Circle the best answer from the bracket (從中括弧中選擇一個最正確的答案) or fill in the blanks along with the SI units after the calculation (底線處請將計算後的答案連同 SI 單位作答). Use of metric prefixes for the units such as nm for nanometer is preferable (建議使用公制前置詞如 nm 表示奈米等單位) (一格 2.5 分；滿分 100 分) ※ 注意：請於試卷內之「非選擇題作答區」作答，並應註明作答之題號。

1. A biological reactions is generally very **[rapid; selective; slow]** to certain chemical (S) known as the **[scaffold; substance; substrate]** of its relating enzyme (E) which is normally a **[carbohydrate; lipid; nucleic acid; protein]**. The chemical binds to the **[allosteric; active; passive]** site of the enzyme to become an ES complex with a/an **[equilibrium; steady; transition]** state of much lower **[activation; catalyzing; kinetic]** energy than the non-catalyzed reaction. The reaction therefore can occur under **[harsh; mild]** conditions (e.g. temperature and pressure) with sufficient efficiency.

2.

$$E + S \xleftarrow{K_1 / K_{-1}} ES \xrightarrow{K_2} E + P$$

Where K_1 and K_{-1} are the forward and backward rate constant for the binding/dissociation equilibrium of the enzyme and substrate, respectively. The rate constant for producing the product (P), K_2, is called the turnover rate of the enzyme having the SI unit of **[$M^{-1}S^{-1}$; S^{-1}; MS^{-1}]**.

In the aforementioned Michaelis-Menten model, the reaction rate (V) is proportional to the concentration of ES complex as the following **[zero; first; second]**-order kinetics.

$$V = -\frac{d[S]}{dt} = \frac{d[P]}{dt} = K_2[ES]$$

In the **[equilibrium; steady; transition]** state of the reaction, the concentration of ES complex can be solved as follows.

$$\frac{d[ES]}{dt} = 0$$
$$K_1[E][S] = (K_{-1} + K_2)[ES]$$
$$\frac{[ES]}{[E]} = \frac{K_1[S]}{K_{-1} + K_2} = \frac{[S]}{K_M}$$
$$\frac{[ES]}{[E]+[ES]} = \frac{[ES]}{[E]_t} = \frac{[S]}{K_M + [S]}$$
$$[ES] = \frac{[S]}{K_M + [S]}[E]_t$$

Where K_M is called the *Michaelis* constant of the enzyme with the same dimension as **[concentration; length; mass; reaction rate]**; $[E]_t$ is total concentration of enzyme which is a **[constant; function; variable]** during the

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reaction. The higher the K_M, the **[lower; higher]** the affinity of the enzyme with the substrate.

An enzymatic reaction rate therefore increases non-linearly with the substrate concentration and reaches a maximum (V_{max}) as the substrate concentration being much higher than **[K_1; K_{-1}; K_2; K_M]**. In most cases the substrate concentration is much lower than K_M, so the reaction rate will be linearly related to the multiplicative product of substrate concentration and enzyme concentration. The reaction turns out to be a **[zero; first; second]**-order kinetics. The relating coefficient, K_2/K_M, is called the catalytic efficiency of the enzyme with the SI unit of **[$M^{-1}S^{-1}$; S^{-1}; MS^{-1}]**. For an enzyme with a high turnover number, the catalytic efficiency will be approximately equal to **[K_1; K_{-1}; K_2; K_M]**.

$$V = K_2[ES] = K_2[E]_t \frac{[S]}{K_M + [S]} = V_{\max} \frac{[S]}{K_M + [S]} \cong \frac{K_2}{K_M}[E]_t[S] \text{ when } [S] << K_M$$

$$V \cong \frac{K_2}{K_M}[E]_t[S] \cong \frac{K_2}{\frac{K_{-1} + K_2}{K_1}}[E]_t[S] \cong K_1[E]_t[S] \text{ when } K_{-1} << K_2$$

3.

For a reaction catalyzed by 1.0 nM enzyme A with K_M=1.0 mM and V_{MAX}= 0.1 mMS^{-1}, the reaction rate will be _____ when the substrate concentration is 9.0 mM. The turnover number of the enzyme is _____. Enzyme B (1.0 nM) has a K_M of 0.1mM and V_{MAX} of 0.05 mMS^{-1}, compared with enzyme A, enzyme B has a **[higher; lower]** affinity with the substrate and **[higher; lower; similar]** catalytic efficiency.

Since the kinetics is not linear, it will be more convenient to analyze the parameters by plotting the **[absolute; logarithmic; reciprocal]** values of the reaction rate against the **[absolute; logarithmic; reciprocal]** of the substrate concentration. The **[intercept; origin; slope]** of the plot will be proportional to the *Michaelis* constant, and the V_{max} can be obtained by the **[intercept; origin; slope]**.

$$\frac{1}{V} = \frac{1}{V_{\max}}\left(\frac{K_M + [S]}{[S]}\right) = \frac{1}{V_{\max}} + \left(\frac{K_M}{V_{\max}}\right)\frac{1}{[S]}$$

4.

The following figures compares the plots with (the dashed line) or without (the solid line) the existence of a/an **[competitive; non-competitive; uncompetitive]** inhibitor which will alter the apparent K_M (the slope of the plot) of the enzyme kinetics without changing the V_{max}.

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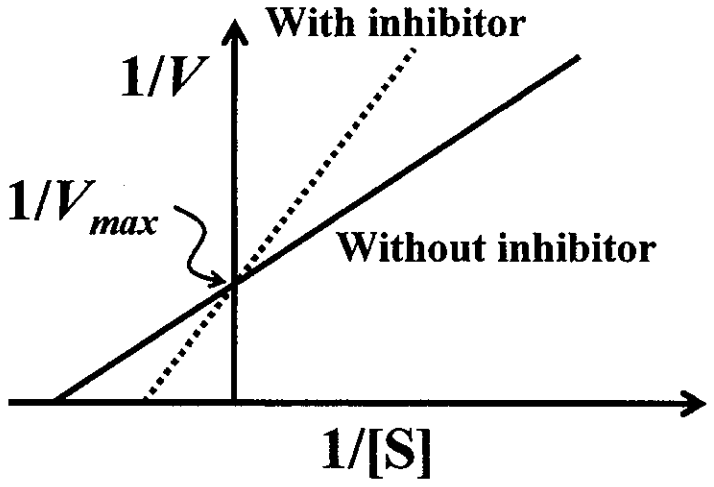

The inhibitor (I) can bind to the enzyme to form EI complex with a **[binding; chelating; dissociation]** constant, K_I. The higher the inhibitor concentration, the **[lower; higher]** the apparent K_M and the **[lower; higher]** the apparent affinity of the enzyme with the substrate since the binding was hindered by the existence of the inhibitor.

$$E + I \xrightarrow{K_I} EI$$

$$K_I = \frac{[I][E]}{[EI]}$$

$$\frac{[ES]}{[E]_t} = \frac{[ES]}{[E]+[ES]+[EI]} = \frac{[E]\times\frac{[S]}{K_M}}{[E](1+\frac{[S]}{K_M}+\frac{[I]}{K_I})} \cong \frac{[S]}{K_M+[S]} \text{ when } [I]\to 0$$

$$V = V_{\max}\times\frac{[E]\times\frac{[S]}{K_M}}{[E](1+\frac{[S]}{K_M}+\frac{[I]}{K_I})}$$

$$\frac{1}{V} = \frac{1}{V_{\max}}(\frac{K_M}{[S]}+1+\frac{[I]K_M}{[S]K_I}) = \frac{1}{V_{\max}}+(1+\frac{[I]}{K_I})\frac{K_M}{V_{\max}}\frac{1}{[S]}$$

With 10 nM competitive inhibitor (K_I =5.0 nM) existing during a catalyzed reaction (K_M=6.0 mM; V_{MAX}= 0.12 mMS^{-1}), the apparent K_M will become _____, while V_{MAX} will be _____.

5.

According to Arrehenius expression, the rate constant (*k*) of an enzyme will be affected by the temperature (*T*) and the activation energy (*Ea*). The reaction for the substrate possesses an *Ea* much **[lower; higher]** than other chemicals, which is one of the origins of the substrate **[priority; specificity]** of the enzyme.

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$$k = Ae^{-E_a/RT}$$

$$V = k[E]_t[S] = Ae^{-E_a/RT}[E]_t[S]$$

$$\ln V = \ln(A[E]_t[S]) - \frac{E_a}{R}(\frac{1}{T})$$

The best way to calculate the activation energy is to multiply the **[intercept; origin; slope]** of the semi-logarithmic plot of V againt $(1/T)$ with the negative value of **[Avogadro; Faraday; gas; Planck]** constant, the plot can be conducted by investigating the change in **[enzyme concentration; substrate concentration; reaction rate; temperature]** at different **[enzyme concentration; substrate concentration; reaction rate; temperature]**.

$$Ethanol + NAD^+ \xrightarrow{ADH} Acetaldehyde + NADH + H^+$$

$$Methanol + NAD^+ \xrightarrow{ADH} Formaldehye + NADH + H^+$$

The alcohol dehydrogenase (ADH) in cod liver catalyzes the oxidation of alcohol with a substrate specificity of ethanol over methanol since formaldehye is highly toxic for the fish. At 37°C (0°K ≅ -273°C), the oxidation rate of ethanol in cod liver is 100-fold higher than that of methanol, the difference in activation energy of the enzymatic oxidation for the two substrates will be _____ (gas constant ≅ 0.08 $atmL^{-1}mol^{-1}K^{-1}$, or 8.0 $J\ mol^{-1}K^{-1}$; ln10≅2.3). As cod lives in cold sea water (e.g. 7°C), the ADH-catalyzed oxidation reaction of ethanol will have a **[higher; lower]** reaction rate but with the substrate specificity **[equal to; higher than; lower than]** that that of 37°C.

試題隨卷繳回