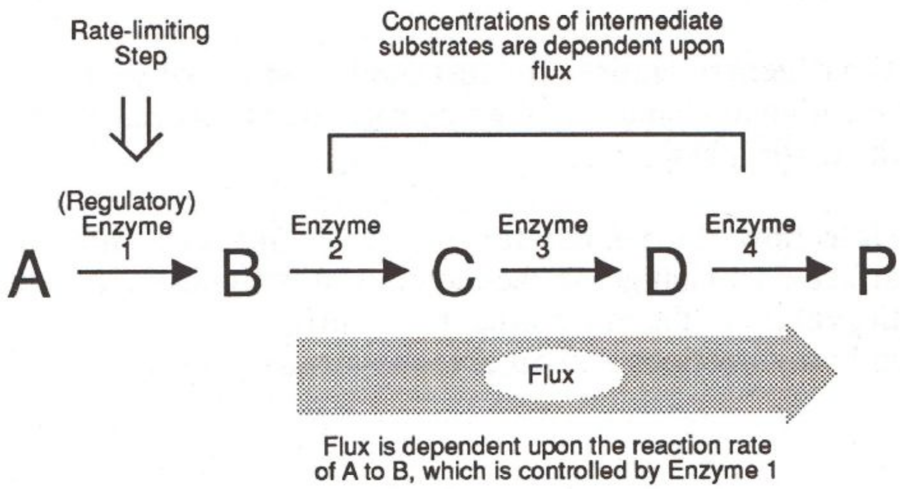
Experiment 5.4

Substrate Concentration as a Function of V_{max}, K_m, and Reaction Velocity

In the laboratory, kinetic experiments with enzymes typically involve measurements of reaction rates at selected substrate concentrations. It is therefore natural to think of substrate concentration as the independent variable and reaction rate as the dependent variable. In fact, the opposite is true for most enzymes in the intact cell.

The flux through a typical reaction sequence or metabolic pathway is controlled by the rate of the first reaction in the sequence. This reaction rate is controlled by the regulatory enzyme at the beginning of the sequence. The reaction rates of intermediate steps of the reaction sequence are governed by the flux through the sequence as a whole, and the concentrations of the intermediates must adjust accordingly (Figure 5.9).

Figure 5.9



Such behavior is readily modeled by simply rearranging the traditional form of the Michaelis equation (Equation 5.26) such that [S] becomes a function of K_m, v, and V_{max} (Equation 5.27).

$$\frac{v}{V_{max}} = \frac{[S]}{K_m + [S]} \tag{5.26}$$

$$[S] = \frac{K_m v}{V_{max} - v} \tag{5.27}$$

Directions

Build and verify the model described in Figure 5.10.

Figure 5.10

Formulas

	A	B
1	Flux =	100
2	Vmax =	200
3	Km =	1
4		
5	[S] =	=(B3*B1)/(B2-B1)

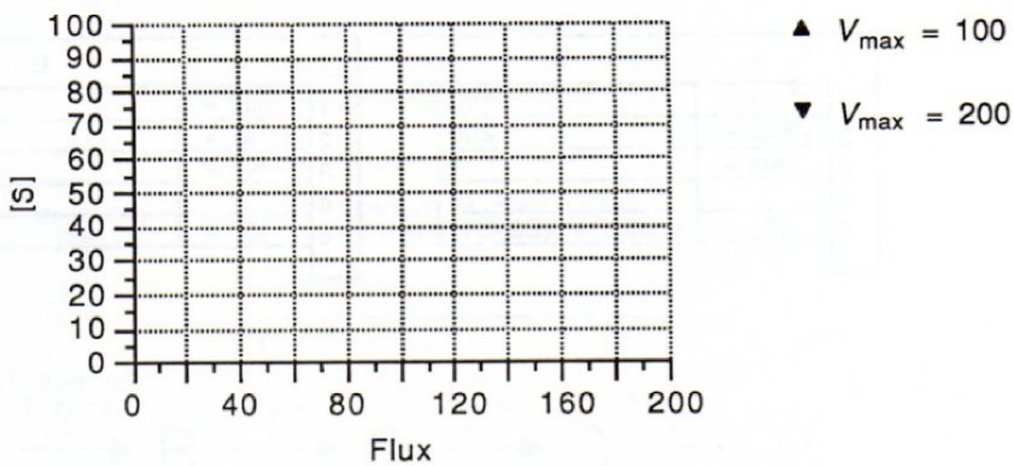
Values

	A	B
1	Flux =	100
2	Vmax =	200
3	Km =	1
4		
5	[S] =	1

Questions

1. Use the model described in Figure 5.10 to complete and graph the table below.

Flux	V_{max} = 100; K_m = 1 [S]	V_{max} = 200; K_m = 1 [S]
0	________	________
20	________	________
40	________	________
60	________	________
80	________	________
90	________	________
95	________	________
99	________	________
95	________	________
99	________	________
120		________
140		________
160		________
180		________
190		________
198		________

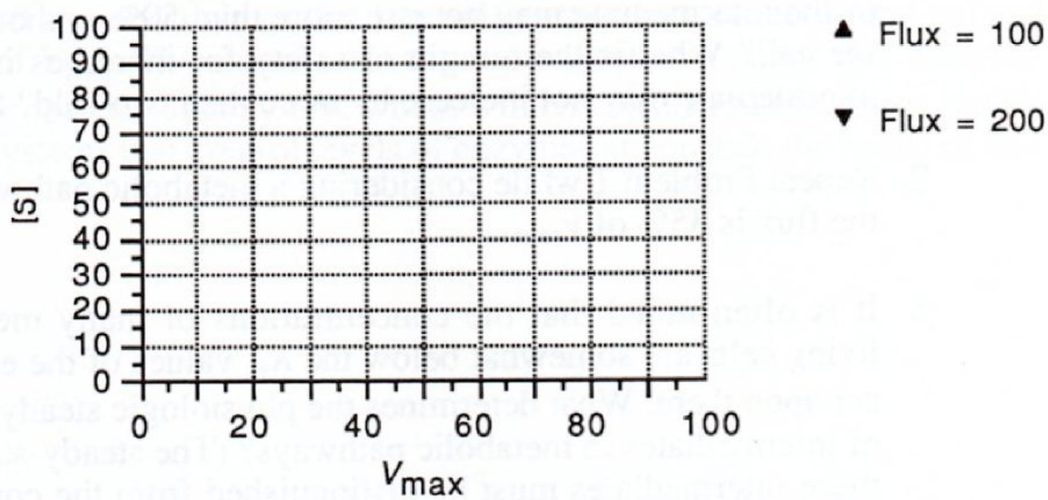


a. When V_{max} = 100, what happens to [S] as flux approaches 100? When V_{max} = 200?

This simulation is relevant to the concentrations of inter - mediates along a metabolic pathway. If the flux were to increase to levels where it exceeds the catalytic capacity of the enzyme, the concentration of the substrate of that enzyme would rise catastrophically without limit unless it was excreted by the cell or converted to a side product (which may well be toxic).

2. Use the model described in Figure 5.10 to complete and graph the table below.

V_{max}	Flux = 100; K_m = 1 [S]	Flux = 200; K_m = 1 [S]
1000	______	______
800	______	______
600	______	______
400	______	______
300	______	______
220	______	______
210	______	______
202	______	______
200	______	
150	______	
110	______	
105	______	
101	______	



a. When flux = 100, what happens to [S] as the enzyme concentration decreases such that V_{max} approaches 100?

This would be the effect of a decreasing enzyme amount on the concentration of an intermediate in a reaction sequence where flux is constant. Do you see the importance of mechanisms that regulate enzyme levels (e.g., induction, repression)?

3. It is often assumed that reactions in intact cells proceed at rates close to the V_{max} of their enzymes. Your study of the previous two questions should already cause you to doubt this assumption.

   a. Use the model described in Figure 5.10 (modify it slightly if you wish) to determine the percent increase in [S] that occurs when a flux of 0.85 of V_{max} increases by 12% to 0.95 of V_{max}.

   b. Now determine the percent increase in [S] that occurs when a flux of 0.30 of V_{max} increases by 12% to 0.34 of V_{max}.

Problems

1. Consider a metabolic pathway in which flux is 85% of V_{max} for an enzyme that catalyzes the reaction of an intermediate in the pathway. What is the margin of safety for increases in the rate at which the initial metabolite enters the sequence if the concentration of the intermediate may not rise more than 50% without damaging the cell? What is the margin of safety for increases in flux if the intermediate may not increase by more than two-fold? five-fold?

2. Repeat Problem 1 while considering a metabolic pathway in which the flux is 35% of V_{max}.

3. It is often noted that the concentrations of many metabolites in living cells are somewhat below the K_m values of the enzymes that act upon them. What determines the physiologic steady-state levels of intermediates in metabolic pathways? (The steady-state levels of these intermediates must be distinguished from the concentrations of branchpoint metabolites and end products that are usually controlled by specific and complex regulatory interactions.)

4. In agreement with the first sentence of Problem 3, the activities of most enzymes seem to be held at levels that result in normal fluxes of between 10% and 35% of V_{max}. By now you should realize that selection pressures in the course of evolution make such values reasonable. What is the concentration of substrate as a function of K_m when flux is 0.30 of V_{max}?

Determine the boundaries on the ratio of [S] to K_m (at "normal" values of flux) if the levels of enzymes are sufficient to allow the flux to increase three-fold above "normal" values without increasing the concentrations of the intermediates by more than a factor of eight.

Determine the boundaries on the ratio of [S] to K_m if the levels of enzymes are sufficient to allow the flux to increase four-fold without increasing [S] by more than a factor of ten?

5. How would you answer someone who argues that it is wasteful for a cell to make more enzyme than just enough to permit the reaction rates required by "normal" conditions?

Conclusion

In this experiment you have seen that it is important to the cell that enzyme levels be sufficiently high so that normal reaction velocities do not exceed about 30% of V_{max}. The amount of enzyme in a cell is a factor that interacts with other properties of enzymes, which will be considered elsewhere in this book. The systems that control levels of enzymes are outside the scope of this book.