Version AJK/1

EGT2
ENGINEERING TRIPOS PART IIA

Monday 6 May 2024   14.00 to 15.40

Module 3G2

MATHEMATICAL PHYSIOLOGY

<u>Answer:</u>

*Answer not more than **three** questions.*

All questions carry the same number of marks.

*The **approximate** percentage of marks allocated to each part of a question is indicated in the right margin.*

*Write your candidate number **not** your name on the cover sheet.*

STATIONERY REQUIREMENTS
Single-sided script paper

SPECIAL REQUIREMENTS TO BE SUPPLIED FOR THIS EXAM
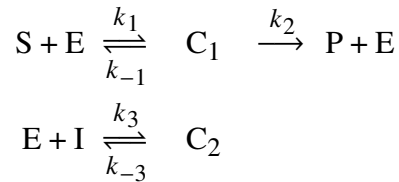CUED approved calculator allowed
Engineering Data Book

10 minutes reading time is allowed for this paper at the start of the exam.

You may not start to read the questions printed on the subsequent pages of this question paper until instructed to do so.

You may not remove any stationery from the Examination Room.

1   Researchers have collected data about the activity of an enzyme E with and without introducing an inhibitor, and would like to identify the mechanism of action of this inhibitor in the creation of a product P. They consider in particular these two mechanisms: competitive inhibition and uncompetitive inhibition.

(a)   Competitive inhibition.

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \overset{k_2}{\longrightarrow} P + E$$

$$E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2$$

Find an expression for the rate of product formation as a function of the substrate concentration [S], the inhibitor concentration [I] and total enzyme concentration E_0.   [35%]

Answer:

The conservation of the number of enzyme molecules implies that $[E] + [C_1] + [C_2] = E_0$. The quasi steady state assumption says that the concentrations of the two complexes do not change, which yields two equations:

$$\begin{aligned} k_1[S][E] &= (k_2 + k_{-1})[C_1] \\ k_3[I][E] &= k_{-3}[C_2] \end{aligned}$$

We define new combinations of the rate constants:

$$\begin{aligned} \frac{[S][E]}{[C_1]} &= \frac{(k_2 + k_{-1})}{k_1} \equiv K_M \\ \frac{[I][E]}{[C_2]} &= \frac{k_{-3}}{k_3} \equiv K_I \end{aligned}$$

Using the conservation equation, we get

$$\begin{aligned} [S](E_0 - [C_1] - [C_2]) &= K_M[C_1] \\ [I](E_0 - [C_1] - [C_2]) &= K_I[C_2] \end{aligned}$$

Rearranging gives the following equation for the C_1

$$[S](E_0 - [C_1] - \frac{[I](E_0 - [C_1])}{K_I + [I]}) = K_M[C_1]$$

yielding the expressions:

(cont.

$$[C_1] \quad = \quad \frac{[S]E_0K_I}{[S]K_I + [I]K_M + K_IK_M}$$
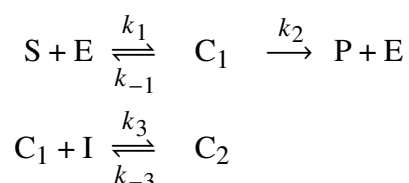
$$[C_2] \quad = \quad \frac{[I]E_0K_M}{[S]K_I + [I]K_M + K_IK_M}$$

Thus the overall rate of the reaction is

$$V \quad = \quad k_2[C_1] = \frac{k_2[S]E_0}{[S] + K_M(1 + [I]/K_I)}$$

$$V \quad = \quad \frac{V_{\max}[S]}{[S] + K'_M} \text{ with } K'_M = K_M(1 + [I]/K_I)$$

(b) Uncompetitive inhibition.

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \quad C_1 \quad \overset{k_2}{\longrightarrow} \quad P + E$$

$$C_1 + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \quad C_2$$

Find an expression for the rate of product formation as a function of the substrate concentration [S], the inhibitor concentration [I] and total enzyme concentration E_0.   [35%]

Answer:

In the case of uncompetitive inhibition, the inhibitor sequesters a fraction of the substrate-enzyme complex C_1 and prevent it to form the product.

We use the following notations: e=[E], i=[I], s=[S], c_1=[C_1], c_2=[C_2].

The rate is $V = k_2c_1$. We therefore need to have an expression of c_1 as a function of s and the total enzyme concentration E_0.

From the steady state assumption, we get:

$$\frac{dc_1}{dt} = 0 = k_1se - k_{-1}c_1 - k_3c_1i + k_{-3}c_2 - k_2c_1$$

$$\frac{dc_2}{dt} = 0 = k_3c_1i - k_{-3}c_2$$

The second equation leads to $c_2 = c_1i/K_I$, with $K_I = k_{-3}/k_3$. We can also obtain a relationship between e and c_1 using the first and second equations:

$$k_1se = k_{-1}c_1 + k_3c_1i - k_{-3}c_2 + k_2c_1 = (k_{-1} + k_3i - k_{-3}i/K_I + k_2)c_1 = (k_{-1} + k_2)c_1$$

Hence:

$$e = c_1K_M/s$$

Since the total enzyme concentration is $E_0 = e + c_1 + c_2$, we get:

$$E_0 = (K_M/s + 1 + i/K_I)c_1$$

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The expression of the rate of product creation V therefore becomes:

$$V = \frac{k_2 E_0 [S]}{[S](1 + [I]/K_I) + K_M}$$

$$V = \frac{V'_{max}[S]}{[S] + K'_M}$$

with $K'_M = \dfrac{K_M}{1 + [I]/K_I}$ and $V'_{max} = \dfrac{V_{max}}{1 + [I]/K_I}$

(c)   Using the data below, identify a likely mechanism for the inhibition process. Justify your answer.   [30%]

Substrate concentration (mmol/L)	Rate of product formation without inhibitor (mmol/L/min)	Rate of product formation with inhibitor (mmol/L/min)
0.710	0.200	0.180
0.400	0.180	0.150
0.310	0.160	0.110
0.098	0.120	0.070
0.066	0.100	0.050
0.040	0.070	0.040

Answer: On a qualitative level, it seems that the concentration of S increases, the rates converge to the same value. This is consistent with a competitive inhibition, which does not change Vmax.

More precisely, for both processes, we would expect the data with and without inhibitor to fall on a line when plotting 1/V as a function of 1/[S].

$$\frac{1}{V} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \frac{1}{[S]}$$

This is indeed the case on a plot.

It is then apparent that the two curves have similar intercept with the y-axis but different slopes. This means that the inhibitor does not change V_{max} but changes the ratio K_M/V_{max}. This is consistent with competitive inhibition and goes against an uncompetitive mechanism which would imply a change of V_{max} but contant K_M/V_{max}.

2   This question is about ion flux at the Nernst potential.

(a)   Give the formula for the Nernst–Planck equation, describing the flux of an ion along an ion channel, and explain the meaning of each symbol in it.   [10%]

Answer:

$$J(x,t) = -D \left(\frac{\partial}{\partial x} c(x,t) + \frac{zF}{RT} c(x,t) \frac{\partial}{\partial x} \phi(x,t) \right) \tag{1}$$

where x is one dimensional space (along the length of a channel), t is time, J is the flux of the ion, c is its concentration, D is its diffusion coefficient, z is its valence, T is temperature, F is the Faraday constant, and R is the universal gas constant.

(b)   Give the formula for the Nernst potential of an ion, and explain the meaning of each symbol in it.   [5%]

Answer:

$$V = \frac{RT}{zF} \ln \frac{c_e}{c_i} \tag{2}$$

where V is membrane potential (electric potential difference between the two ends of the channel), c_e and c_i are the concentrations of the ion at the extra- and intracellular ends of the channel, respectively, z is the valence of the ion, T is temperature, F is the Faraday constant, and R is the universal gas constant.

(c)   Prove that whenever the membrane potential is equal to the Nernst potential, the following equation holds: [25%]

$$\int_0^L \frac{J(x,t)}{c(x,t)} \, dx = 0$$

where x is the position along the channel, t is time, J is the flux of the ion, c is its concentration, and L is the length of the channel.

Answer:

$$
\begin{aligned}
V(t) &= \frac{RT}{zF} \ln \frac{c_e(t)}{c_i(t)} \\
\phi(L,t) - \phi(0,t) &= -\frac{RT}{zF} \left(\ln c(L,t) - \ln c(0,t) \right) \\
\int_0^L \frac{\partial}{\partial x} \phi(x,t) \, dx &= -\int_0^L \frac{RT}{zF} \frac{\partial}{\partial x} \ln c(x,t) \, dx \\
\int_0^L \left(\frac{\partial}{\partial x} \phi(x,t) + \frac{RT}{zF} \frac{\frac{\partial}{\partial x} c(x,t)}{c(x,t)} \right) dx &= 0 \\
\int_0^L \frac{\frac{\partial}{\partial x} c(x,t) + \frac{zF}{RT} c(x,t) \frac{\partial}{\partial x} \phi(x,t)}{c(x,t)} \, dx &= 0 \\
\int_0^L \frac{J(x,t)}{c(x,t)} \, dx &= 0 \tag{3}
\end{aligned}
$$

(d)   Based on the equation in part (c), explain with reasons if the membrane potential being equal to the Nernst potential of the ion implies that the flux of the ion is zero everywhere inside the channel.   [10%]

Answer: No, it doesn't imply that, because $J(x, t)$ could be negative at some x and positive at some other x, so that they cancel out in the integral.

(e)   Answer if each of the following statements is always true for the steady state of an ion channel. (You can assume that the channel is only permeable to a single ion.)   [20%]

>   (i)   No individual ions move along the channel;
>
>   Answer: Not always true.
>
>   (ii)   There is no net movement of ions along the channel;
>
>   Answer: Not always true.
>
>   (iii)   The flux of the ion is constant as a function of space along the channel;
>
>   Answer: Always true.
>
>   (iv)   The flux of the ion is zero everywhere along the channel.
>
>   Answer: Not always true.

(f)   The equation in part (c) holds at any time (when the condition from which it was derived holds). What is its form when the channel is in steady state? Describe the meaning of any symbols that are different from those in the equation in part (c) and explain how they depend on time and space.   [20%]

Answer:
$$\int_0^L \frac{J^\star}{c^*(x)}\, dx = 0 \tag{4}$$
$J^\star$ is steady-state flux, which is neither a function of time, because the channel is in steady-state, nor a function of space, because at steady-state the flux is constant (though not necessarily zero). $c^*(x)$ is steady-state concentration, which is not a function of time, because the channel is in steady-state, but it can be a function of space, because at steady-state the concentration may not be constant (let alone zero).

(g)   Prove that whenever the membrane potential is equal to the Nernst potential, and the channel is in steady state, the flux of the ion is zero everywhere inside the channel.   [10%]

Answer: In this case, Equation 4 holds. Because $J^\star$ does not depend on x, we can rewrite equation 4 as

$$J^\star \int_0^L \frac{1}{c^*(x)}\, dx = 0 \tag{5}$$
$$J^\star = 0 \tag{6}$$

3   Consider a Bingham fluid flowing in a cylindrical rigid vessel of radius R and length L, in steady state. A Bingham fluid has a rheology defined by the following constitutive equation:

$$\tau = \tau_y + \eta \dot{\gamma}$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, and τ_y and η are constants. The following equations characterise force balance at the steady state in a cylindrical vessel in polar coordinates, where x is the coordinate along the flow direction, and $p(x, r)$ is the pressure field in the fluid.

$$\frac{\partial p}{\partial x} = \frac{1}{r} \frac{\partial (r\tau)}{\partial r}$$

$$\frac{\partial p}{\partial r} = 0$$

(a)   What is the minimum pressure drop ΔP_c across the vessel required to have a non-zero flow rate through the vessel?   [25%]

Answer: The second force balance equation indicates that the pressure does not depend on r. So we can use the separation of variable to solve the first one, indicating that dp/dx is constant (and negative here, as pressure drops). We can then integrate the first equation to find $\tau(r)$.

$$\frac{\partial (r\tau)}{\partial r} = \frac{\partial p}{\partial x} r$$

$$r\tau = \frac{1}{2} \frac{\partial p}{\partial x} r^2 + C$$

$$\tau = \frac{1}{2} \frac{\partial p}{\partial x} r + \frac{C}{r}$$

Now the stress must be finite at $r = 0$, so C must be zero.

$$\tau = \frac{1}{2} \frac{\partial p}{\partial x} r$$

Note that here τ must be negative too. A flow will develop as soon as the stress at its largest point, i.e. at the boundary, has a magnitude larger than the yield stress τ_y. We assume here that ΔP is defined as positive (magnitude of the pressure drop), we have $\frac{\partial p}{\partial x} = -\frac{\Delta P}{L}$ (other sign conventionsare fine as long as they are consistent). If there is a flow, we therefore have:

$$\frac{1}{2} \frac{\Delta P}{L} R > \tau_y$$

$$\frac{\Delta P}{L} > \frac{2\tau_y}{R}$$

Hence:

$$\Delta P_c = \frac{2L\tau_y}{R}$$

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(b) Find a mathematical expression of the flow profile in the case where the material is flowing, as a function of the pressure drop ΔP and other parameters defined above. [50%]

Answer: Considering that the flow is directing towards positive x, the velocity profile $u(r)$ is positive and maximum at the centre and decreases with r. du/dr and the shear stress are therefore negative, and the constitutive equation writes here:

$$\tau = -\tau_y + \eta \frac{\partial u}{\partial r}$$

This expression can then be introduced in the equation for $\tau(r)$ obtained in the previous section and solve for $u(r)$

$$\frac{\partial u}{\partial r} = \frac{1}{2\eta} \frac{\partial p}{\partial x} r + \frac{\tau_y}{\eta}$$

$$u = \frac{1}{4\eta} \frac{\partial p}{\partial x} r^2 + \frac{\tau_y}{\eta} r + D$$

D is determined using the no-slip boundary condition: $u(R) = 0$.

$$D = -\frac{1}{4\eta} \frac{\partial p}{\partial x} R^2 - \frac{\tau_y}{\eta} R$$

(c) Sketch the flow profiles for the following conditions: $\Delta P = \Delta P_c/2$, $\Delta P = 2\Delta P_c$, $\Delta P \gg \Delta P_c$. [25%]

Answer: For $\Delta P = \Delta P_c/2$, there is no flow; $u(r) = 0$. For $\Delta P = 2\Delta P_c$, the flow profile is flat (plug-like) between $r = 0$ and $R/2$, and parabolic between $R/2$ and R. For $\Delta P \gg \Delta P_c$, the plug region is negligible and the flow is approximately parabolic.

4   (a)   The Krogh cylinder model allows us to estimate the oxygen concentration c in tissues as a function of the distance to the closest capillary. Explain the interpretation of the Krogh cylinder radius R_0 in this model.   [10%]

Answer: The Krogh cylinder radius represents half of the typical distance between two blood capillaries. In the Krogh model, space is segmented to allocate each point in the tissue to its closest capillary, defining domains that have approximately the shape of cylinders.

(b)   Using appropriate assumptions, show that the concentration profile takes the following form:

$$\frac{c(r)}{c_c} = 1 + \Phi \left(\frac{r^2}{R_0^2} - R^{*2} - 2 \ln \left(\frac{r}{R_0 R^*} \right) \right)$$

where r is the distance to the centre of the closest capillary, c_c is the oxygen concentration in the capillary, and Φ and R^* are constants. Find expressions for Φ and R^* as a function of the radius R_0 of the Krogh cylinder, the radius R_c of the capillary, the concentration c_c of oxygen in the capillary, the rate of oxygen consumption per unit time ρ and the coefficient of diffusion D of the oxygen in the tissue.   [50%]

Answer: In the steady state and cylindrical geometry:

$$\frac{D}{r} \frac{d}{dr} \left(r \frac{dc}{dr} \right) = \rho$$

The general solution is:

$$c(r) = \frac{\rho}{4D} r^2 + A \ln(r) + B$$

with the following boundary conditions:

$$c(R_c) = c_c \text{ and } \frac{dc}{dr}(R_0) = 0$$

We obtain:

$$\frac{c(r)}{c_c} = 1 + \frac{\rho R_0^2}{4 c_c D} \left(\frac{r^2}{R_0^2} - \frac{R_c^2}{R_0^2} - 2 \ln(r/R_c) \right)$$

We denote $\Phi = \frac{\rho R_0^2}{4 c_c D}$ (dimensionless reaction rate). We define as well the dimensionless geometrical parameter: $R^* = R_c / R_0$:

$$\frac{c(r)}{c_c} = 1 + \Phi \left(\frac{r^2}{R_0^2} - R^{*2} - 2 \ln \left(\frac{r}{R_0 R^*} \right) \right)$$

(c)   Figure 1 shows the line defined by:

$$\Phi \left(R^{*2} - 2 \ln \left(R^* \right) - 1 \right) = 1$$

Explain the significance of this line and show how it is derived mathematically.   [20%]
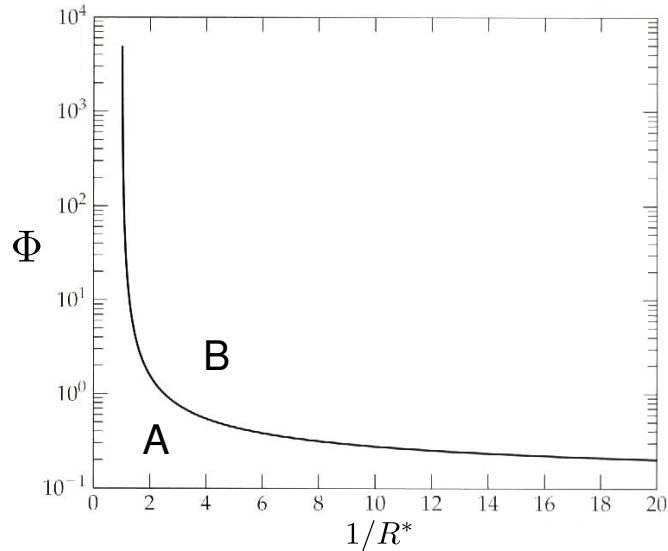

Fig. 1

<u>Answer:</u> This line corresponds to the condition $c(r = R_0) = 0$, i.e. the situation where there is just enough oxygen to supply the whole volume of the cylinder.

(d)   Sketch the oxygen concentration profiles for two tissues whose oxygenation states are in domain A and domain B respectively.   [20%]

<u>Answer:</u> In case A, the consumption is low enough to have a concentration always above zero. It starts at c_c at the radius of the capillary, and ends with a zero gradient at $r = R_0$.

In case B, there is not enough oxygen, and the concentration reaches zero before $r = R_0$, and a concentration equal to zero beyond this point, indicating a region where the tissue would die.

END OF PAPER

Comments from examiners

Q1: This was a very popular question. It was a straight forward application of what students had covered in the course, and students did overall very well indeed. When comparing models to data, it is important to discuss both model. Show that one of them works doesn't mean that the other one doesn't, and vice-versa.

Q2: This was a moderately popular question, with some excellent solutions, and a couple of poor attempts. Some responses were outright dimensionally inconsistent (in a or b). Typical errors included misunderstanding the somewhat paradoxical nature of steady-state in an ion channel (e/ii), where concentrations do not change over time, and yet there may be constant uniform flux across the channel (because concentrations are assumed to be maintained at the two ends "externally"), and not recognising that at steady state the flux is not only time-independent (i.e. constant), but also space-independent (i.e. uniform).

Q3: Bingham fluid. This was a very popular question, but a number of students struggled with it nonetheless. Although a similar question (with a different constitutive equation) is part of the examples paper, some students had issues understanding how the presence of a yield stress affects the flow profiles. One of the most common issues concerned the consistency of the signs of the shear stress and pressure drop or gradient.

Q4: Krogh model. The question was fairly popular, and generally well answered. Many students could write and solve the reaction diffusion equation properly in cylindrical coordinates, and use the right boundary conditions. The graph was also well understood and well interpreted.