{C}\sim$$
$$|$$
$$CH_2-OPO_3^{2-}$$

14. The change in free energy for hexokinase and phosphofructokinase-1 is essentially identical, yet PFK-1 is more highly regulated. Why do you think this is?

Regulating PFK-1 allows levels of glucose-6-phosphate to build-up, which can be diverted to many other pathways (building up glucose is not as useful).

15. What is the name of the following molecule and where are you most likely to find it in the cell?

$$CH_3-N^+-CH_2-CH-CH_2-COO^-$$

with CH_3 groups on the nitrogen and OH on the middle carbon
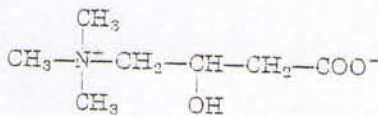
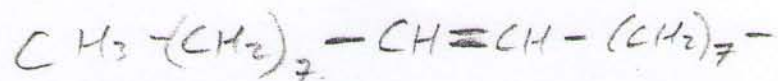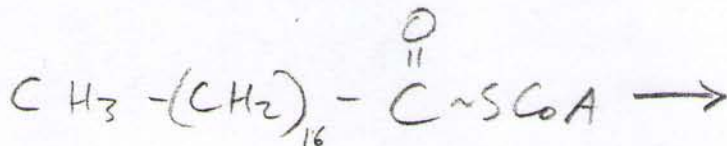Carnitine / mitochondrial (or mitochondrial membrane).

16. A naturally occurring mutant form of apolipoprotein CII was examined. In which type of particle do you find this apolipoprotein?

Chylomicron

Interestingly, individuals expressing the mutant exhibited lower levels of the gene encoding an enzyme that oxidizes stearoyl-CoA, which normally makes an important cellular lipid. Draw the reaction that you think might be catalyzed by this enzyme:
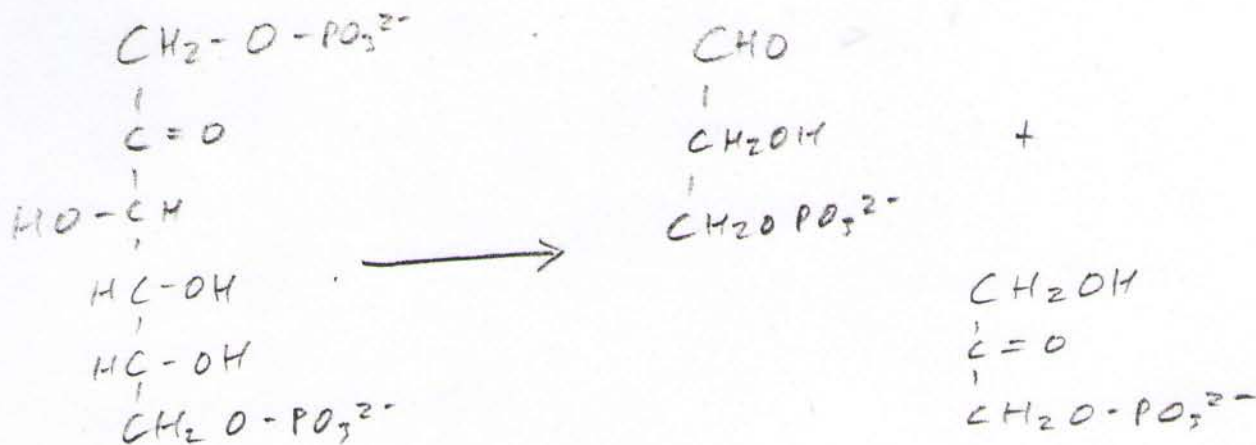
(C-18)

Stearoyl-CoA

$$CH_3-(CH_2)_{16}-\overset{O}{\overset{||}{C}}\sim SCoA \longrightarrow$$

$$CH_3-(CH_2)_7-CH=CH-(CH_2)_7-\overset{}{C}\sim SCoA$$
with $\overset{||}{O}$

(oleic acid)

6

17. Draw the reaction—including the structures of the reactant(s) and product(s)—that represents the most unfavorable reaction in glycolysis: (aldolase)
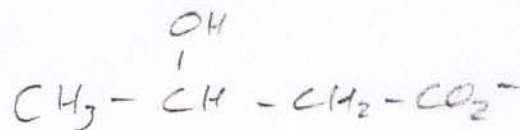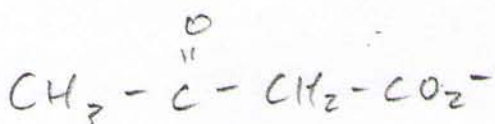
$$CH_2 - O - PO_3^{2-}$$
$$|$$
$$C = O$$
$$|$$
$$HO - CH$$
$$|$$
$$HC - OH$$
$$|$$
$$HC - OH$$
$$|$$
$$CH_2 O - PO_3^{2-}$$

$\longrightarrow$

$$CHO$$
$$|$$
$$CH_2OH$$
$$|$$
$$CH_2 O PO_3^{2-}$$

$+$

$$CH_2OH$$
$$|$$
$$C = O$$
$$|$$
$$CH_2 O \cdot PO_3^{2-}$$

18. The generation of ketone bodies is accelerated in response to fasting. Draw the structure of one of the two ketone bodies that is an acid and state its name:
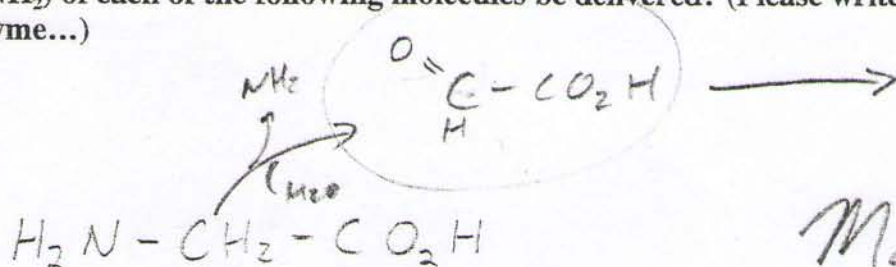
acetoacetate

$$CH_3 - \overset{O}{\overset{||}{C}} - CH_2 - CO_2^-$$

β-hydroxybutyrate

$$CH_3 - \overset{OH}{\overset{|}{CH}} - CH_2 - CO_2^-$$

19. Into which metabolic enzyme that we discussed might the breakdown products (i.e., loss of NH_2) of each of the following molecules be delivered? (Please write the full name of the enzyme...)

A.

$$O = \overset{NH_2}{\underset{H}{\overset{}{C}}} - CO_2 H \longrightarrow$$

$H_2 N - CH_2 - CO_2 H$

Malate Synthase.

B.

$$CO_2 H$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$H_2 N - CH - CO_2 H$$

$$CO_2 H$$
$$|$$
$$CH_2$$
$$|$$
$$CH_2$$
$$|$$
$$O = C - CO_2 H$$
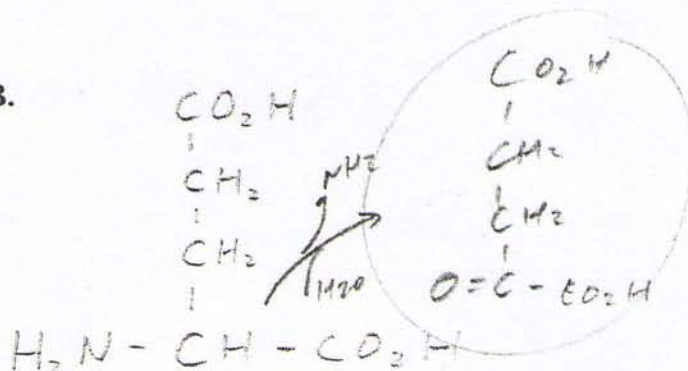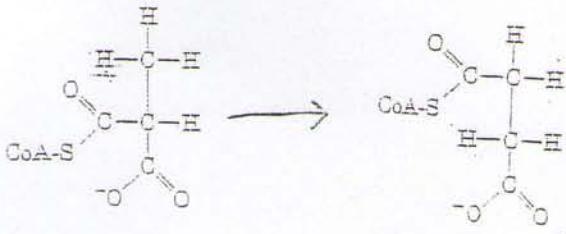
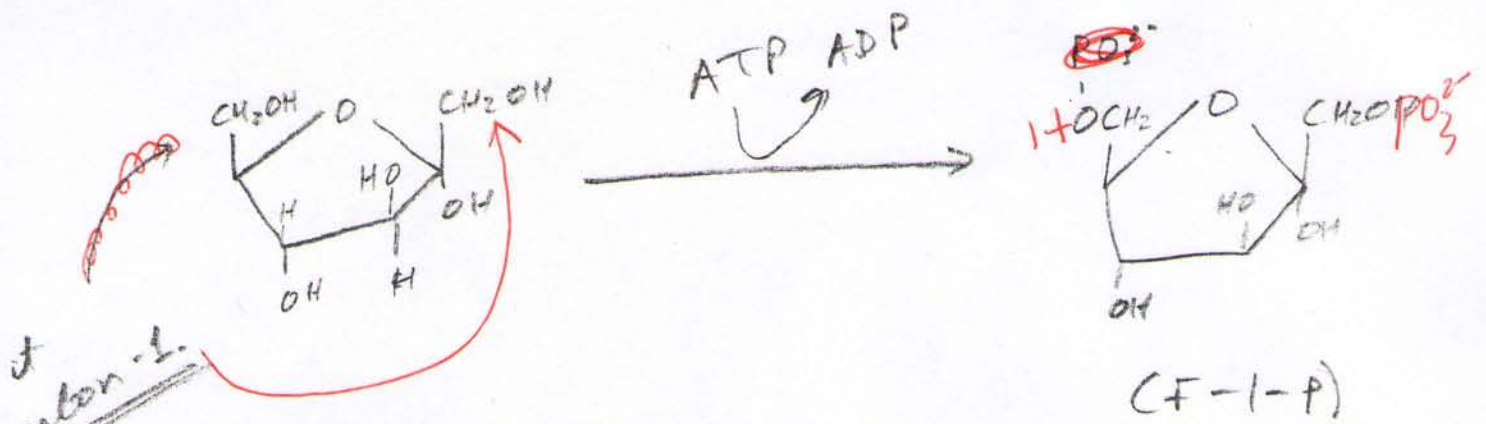α-Ketoglutarate dehydrogenase.

7

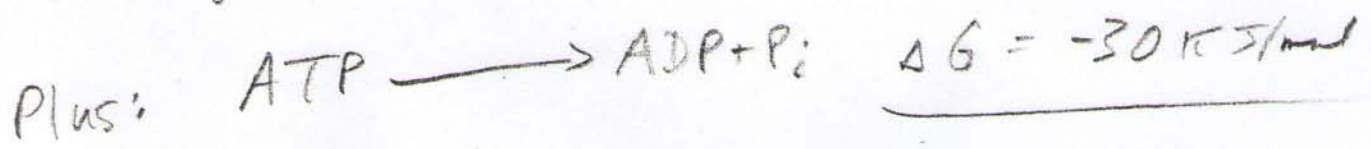20. Which vitamin is needed for the following reaction?

Vitamin
B12

21. Draw the structures of the reactants and the products of the reaction catalyzed by fructokinase (please draw the structure of the product in the furanose conformation):

(F-1-P)

22. The $\Delta G°$ for the hydrolysis of phosphate from a singly phosphorylated form of fructose is ~ -16 kJ/mol. What is the standard free energy change for the reaction catalyzed by fructokinase?

$$F-1-P \longrightarrow P_i + F \qquad \Delta G° = -16 \, kJ/mol$$

$$So, \quad P_i + F \longrightarrow F-1-P \qquad \Delta G = +16 \, kJ/mol$$

$$Plus: \quad ATP \longrightarrow ADP + P_i \qquad \Delta G = -30 \, kJ/mol$$

$$-30 + 16 = \sim -14 \, kJ/mol.$$

23. The following is an intermediate in the reaction catalyzed by which enzyme?

$$
\begin{array}{c}
\text{O} \qquad \text{O} \\
\| \qquad \| \\
\text{C} \sim \text{O} - \text{P} - \text{O}^- \\
| \qquad\quad | \\
\text{CH}_2 \qquad \text{O}^- \\
| \\
\text{CH}_2 \\
| \\
\text{COO}^-
\end{array}
$$
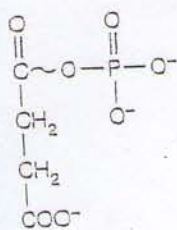
Succinyl - CoA
Synthetase

24. The complete oxidation of glucose generates 6 molecules of carbon dioxide. Name the enzymes that catalyze the release of CO_2 from carbohydrates during this process:

- Pyruvate dehydrogenase

- α keto glutarate dehydrogenase

- Isocitrate dehydrogenase

25. Assuming that each NADH generates 3 ATPs and each $FADH_2$ generates 2 ATPs, how many ATPs would be made by the complete oxidation of the following molecule?

$$
\text{CH}_3 - \text{CH}_2 - \text{CH}_2 - \overset{\displaystyle \overset{O}{\|}}{C} \sim \text{SCoA}
$$

only
need 1
"cut"

1 turn oF   ATPs.
FA β oxdn cycle: 1 $FADH_2$ =   2
                              1 NADH   =   3

2 acetyl CoA (×12) =   24

                                                        29 ATPs.

26. A scientist fed radioactive molecules of inorganic phosphate into the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase. What is the name of the enzyme in the glycolytic pathway that would remove this radioactive molecule and transfer it to ADP via a substrate level phosphorylation?

Phosphoglycerate Kinase

(next step in pathway).

27. An increase in the activity of phosphofructokinase-2 would have what effect on glycolysis?

This would lead to an increase in

F-2,6-Bis Phosphate,

which activates

glycolysis. (via ↑PFK1).

(via ↓ FBPase).

incorrect:

/37

correct x2 =   /75

HAVE AN AWESOME AND WELL-DESERVED SPRING BREAK!!!