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Guided Inquiry Activity
Enzyme Kinetics, Part 2
The Lineweaver Burk transformation

Why? It is not always possible to determine V_{max} from reaction data. The Lineweaver Burk method transforms the hyperbolic Michaelis Menton equation to a linear equivalent, from which the V_{max} and Km parameters can always be determined.

Suppose you obtain the following data for an enzyme catalyzed reaction. (From http://dwb.unl.edu/calculators/pdf/Enzyme-Calc.pdf) Enter the data into a spreadsheet as follows. You can adjust the column width by selecting format, column, autofit.

A	B	C
2	[substrate], uM	velocity, uM/min
3	0.02	0.18
4	0.025	0.22
5	0.04	0.32
6	0.1	0.59
7	0.2	0.83
8	0.5	1.17

Highlight cells B3 through C8. Create a graph of velocity vs. substrate by selecting the graph icon, XY scatter plot with the dots connected, then add appropriate titles and axis labels.

Critical Thinking Questions

1. What is V_{max}? What is Km? Can you determine them from this data? Why or why not?

The Lineweaver Burk "double reciprocal" plot allows for analysis of enzymatic data when V_{max} has not been clearly reached. The derivation is as follows:

The Michaelis Menton equation:

$$v = \frac{V\max[S]}{Km + [S]}$$

Take the reciprocal of both sides:

$$\frac{1}{v} = \frac{Km + [S]}{V\max[S]}$$

Split terms:

$$\frac{1}{v} = \frac{Km}{V\max[S]} + \frac{[S]}{V\max[S]}$$

Canceling to obtain the final transformed equation:

$$\boxed{\frac{1}{v} = \frac{Km}{V\max[S]} + \frac{1}{V\max}}$$

Critical Thinking Questions:

1. What is the equation of a straight line?

2. What is the slope? What is the y intercept?

3. Remember that V_{max} and Km are constants. In the transformed equation (which represents a line), what would the y term be?

4. The x term?

5. What is the y intercept?

6. The slope?

Return to the original spreadsheet. Now add a column to take the reciprocals of both the substrate and the velocity. Create and copy appropriate formulas to take the reciprocals.

A	B	C	D	E
2	[substrate], uM	velocity, uM/min	1/[S]	1/v
3	0.02	0.18	50	5.555556
4	0.025	0.22	40	4.545455
5	0.04	0.32	25	3.125
6	0.1	0.59	10	1.694915
7	0.2	0.83	5	1.204819
8	0.5	1.17	2	0.854701

Create a graph of 1/v vs. 1/[S]. Highlight cells D3 through E8. Select the graph icon, choose an XY scatter plot with the lines connected, and add appropriate titles and axis labels.

Excel will display the equation of the line on your graph, which will provide you with a value for the y intercept. (Why do you want this?)

Click on your completed graph, and select “add trendline” from the chart menu. Make sure that the linear box is highlighted under the “type” tab. Click on the “options” tab, then select “display equation on chart” and click OK.

Critical thinking questions

1. What is the value displayed for the y intercept? This represents $1/V_{max}$. How could you get V_{max} from this?

2. The slope is $\frac{Km}{V\max}$. How could you calculate a value for Km? What is it?

3. Another, often easier, way to get a value for Km is as follows. As you recall, at the x intercept, the y term is equal to zero. Let's make the assumption that we are at the x intercept on the line. You can then set the $\frac{1}{v}$ term equal to zero in the transformed linear equation:

$$0 = \frac{Km}{V\max} \text{ x } \frac{1}{[S]} + \frac{1}{V\max}$$

   Now solve for $\frac{1}{[S]}$. What does this term equal at the x intercept?

4. Thus, the x value ($\frac{1}{[S]}$) at the x intercept is equal to - $\frac{1}{Km}$. You can expand the scale on your graph to see the x intercept as follows:
   Double click on any number on the x axis. Select the scale tab and set the minimum to about -10. You can extend the trendline back to the x axis by double clicking on the trendline, selecting format trendline, and then choosing "forecast backward" in the options menus. You can see that the x intercept will fall at about -8, so forecast backward maybe 9 units. What is the value of the x intercept? How could you get Km from this? How does it compare to the value you obtained in question 2?