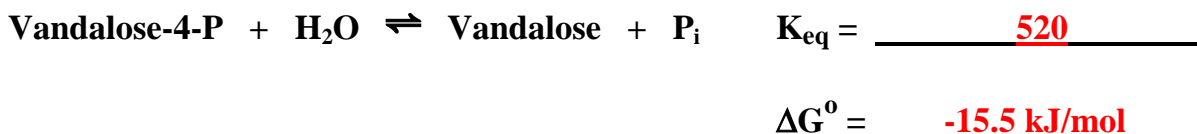


Homework is due one week after assignment. This homework is due 5:30 pm, Wed Sept 26. It is recommended that you convert to molarity (M) for your calculations.

(6,000 pts)

1. An enzymatic hydrolysis of a new phosphorylated molecule, vandalose-4-P (the '4' denotes position of the phosphate), was allowed to proceed to equilibrium at 25°C. The original starting concentrations of vandalose-4-P, vandalose and P_i were 25.000 mM, 0.000 mM and 0.000 mM, respectively. After the system reached equilibrium, the concentration of vandalose-4-P was only 1.20 μM. Calculate the equilibrium constant for this reaction and the standard free energy of hydrolysis of vandalose-4-P. Even though it is a reactant, the concentration of H₂O should not be used in your calculations.



The key here is to determine the equilibrium concentrations for V-4-P, V & P_i

The equil concentration of V-4-P is given, [V-4-P] = 1.20 μM = 1.20 x 10⁻⁶ M

For every V-4-P hydrolyzed, you get one V & one P_i; [V] = [P_i] = 25.000 - 0.0120 mM

[X] = [P_i] = 24.988 mM = 2.4988 x 10⁻² M

if we are at equilibrium, then $K_{\text{eq}} = \frac{[\text{V}][\text{P}_i]}{[\text{V-4-P}]} = \frac{(24.988 \times 10^{-2})^2}{1.20 \times 10^{-6}} = 520.3$

At equilibrium, ΔG = 0

Thus, ΔG = 0 = ΔG° + 5700 J/mol log [V][P_i]/[V-4-P]

ΔG° = -5700 J/mol log 520.3 = (-5700 J/mol)(2.72) = -15,480 J/mol = -15.48 kJ/mol

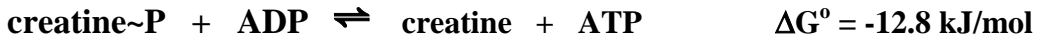
b) Compare the high energy phosphate bond of Vandalose-4-P and the gamma phosphate of ATP. Under standard conditions, which has the "higher energy phosphate bond?" Explain briefly; include a definition of "higher energy bond."

A high energy bond refers to how much energy is released upon hydrolysis of a specific bond. Our text (pg 501) states that hydrolysis of a high energy phosphate bond must release at least 25 kJ/mol. If it releases less than this, it is considered a 'low-energy' phosphate bond. This is arbitrary; some sources quote ΔG°' = -15 kJ/mol as the cutoff. For hydrolysis of the gamma phosphate in ATP, ΔG°' = -30.5 kJ/mol. This is definitely a higher energy phosphate bond than V-4-P. To determine this, one compares the energy yield from hydrolysis of the phosphate bonds in question.

Hydrolysis of ATP to ADP under standard conditions releases 30.5 kJ of energy for every mole hydrolyzed. Hydrolysis of V-4-P to vandalose under standard conditions releases 15.48 kJ of energy for every mole hydrolyzed.

(8,000 pts)

2. Your muscles use the chemical creatine phosphate (creatine~P) to maintain reasonably high ATP levels. The following reaction is catalyzed by the enzyme, creatine kinase (creatine phosphokinase, CPK or CK):



a) In a muscle tissue sample from a diseased individual A at rest, [ATP] = 5.5 mM, [ADP] = 0.52 mM, [creatine~P] = 1.33 mM, and [creatine] = 39.6 mM. Calculate the free energy of this reaction and determine if ATP is likely to be generated using creatine~P under these conditions.

$$\Delta G = \Delta G^\circ + [5700 \text{ J/mol}][\log\{\frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine~P}]}\}]$$

$$\Delta G = -12,800 \text{ J/mol} + (5700 \text{ J/mol}) \log\{ (.0055)(0.0396)/(0.00052)(0.00133)\}$$

$$\Delta G = -12,800 \text{ J/mol} + (5700 \text{ J/mol}) \log (314.9) = -12,800 \text{ J/mol} + 14,240 \text{ J/mol} = +1.44 \text{ kJ/mol}$$

Alternatively, $T = 37^\circ\text{C}$ $\Delta G = \Delta G^\circ + RT \ln\{\frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine~P}]}\}$

$$\Delta G = -12,800 \text{ J/mol} + 8.314 \text{ J K}^{-1}\text{mol}^{-1} (310 \text{ K}) \ln (314.9) = -12,800 + 14,800 \text{ J/mol} = +2.0 \text{ kJ/mol}$$

Since $\Delta G > 0$, the reaction *will not proceed* spontaneously

This person has very little creatine~P but they have plenty of ATP; they may have a disease that significantly inhibits the conversion of creatine to creatine~P.

b) In a sample from a individual B, [ATP] = 5.5 mM, [ADP] = 0.65 mM, [creatine~P] = 40.0 mM, and [creatine] = 1.5 mM. Calculate the free energy of this reaction and determine if ATP could be generated using creatine~P under these conditions.

$$\Delta G = \Delta G^\circ + [5700 \text{ J/mol}][\log\{\frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine~P}]}\}]$$

$$\Delta G = -12800 \text{ J/mol} + (5700 \text{ J/mol}) \log\{ (0.0055)(0.0015)/(0.00065)(0.0400)\}$$

$$\Delta G = -12800 \text{ J/mol} + (5700 \text{ J/mol}) \log 0.317 = (-12800 + -2840) \text{ J/mol} = -15640 \text{ J/mol}$$

$$\Delta G = -15.6 \text{ kJ/mol}$$

Alternatively, $T = 37^\circ\text{C}$ $\Delta G = \Delta G^\circ + RT \ln\{\frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine~P}]}\}$

$$\Delta G = -12,800 \text{ J/mol} + 8.314 \text{ J K}^{-1}\text{mol}^{-1} (310 \text{ K}) \ln (0.317) = -12,800 - 2960 \text{ J/mol} = -15.8 \text{ kJ/mol}$$

Since ΔG is a fairly large negative number, these conditions clearly favor production of ATP using creatine~P as the source of high energy phosphate.

- c) At a different point in time, a second sample is taken from individual B where
 [ATP] = 0.44 mM, [ADP] = 5.25 mM, [creatine-P] = 0.025 mM, and [creatine] = 53.0 mM.
 Calculate the free energy of this reaction and determine if ATP is likely to be generated using creatine~P under these conditions.

$$\Delta G = \Delta G^\circ + [5700 \text{ J/mol}] \frac{\log\{ \frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine}\sim\text{P}]} \}}$$

$$\Delta G = -12800 \text{ J/mol} + (5700 \text{ J/mol}) \log\{ (0.00044)(0.0530)/(0.00525)(0.000025) \}$$

$$\Delta G = -12800 \text{ J/mol} + (5700 \text{ J/mol}) \log 177.7 = (-12800 + 12820) \text{ J/mol} = +20 \text{ J/mol}$$

$$\Delta G = 0.0 \text{ kJ/mol}$$

Alternatively, $T = 37^\circ\text{C}$ $\Delta G = \Delta G^\circ + RT \ln\{ \frac{[\text{ATP}][\text{creatine}]}{[\text{ADP}][\text{creatine}\sim\text{P}]} \}$

$$\Delta G = -12,800 \text{ J/mol} + 8.314 \text{ J K}^{-1}\text{mol}^{-1} (310 \text{ K}) \ln (177.7) = -12,800 + 13,350 \text{ J/mol} = +0.55 \text{ kJ/mol}$$

Since ΔG is close to 0.0 kJ, this reaction is close to equilibrium.

- d) Explain the difference between your answers for parts b & c. If individual B is a normal healthy human, why did you come up with such different ΔG values? Your answer should include differences in substrate and product concentrations.

Individual B in part c has depleted both his creatine~P AND his ATP, likely due to intense exercise. The healthy person in part b may be at rest or low activity because creatine~P AND ATP are both high (actually they are both normal for a person at rest).

(6,000 pts)

3. Within 24 h of a suspected heart attack, a male patient's blood (serum) will be tested for creatine kinase (CPK) activity. This is because a heart attack usually results in significant leakage of CPK from the cardiac muscle. A normal male would expect to have a CPK activity of 55-170 units/L serum (tested at 25°C). As a Medical Technician, you test 2.0 ml of a patient's serum and find that it exhibits a CPK activity of 6.22×10^{-9} mol reactions per second. If a unit of CPK activity is defined as 1 μmole of reaction per minute (at 25°C), determine if this patient's serum CPK activity is below, within, or above normal levels.

A unit = 1 $\mu\text{mole rxn/min}$

You found $7.55 \times 10^{-9} \text{ mol rxns ml}^{-1} \text{ sec}^{-1}$

$$\frac{6.22 \times 10^{-9} \text{ mol rxns}}{2.0 \text{ ml sec}} \frac{(1000 \text{ ml})}{1.0 \text{ L}} \frac{(10^6 \mu\text{mol rxns})}{\text{mol rxns}} \frac{(60 \text{ sec})}{\text{min}} = 187 \text{ units/L serum}$$

187 units per liter is above (but not by much) the 55-170 units/L observed in normal males.

**Note that when a doctor is trying to determine if a patient has had a heart attack, they will use elevated CPK activity as one of multiple indicators. It turns out that other tissues can also release CPK into the bloodstream. For example, damaged skeletal muscle tissue (maybe a torn muscle) will definitely release CPK. Normal CPK levels (or close to it) is a good sign that muscles, including the heart, have not been damaged.