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Biomedical Engineering E3500x

Solution to Mid-Term Examination (Maximum possible score is 89 pts.)

1. (35 pts., 7 for each part.) An experimental "construct" in the form of a small oil-covered water droplet (liposome) is proposed for the regeneration of ATP from ADP and P_i by using the free energy liberated in the oxidation of 3-phosphoglyceraldehyde (RCOH) to 3-phosphoglycerate (RCOOH). (The extra oxygen is obtained from water.) The ATP produced would be used subsequently to 'drive' another biochemical reaction in another construct. The reaction has a $\Delta G^{0'}$ of –7.3 kcal/gmole. In the exclusive (no other enzyme capable of reaction with the aldehyde) presence of *3 phosphoglycerate kinase* the reaction occurs with the involvement of a mole each of P_i and ADP for each mole of aldehyde, to form ATP in addition to the acid. The observed overall $\Delta G^{0'}$ is 0.0.

(a) Calculate the equilibrium constant for the overall reaction. Give the units of your answer.

Use $\Delta G^{0'} = 0 = -RT \ln K$. Thus $K = e^0 = 1$. The units are (moles/liter)$^{-1}$.

A suspension of constructs is placed in an environment that has the following concentrations:

P_i	RCOH	RCOOH	ATP	ADP
0.100 M	0.020 M	0.004 M	0.007 M	0.022 M

(b) Assume the membrane is freely permeable to all participants in the reaction and calculate the reaction's molar ΔG.

Use $\Delta G = \Delta G^{0'} + RT \ln \frac{[ATP][RCOOH]}{[P_i][RCOH][ADP]}$. I calculated a value of -0.451 *RT*. Equal credit whether or not you substituted numbers for *RT*.

(c) It is proposed to make the reaction more efficient (reduce its -ΔG) by introducing transport resistance into the membrane of the construct. Assume that the transport resistance will be either to ATP or ADP. (The other reactants and products will continue to move freely.) Choose one of these answers and explain it in not more than 25 words:

i. The resistance must be introduced for ATP
ii. The resistance must be introduced for ADP
iii. The resistance must be introduced for both ATP and ADP in a definite ratio.
iv. The resistance can be introduced by any of the methods proposed above and can be used to make the reaction ΔG essentially zero.

The correct answer is iv. The slower the reaction is made (by reducing the actual free energy change) the more efficient the reaction. However the overall process is not made more efficient. The free energy is consumed by one or the other substance needing to overcome a resistance in the membrane.

(d) Give the principal reason for not running biochemical reactions with ΔG equal to zero in not more than 25 words.

When the free energy change is zero, the reaction is at equilibrium and there is no net conversion. If product is to be made at a finite rate there must be a finite free energy change.

(e) The enzyme reaction described above can be described by which of the following kinetic equations:

i. Michaelis-Menten equation
ii. The more elaborate form of the Michaelis-Menten equation that allows for reversibility, without which there will be big errors in rate calculations.
iii. One of the above, but more data is needed to decide which.

You may explain your answer in not more than 25 words.

The correct answer is (ii). The Michaelis-Menten equation calculates only the forward, not the net rate. Since we already know the reaction is near equilibrium we know there will be a finite reverse reaction to account for.

2. (24 pts., 6 for each part.) The original method for treating a person with the artificial kidney was to flow his blood over a membrane that was washed by a large volume of a well-mixed buffer solution on its opposite side. The original model to explain urea transfer from patients subjected to this treatment considered the body of an adult male to consist of a single lumped compartment that contained 50 L of water. Remember that one L is 1000 cm^3. If the permeability of the membrane to urea is 10^{-4} cm/sec and the area is 5 10^3 cm^2, answer the following:

(a) If the volume of buffer solution is taken to be infinite, how long will it take for the blood concentration of urea to drop to 37% of its original value?

We write $V\frac{dc}{dt} = -PA(c-0)$ which integrates to $\ln\frac{c}{c_0} = -\frac{PA}{V}t$. The 37% value corresponds to a drop of $1/e$ or a time equal to v/PA, 100,000 sec. or 27.7 hours.

(b) If (as was actually true) the volume is 100 L (not infinite), how long will it take for the blood concentration of urea to drop to 37% of its original value?

In this case we must first make a material balance to find what the buffer concentration is when the blood concentration has falled to 0.37 C_{B0}: $C_D = (V_B/V_D)\, C_{B0}\, (1-0.37)$. This gives $C_D/C_{B0} = 0.315$ and the final concentration difference is very small. 0.055 C_{B0}. As derived in class the natural log of the concentration difference between body and buffer equals $-(PA*(1/V_B + 1/V_D)\, t$. When the blood concentration has fallen to 37%, the buffer concentration has risen to 18.5% (since the volume ratio is 2:1) and the final difference is 18.5% of c_0. Thus:

$$-\ln(0.055) = (10)^{-4}\, 5\,(10)^3\,(1/50000 + 1/100000)\,t$$

which I calculate to give a t of 193,400 sec., much longer than before because the finite size of the buffer reduces the concentration driving force.

(c) If (as was also actually true) the 100 L of washing buffer were replaced by fresh buffer after about 1 hr. and 33 min, to what fraction of its original value will the blood concentration of urea have been dropped after another 1 hr. and 33'? If you do not have time to calculate this value, estimate it in terms of the values calculated in parts (a) and (b).

Again the difference formula is used: For no replacement:

$$\frac{c_B}{c_{B0}} = \frac{(1+2e^{-Kt})}{3} \text{ where } K = PA\left(\frac{1}{V_B}+\frac{1}{V_D}\right)\text{, a fixed number in this problem}$$

and the difference between changing solution and not changing solution is

$$\frac{(1+2e^{-Kt})}{3} \text{ and } \left[\frac{(1+2e^{-Kt/2})}{3}\right]^2$$

a small numerical difference in this problem because the dialysis times proposed were very short.

(d) A small organ in an artificial kidney patient who is treated as described above has the following characteristics: volume: 50 cc, ‘effective’ area: 500 cm^2, ‘effective’ permeability between the urea in the organ and that in the blood bathing it (which is the organ’s environment) 300 cm/sec. Is the behavior of this organ ‘quasistatic’, or does it barely reflect the changes going on around it, or is its behavior midway between these extremes?

Compare the characteristic time of the small organ *(V/(PA))* with that of the system. The characteristic time is 3.3 10^{-4} sec, very fast compared to the values above. The organ behaves absolutely quasistatically.

3. (18 pts. 6 for each part.) A cylinder of tissue consumes oxygen at the spatially uniform rate of 10^{-17} g-moles/cm^3-sec. A cylinder of this tissue is placed inside an impermeable glass tube. The tube is 6 cm long and 0.1 cm in radius. The tissue is perfused with blood (its oxygen source) through a cylindrical hole of diameter 0.05 cm that is centered on, and runs along, the axis of the tube. Calculate the steady-state oxygen flux:

(a) At the blood-glass boundary.

For <u>any</u> radius r, $r_O \geq r \geq r_i$ one can write

$$2\pi rL\, J_{O2} = \pi L\left(r_o^2 - r^2\right) R_{O2}$$

There is an error in the problem statement: "blood-tissue" boundary was meant. There is no blood-glass boundary. For r equal to 0.05, I calculate J to be 2.36 $(10)^{-18}$.

(b) At a radius of 0.075 cm.

Here I calculated, using the same formula, 0.916 $(10)^{-18}$.

(c) At the edge of the cylindrical hole.

.Here the answer is clearly zero. No oxygen can go through the glass. If you use the formula you get the same answer.

4. (12 pts., 6 each part.) In old-fashioned blood oxygenators, blood flowed down a vertical wall of stainless steel, with one side of the film of flowing blood in contact with the wall and the other in contact with gas. If the direction of flow is –z, and the direction perpendicular to the wall is x (with x=0 at the wall), the velocity is $v_z(x)$ and it varies with x as follows:

$$v_z(x)=10\,[x/\delta]^2$$

where δ is the thickness of the film and the units of v are cm/sec.

The concentration in mM/L of oxygen is zero at z=0 and, in one experiment, is measured to be $3[1-(x/\delta)]$ at z= 10 cm. Calculate:

(a) the oxygen concentration in a container of blood collected from the flow at z= 10 cm.

Take the width to be any convenient value, say, 1 cm. The concentration equals the amount of oxygen flowing, divided by the volume of blood flowing:

$$\delta\int_0^1 10\left(\frac{x}{\delta}\right)^2 3\left(1-\left(\frac{x}{\delta}\right)\right) d\left(\frac{x}{\delta}\right) \div \delta\int_0^1 10\left(\frac{x}{\delta}\right)^2 d\left(\frac{x}{\delta}\right)$$

The oxygen flow is 5 $(10)^{-3}$ ***d*** mM/sec. The flow is 10 ***d*** cc/sec. Thus the concentration is 5 $(10)^{-4}$ mM/cc. (Note that the concentration given in the problem was in mM per liter.) Note that ***d*** cancels in this calculation.

(b) the oxygen flux across the blood-gas interface between z=0 and z=10 cm.

Since there was no oxygen in the inflow, the flux must be the amount of oxygen flowing out in the blood divided by the area. Consider a width of 1 cm^2, an area of 10 cm^2. The flux is then just 5 $(10)^{-4}$ ***d*** mM/cm^2-sec. The value of ***d*** was not specified.