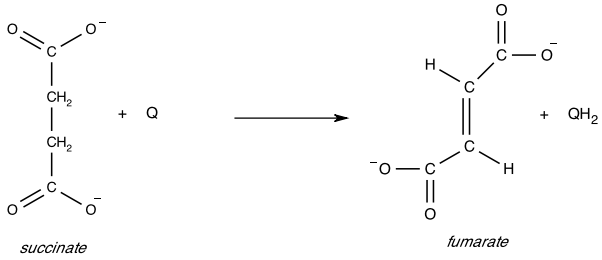
Name Key

Chem 352 - Fall 2018
Quiz 2

$R = 8.314\ J/(mol \cdot K) = 0.08206\ (L \cdot atm)/(mol \cdot K)$

1. Succinate dehydrogenase is an enzyme that catalyzes one of the reactions in the citric acid cycle where succinate is converted to fumarate.

(Because hydrogens are being removed to form the double bond, this is an oxidoreductase (Class 1) and not a lyase (Class 4).)



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a. What class of enzyme-catalyzed reaction does this belong to? 2 Oxidoreductase (Class 1)

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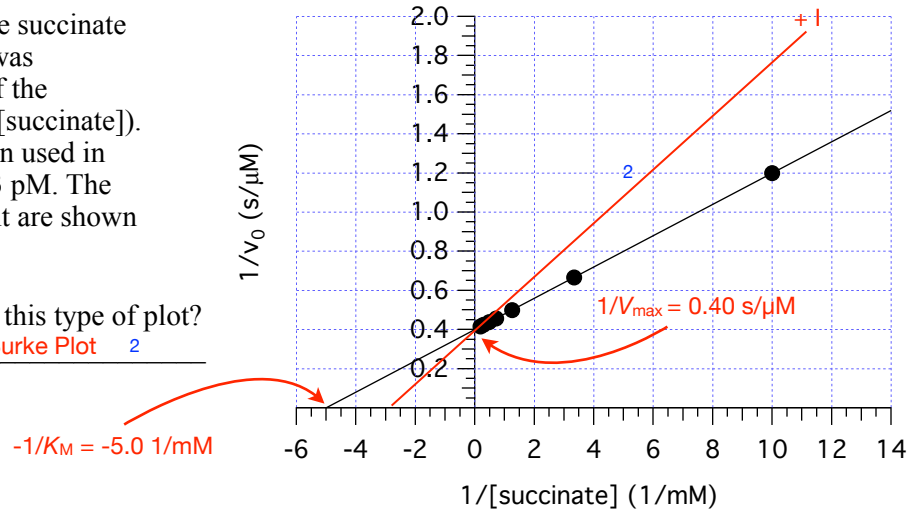
The initial rate (v_o) for the succinate dehydrogenase reaction was measured as a function of the succinate concentration ([succinate]). The enzyme concentration used in these experiments was 53 pM. The results for this experiment are shown in the graph to the right.

b. What is the name for this type of plot?
Lineweaver-Burke Plot 2



c. When fully saturated with succinate, how may succinate molecules does each succinate dehydrogenase molecule convert to fumarate per second? (Show calculations) 47,000 molecules/s

It is the catalytic rate constant, k_{cat}, that provides us with this information. k_{cat} is determined from the V_{max} and the total enzyme concentration, $[E]_{total}$. The V_{max} can be determine from the y-intercept of the Lineweaver-Burke plot, which is equal to $1/V_{max}$. It is important to have k_{cat} have dimensions of 1/time:

$$V_{max} = k_{cat}\left[E\right]_{total} \; ; \; k_{cat} = \frac{V_{max}}{\left[E\right]_{total}}$$

6

$$\frac{1}{V_{max}} = 0.40\ \frac{s}{\mu M} \; ; \; V_{max} = \frac{1}{0.40\ \frac{s}{\mu M}} = 2.5\ \frac{\mu M}{s}$$

converting to units of $\frac{M}{s}$, $V_{max} = 2.5\ \frac{\mu M}{s}\left(\frac{1\ M}{10^6\ \mu M}\right) = 2.5 \times 10^{-6}\ \frac{M}{s}$

$\left[E\right]_{total} = 53\ pM$

converting to units of M, $\left[E\right]_{total} = 53\ pM\left(\frac{1\ M}{10^{12}\ pM}\right) = 53 \times 10^{-12}\ M$

$$k_{cat} = \frac{V_{max}}{\left[E\right]_{total}} = \frac{2.5 \times 10^{-6}\ \frac{M}{s}}{54 \times 10^{-12}\ M} = 4.7 \times 10^{4}\ \frac{1}{s}$$

d. Under these condition, is succinate dehydrogenase displaying catalytic perfection? Yes
What is the evidence for this claim?
An enzyme is considered "catalytically perfect" if its catalytic efficiency, k_{cat}/K_M is greater than 10^8 1/(M•s)

$$\frac{1}{K_M} = 5.0\ \frac{1}{\text{mM}}\ ;\ K_M = \frac{1}{5.0\ \frac{1}{\text{mM}}} = 0.20\ \text{mM}$$

converting to units of M, $K_M = 0.20\ \text{mM}\left(\frac{1\ \text{M}}{10^3\ \text{mM}}\right) = 0.20 \times 10^{-3}\ \text{M}$

6

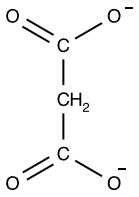
$$\frac{k_{cat}}{K_M} = \frac{4.7 \times 10^4\ \frac{1}{\text{s}}}{0.20 \times 10^{-3}\ \text{M}} = 2.4 \times 10^8\ \frac{1}{\text{M•s}}$$

e. In words, describe what it mean for an enzyme to be *catalytically perfect*.

It means that no more evolutionary tinkering can be done to the enzyme to make it more efficient, because the rate of the reaction has become dependent on rate which a substrate diffuses into the the active site of the enzyme, which is out of the enzyme's control. The reaction is said to be diffusion rate limited.

3

f. Malonate is a competitive inhibitor of succinate dehydrogenase.



malonate

i. As a competitive inhibitor does malonate affect the apparent V_{max}, the apparent K_M, or both? K_M 2

(The inhibitor is competing for the same binding site on the enzyme as the substrate so it will require a higher substrate concentration to saturate the enzyme, but since it can be saturated, the V_{max} remains unchanged. $1/K_M$, however, which is a measure of an enzyme's affinity for the substrate, will be lowered (the apparent K_M will increase).

ii. The follow reaction equation is used to describe enzyme-catalyzed reactions that adhere to the Michaelis-Menton model for enzyme kinetics. Using the letter "I" to represent the inhibitor malonate, modify this reaction equation to account for malonate as a competitive inhibitor.
Because this is a competition inhibitor, the enzyme can either bind substrate (S) tor form an enzyme-substrate complex (ES), or it can bind inhibitor (I) to form an enzyme-inhibitor complex (EI). But it cannot do both.

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

+

2 I

⇓

EI

25/25

iii. On the graph shown on the previous page, sketch a line that shows the expected effect that the presence of malonate should have on the observed kinetics of the reaction catalyzed by succinate dehydrogenase.