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CHEMISTRY II

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SOLUTIONS OF BIOPHYSICS AND CATALYSIS EXERCISES

Exercise 1. Utilize the Michaelis-Menten equation to illustrate the following assessments:

a) When the substrate concentration [S] significantly surpasses the Michaelis constant (K_m), the initial velocity (V_o) becomes independent of [S].

b) When the substrate concentration [S] is considerably less than the Michaelis constant, the reaction is first order with respect to S.

c) At the point where the initial velocity (V_o) is half of the maximum velocity (V_{max}), the substrate concentration ([S]) equals the Michaelis constant (K_m).

SOLUTION

a) When [S] >> K_m, K_m + [S] ≈ [S]. Substrate concentration has no effect on velocity, and $V_0 = V_{max}$, as shown in the upper part of the curve:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \approx \frac{V_{max}[S]}{[S]} = V_{max}$$

b) When [S] << K_m, K_m + [S] ≈ K_m, and the Michaelis-Menten equation simplifies to:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \approx \frac{V_{max}[S]}{K_m}$$

Velocity is related to [S] by a constant value, and the reaction is first order with respect to S, as shown in the lower part of the curve.

c) When $V_0 = V_{max}/2$, K_m = [S]:

$$V_0 = \frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

$$K_m + [S] = 2[S]$$

$$K_m = [S]$$

Exercise 2. Initial velocities have been determined for the interaction between α-chymotrypsin and tyrosine benzyl ester substrate ([S]) across a range of six distinct substrate concentrations. Employ the provided data to formulate an estimation of both the maximum reaction velocity (V_{max}) and the Michaelis constant (K_m) for this specific substrate.

[S] (mM)	0.00125	0.01	0.04	0.10	2.0	10
V_0 (mM/min)	14	35	56	66	69	70

SOLUTION

The initial speeds tend to stabilize at elevated substrate concentrations, allowing us to approximate the V_{max} at 70 mM/min. As K_m represents the substrate concentration [S] needed to achieve half the maximum speed, we can approximate the K_m at 0.01 mM based on the initial velocity value of 35 mM/min.

Exercise 3. An enzyme named strongase is identified, catalyzing the chemical reaction:

$$WEAK \rightleftarrows STRONG$$

A dedicated team of researchers conducts extensive studies on strongase, discovering its catalytic efficiency (k_{cat} = 600 s^{-1}). In further experiments, they set the enzyme concentration [E_t] to 10 nM and measure a reaction velocity (V_0) of 3 µM s^{-1}. What is the substrate concentration ([S]) employed in this specific experiment?

Data: K_m = 10 µM.

SOLUTION

For this enzyme concentration, the V_{max} stabilizes at 6 µM s^{-1} ($V_{max} = K_{cat} \times [E]_{total}$), with V_0 exactly half of Vmax. In accordance with the definition of K_m, which corresponds to the substrate concentration [S] at $V_0 = ½V_{max}$, the estimated [S] is 10 µM.

If V_0 deviates from $½V_{max}$, the expression $V_0/V_{max} = [S] / (K_m + [S])$ can be employed for [S] determination.

Exercise 4. An enzyme operating under Michaelis-Menten kinetics exhibits a K_m value of 1 µM. At a substrate concentration of 100 µM, the initial velocity is measured to be 0.1 µM min^{-1}. Determine the initial velocity when the substrate concentration ([S]) is (a) 1 mM, (b) 1 µM, or (c) 2 µM.

SOLUTION

[S] >> K_m when [S] = 100 µM, therefore, $V_0 = V_{max}$ = 0.1 µM min^{-1}.

(a) At concentrations greater than 100 µM, $V_0 = V_{max}$ = 0.1 µM min^{-1}.

(b) [S] = K_m, then $V_0 = V_{max}/2$ = 0.05 µM min^{-1}.

(c) Michaelis-Menten equation must be applied:

$V_0 = V_{max} \times [S] / (K_m + [S]) = (0.1 \times 2) / (1 + 2)$ = 0.067 µM min^{-1}.

Exercise 5. An enzyme facilitates the conversion of substrate A to product B. With an enzyme concentration of 2 nM and a V_{max} of 1.2 µM s^{-1}, and a K_m of 10 µM for substrate A, determine the initial velocity (V_0) at the following substrate concentrations: (a) 2 µM, (b) 10 µM, (c) 30 µM.

SOLUTION

Initial velocities are determined through Michaelis-Menten equation:

$$V_0 = V_{max} \times [S] / (K_m + [S])$$

(a) 0.2 µM s^{-1}; 0.6 µM s^{-1}; 0.9 µM s^{-1}.

Exercise 6. The enzymatic activity of an intestinal peptidase with glycylglycine as the substrate was investigated, and the following experimental data were gathered:

$$Glycylglycine + H_2O \rightarrow 2\ glycine$$

[S] (mM)	1.5	2.0	3.0	4.0	8.0	16.0
Product formed (μmol min^{-1})	0.21	0.24	0.28	0.33	0.40	0.45

Utilize graphical analysis to ascertain the V_{max} and K_m values for this particular enzyme-substrate combination.

SOLUTION

Lineweaver-Burk equation is the reciprocal of the Michaelis-Menten equation and it is more useful in plotting experimental data:

$$1/V_0 = (1/V_{max}) + K_m / (V_{max} \times [S])$$

Graphical representation of the data gives a y-intercept of 2.1 μM min^{-1} ($1/V_{max}$) and a slope of 4.13 mM min μM^{-1} (K_m/V_{max}). Therefore, V_{max} = 0.48 μM min^{-1} and K_m = 2 mM.

Exercise 7. Human immunodeficiency virus 1 (HIV-1) encodes a protease with a molecular weight of 21,500 g mol^{-1}, crucial for the virus's assembly and maturation. The protease facilitates the hydrolysis of a heptapeptide substrate with a k_{cat} of 1000 s^{-1} and a K_m of 0.075 M.

a) Determine the V_{max} for substrate hydrolysis when HIV-1 protease is present at 0.2 mg mL^{-1}.

b) In an experiment where the -C(O)NH- of the heptapeptide is replaced by -CH_2NH-, the resulting derivative acts as an inhibitor, rendering it incapable of being cleaved by HIV-1 protease. Under the same conditions as in part (a), but with the presence of 2.5 μM inhibitor, the observed V_{max} is 9.3×10^{-3} M s^{-1}. Identify the type of inhibition occurring and discuss whether this type of inhibition is expected for a molecule with this structure.

SOLUTION

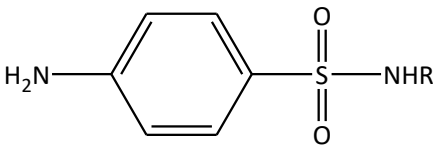
a) First, $[E_t]$ is determined and then V_{max} is calculated:

$$[E]_{total} = 0.2 \text{ g } L^{-1} / 21{,}500 \text{ g } mol^{-1} = 9.3\times10^{-6} \text{ M}$$

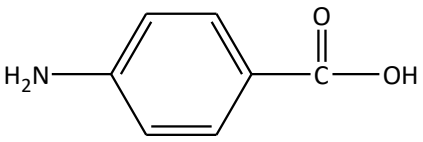
$$V_{max} = k_{cat} \times [E]_{total} = 1000 \text{ } s^{-1} \times 9.3\times10^{-6} \text{ mol } L^{-1} = 9.3\times10^{-6} \text{ M } s^{-1}$$

b) V_{max} is unchanged; therefore, competitive inhibition is taken place. That is the case when inhibitor closely resembles the substrate.

Exercise 8. Sulfonamides, such as sulfanilamide, act as antibacterial drugs by inhibiting the enzyme dihydropteroate synthase (DS), crucial for bacterial folic acid synthesis. Predict the type of inhibition for the bacterial enzyme in the presence of sulphonamides if *p*-aminobenzoic acid (PABA) serves as a substrate for DS. To visualize this, build a double reciprocal plot (Lineweaver-Burk plot) with the x-axis representing the inverse of substrate concentration (1/[S]) and the y-axis representing the inverse of reaction velocity ($1/V_0$).



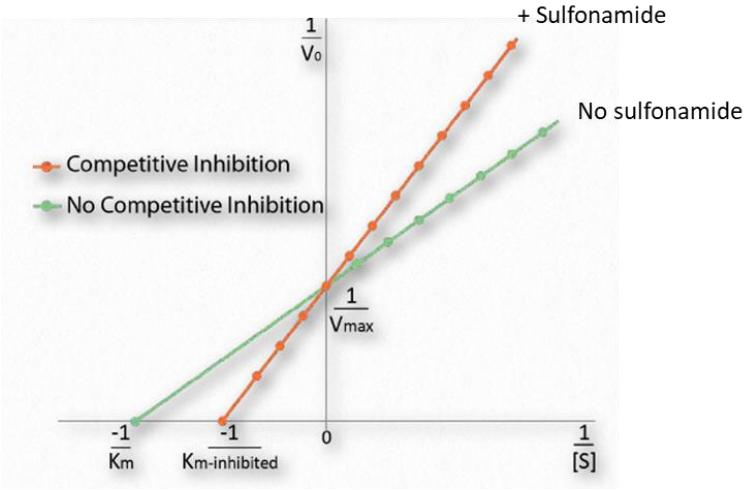
Sulfonamides
(R = H; sulphanilamide)



p-Aminobenzoic acid

SOLUTION

Inhibitor sulfonamides structurally match the *p*-aminobenzoic acid substrate. Hence, it can be predicted that they act as competitive inhibitors; sulfonamides bind to the enzyme site.



Exercise 9. (a) Draw a Lineweaver-Burk plot using the reciprocal values of substrate concentration (1/[S]) and initial velocity ($1/V_0$) to determine the V_{max} and K_m values for the fumarase-catalyzed reaction, given fumarate concentrations and initial velocities:

Fumarate (mM)	2.0	3.3	5.0	10.0
Rate (mmol min^{-1})	2.5	3.1	3.6	4.2

(b) Fumarase, with a molecular weight of 194,000 g/mol and comprising four identical subunits, each housing an active site, has an enzyme concentration of $1x10^{-8}$ M in the experiment from part (a). Calculate the k_{cat} value for the fumarase reaction with fumarate, noting that k_{cat} units are reciprocal seconds (s^{-1}).

SOLUTION

(a) Before plotting the kinetic data for fumarase, compute the reciprocals of substrate concentrations and the initial rates of product formation. Plot $1/V_0$ *versus* 1/[S]. Graphical representation of the data gives a y-intercept of 0.2 mmol L^{-1} min^{-1} ($1/V_{max}$) and a x-intercept of – 0.5 mM^{-1} (K_m/V_{max}). Therefore, V_{max} = 5.0 mM min^{-1} and K_m = 2.0 mM.

(b) The k_{cat} signifies the quantity of reactions executed per second by a single active site of the enzyme. Despite the enzyme concentration being 1×10^{-8} M, fumarase exists as a tetramer, possesses four active

sites per molecule. Consequently, the collective concentration of enzyme active sites, denoted as $[E]_{total}$, amounts to 4×10^{-8} M. From the equation:

$$V_{max} = k_{cat} \times [E]_{total} \Rightarrow k_{cat} = 5.0 \text{ mM min}^{-1} / (4\times10^{-5} \text{ mM}\times60 \text{ s min}^{-1}) = 2\times10^{-3} \text{ s}^{-1}$$

Exercise 10. The cytochrome P450 family of monooxygenase enzymes plays a crucial role in eliminating foreign compounds, including drugs, from our body. P450s are present in various tissues such as the liver, intestine, nasal tissues, and lungs. Pharmaceutical companies, seeking approval for human use by the Federal and Drug Administration, must investigate the drug metabolism by cytochrome P450 for every approved drug. Many drug-drug interactions are associated with interactions with cytochrome P450 enzymes. P450 3A4, a significant member of these enzymes, is responsible for metabolizing a substantial portion of drugs. Human intestinal P450 3A4, for instance, is involved in the metabolism of midazolam, a sedative, producing a hydroxylated product known as 1'-hydroxymidazolam. The provided kinetic data are for the reaction catalyzed by P450 3A4:

Midazolam (μM)	Rate of product formation (pmol L^{-1} min^{-1})	Rate of product formation in the presence of 0.1 μM ketoconazole (pmol L^{-1} min^{-1})
1	100	11
2	156	18
4	222	27
8	323	40

(a) Analyzing the initial two columns, calculate the K_m and V_{max} for the enzyme through a Lineweaver-Burk plot.

(b) Investigate the impact of ketoconazole, an antifungal agent, on the P450-catalyzed hydroxylation of midazolam using the provided data. Determine the type of inhibition exerted by ketoconazole in this context.

SOLUTION

(a) Before plotting the kinetic data for P450 3A4, determine the reciprocals of substrate concentrations and the initial rates of product formation. Plot $1/V_0$ *versus* 1/[S]. Graphical representation of the data gives a y-intercept of 0.0025 pmol L^{-1} min^{-1} ($1/V_{max}$) and a x-intercept of $-$ 0.3 μM^{-1} (K_m/V_{max}). Therefore, V_{max} = 400 pM min^{-1} and K_m = 3.3 μM.

(b) The double reciprocal plot depicts an elevated y-intercept with no discernible alteration in the x-intercept. Analysis of the double reciprocal plot suggests that ketoconazole functions as a noncompetitive inhibitor. In this inhibitor type, V_{max} appears to decrease ($1/V_{max}$ increases), while K_m remains unchanged.

IMAGE CREDITS

- Graph of exercise 8: Biochemistry: Free For All, 2018. Kevin Ahern, Indira Rajagopal, and Taralyn Tan, Oregon State University. https://open.umn.edu/opentextbooks/textbooks/866.
- Images of compounds in exercise 8 were made by authors.