Simulation of Metabolic Reactions Using Excel

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Microsoft Excel can rapidly create simple simulations. In this exercise, students learn simulation skills by modeling the equilibrium reaction between dihydroxyacetone phosphate (DHAP) and glyceraldehyde-3-phosphate (G3P) in glycolysis. First, they create a two-compartment model with constant, literature-based transfer coefficients. Then they add the effect of temperature to their model. Finally, they download a spreadsheet that allows students to vary standard delta G of the reaction. Students are asked to make predictions of how the simulation will turn out for a variety of starting conditions and standard delta Gs, and then test their predictions with the model.

Keywords: Excel, simulation, thermodynamics

Introduction

Microsoft Excel is available to virtually all students, and it can be used to teach students simulation skills while they explore topics of biological interest. This exercise allows students to build a simulation of one of the reactions of glycolysis, the isomerization reaction between glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). Each molecule has a probability of transforming into the other, so the model essentially simulates the transfer of material between two compartments. The molecule that is more stable (has a lower transition probability) will be more abundant at equilibrium. Therefore, the relative stability of the molecules establishes the equilibrium mixture. The students build this basic model first (using literature-based transfer coefficients), and then introduce the effect of temperature (which is principally to speed up or slow down the approach to equilibrium). Finally they download a more complex model that allows them to vary the standard delta G ($\Delta G'^\circ$) of the reaction. This is the delta G of a reaction starting with 1 M concentrations of both reactants and products. No matter what the starting concentrations are, negative $\Delta G'^\circ$ values come to equilibrium with a predominance of products, positive $\Delta G'^\circ$ values produce equilibria dominated by reactants, and $\Delta G'^\circ$ values of zero produce equal concentrations of reactants and products.

The student outline has several places where blanks must be filled in; a filled-in version of the student exercise and a more extensive instructor's section can be downloaded from a URL at the end of the *Notes for the Instructor*.

Student Outline

In this exercise, you will use Microsoft Excel to construct a computer model of one of the chemical reactions of glycolysis. After exploring the behavior of your model, we will give you some more elaborate computer models to help you review thermodynamics and reaction kinetics.

Our Reaction

At one point in glycolysis, fructose 1,6-bisphosphate splits into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). The overall structure of the reactions is as follows:
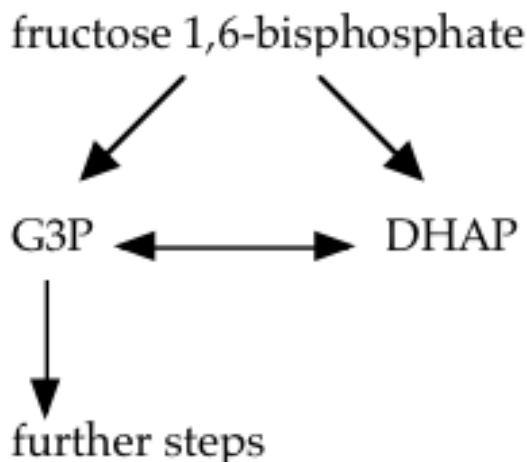
fructose 1,6-bisphosphate

G3P ⟷ DHAP

further steps

Figure 1. The place of the G3P <–>DHAP reaction in glycolysis.

Notice that G3P can transform into DHAP, and DHAP can transform into G3P. This reaction is catalyzed by the enzyme triose phosphate isomerase. Also note that although fructose 1,6-bisphosphate splits into both G3P and DHAP, only G3P continues on to the next reaction. The only way the DHAP can become pyruvate is if it is first transformed into G3P. Therefore, the isomerization reaction between G3P and DHAP is an important part of glycolysis.

Therefore, let us consider the isolated reaction between G3P and DHAP:
$$G3P \leftrightarrow DHAP$$
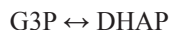
The equilibrium reaction mixture does not contain equal amounts of these two molecules. The reaction comes to equilibrium with about 21 molecules of DHAP for every molecule of G3P (Nelson *et al.*, 2000: 534). This implies that G3P is _____ (more or less) stable than DHAP, and therefore the probability that a molecule of G3P will transform to DHAP is _____ (higher or lower) than the probability that a molecule of DHAP will transform into G3P.

Dynamical Modeling of Chemical Reactions

Chemical reactions can be easily simulated by a technique called dynamical modeling, which is based on the changes that occur in each time interval. Say that each msec, there is a probability of 0.1 that a G3P molecule will form a DHAP molecule, and there is a probability of 0.00475 that a DHAP molecule will form a G3P molecule. Then, each msec, the amount of G3P present is the amount present during the last msec plus the net formation or loss rate of G3P. This net formation or loss rate ($\Delta G3P$) is given by:

$$\Delta G3P = 0.00475*DHAP - 0.1*G3P \tag{1}$$

where G3P and DHAP are the concentrations of those molecules. Likewise, the net rate of change of DHAP is given by

$$\Delta DHAP = 0.1*G3P - 0.00475*DHAP \tag{2}$$

In an isolated reaction, the concentrations of G3P and DHAP will automatically adjust themselves until $\Delta G3P = \Delta DHAP$. Then G3P and DHAP will be at their equilibrium ratio.

Modeling the G3P-DHAP Reaction with Excel

Simulation of the DHAP/G3P system is easy with Excel. In dynamical modeling, we put down an initial condition (like G3P concentration) at the top of a column, and then add changes to it to produce the next concentration, which will be in the cell below the first value. This set of computations is called an iteration, so the time course of the G3P concentration (for example) can be read by tracing and graphing the values that appear in one column. For small simulation problems that don't contain more than a few hundred iterations, Excel can give quick and reliable results.

We will start with a simple version of the G3P<–> reaction.

1. Boot up your laptop, open Excel, and ask for a new Excel "Workbook."

2. Every model contains two kinds of numbers—constants (called "parameters") and numbers that vary as the simulation proceeds (called "state variables"). Excel works from left to right within a line and top to bottom between lines, so to make sure the model "knows" about the values of all parameters, we put those on the top left. In cell A1 of the spreadsheet, put in some title like "G3P-DHAP Simulation," skip a line, and write the following titles in cells in line 3 and the following initial values under them in line 4. Note that "G to D" and "D to G" are parameters and "Time," "G3P," and "DHAP" are state variables.

Figure 2. Column titles and initial values in the G3P/DHAP simulation.

3. The first line of the simulation was initial conditions, but the second line actually calculates values. "G to D" and "D to G" are constants, so all we have to do there is state that they will retain those values in every following line. Click on cell A5 and type in "=A4" there (don't include the quotation marks). Type in "=B4" in cell B5. Type in "=C4+1" in cell C5 because time increases as the simulation advances. You should now see:

Figure 3. The initial (and simple) part of line 5.

4. The calculations will be in the next two cells. G3P loses 0.1*G3P every iteration, and DHAP loses 0.00475*DHAP every iteration. However, what is lost from one quantity is added to the other. Therefore, the D values of G3P and DHAP will be:

$$\Delta G3P = -0.1 \cdot G3P + 0.00475 \cdot DHAP \quad\quad (3)$$
$$\Delta DHAP = -0.00475 \cdot DHAP + 0.1 \cdot G3P \quad\quad (4)$$

Putting this in Excel notation, we would write these equations in lines D5 and E5:

Cell D5 should have: = D4 – A4*D4 + B4*E4
Cell E5 should have: = E4 – B4*E4 + A4*D4

Notice that line 5 is defined by changes to line 4. Line 5 should not refer to itself. Put these equations into cells D5 and E5. When you're finished, the first calculated line should look like this. Recheck your equations or seek your TA's help if you don't get this result.

	A	B	C	D	E
1	G3P-DHAP Simulation				
2					
3	G to D	D to G	Time	G3P	DHAP
4	0.1	0.00475	0	0.5	0.5
5	0.1	0.00475	1	0.452375	0.547625

Figure 4. The simulation calculates its first G3P and DHAP values.

5. While the last step may have seemed laborious, the next one is easy. Now we're going to take this single line of calculation and repeat it as many times as we like to see what the model does. Let's try 40 iterations. Select and copy line 5 from A5 to E5. Then select the blank rectangular area from A6 to E44 and copy line 5 into this area. The results should start off:

	A	B	C	D	E
1	G3P-DHAP Simulation				
2					
3	G to D	D to G	Time	G3P	DHAP
4	0.1	0.00475	0	0.5	0.5
5	0.1	0.00475	1	0.452375	0.547625
6	0.1	0.00475	2	0.40973872	0.59026128
7	0.1	0.00475	3	0.37156859	0.62843141
8	0.1	0.00475	4	0.33739678	0.66260322
9	0.1	0.00475	5	0.30680447	0.69319553
10	0.1	0.00475	6	0.2794167	0.7205833

Figure 5 .Results of copying and pasting line 5 into lines below.

6. Graphing this is also easy. Select the cells from C3 to E44 (that is, the three state variables plus their titles) and then indicate that you want to insert a chart. Select a "scatter chart" with straight lines and no markers. This will produce a "chart" that is not in the scientific style, but it will be good enough for this exercise because all we want to see is the behavior or the system. Your chart should look like this:
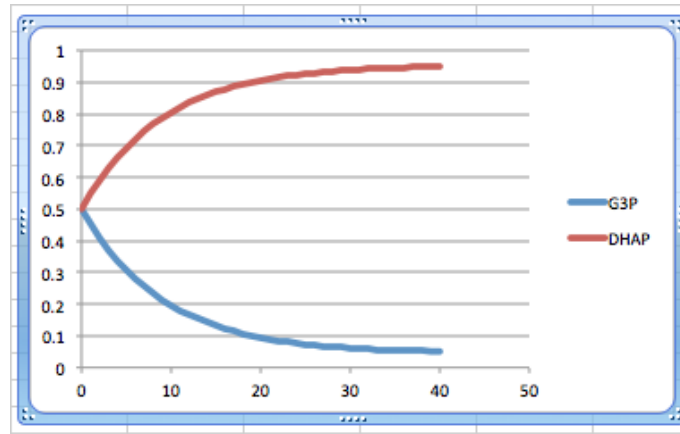
Figure 6. Excel "chart" showing simulated values of G3P and DHAP out to 40 iterations (40 msec).

7. If the probability of G3P --> DHAP is 0.1 and the probability of DHAP --> G3P is only 0.00475, which is more stable? Therefore, which should be more abundant at equilibrium? Are the results above expected?

    _____

    _____

8. You started the concentrations of both G3P and DHAP at 0.5 mM, a 1:1 ratio. If you started with all G3P, for example, would the final equilibrium be different? Go to cells D4 and E4 and change the starting concentrations to 1 and 0. Then change them to 0 and 1.

    What is the effect of initial concentrations on the ratio of products to reactants at equilibrium?

    _____

    _____
    Why does this outcome occur? Hint: What determines relative equilibrium concentrations, and is this influenced by initial concentrations?

    _____

    _____

9. Return the G3P and DHAP numbers to 0.5 and 0.5. In the results above, DHAP only had a transition probability of 0.00475 per time interval. What would happen if we made it 0.0475 instead? Predict the results, and then make this change in cell B4 by selecting the cell and then typing in the new number. When you press "Enter," that cell will change, but that will cause the change in every cell below that in the B column because every cell is defined as equal to the cell above it. Do the results on the graph match your prediction? Return the "D to G" to 0.00475.

    _____

    _____

10. The relative stability of reactants and products determines where the equilibrium point of a reaction lies, but it does not determine how fast that equilibrium is attained. The G3P <--> DHAP reaction is catalyzed by an enzyme, and one of the major influences on its rate of activity is temperature. You will now incorporate the effect of temperature into your model.

11. Incorporating temperature requires you to add two columns to your model. When you model, having to add new elements is a common problem. Change the title of the sheet to "G3P-DHAP Simulation with Temperature." Select the whole Time column (including the heading) and then ask to insert a column. A blank column will appear to the left of the Time column. Then ask to insert another column in the same place. Label these two columns "Temperature" and "Temp Effect." Enter a 25 in cell C4 to indicate the initial temperature; 25° C is a standard temperature in biochemistry. You should now have:

	A	B	C	D	E	F	G
1	G3P-DHAP Simulation withTemperature						
2							
3	G to D	D to G	Temperature	Temp Effect	Time	G3P	DHAP
4	0.1	0.0475	25		0	0.5	0.5
5	0.1	0.0475			1	0.47375	0.52625
6	0.1	0.0475			2	0.45137188	0.54862813
7	^ ^	^ ^475			^ ^ ^^^^^^^	^ ^^^^^^

Figure 7. Insertion of two columns for temperature effects.

12. Now we must compute the effect of temperature. Within small ranges around room temperature, reaction rates tend to double for each 10° rise in temperature and tend to fall by half if the temperature drops by 10°. Thus, we say $Q_{10} = 2$. Fill in the following in cell D4:

$$= 2\^((C4-25)/10)$$

Be sure to get the parentheses in the right spots. If temperature changes from 25°C to 35°C, can you see how this number will 2^1, or 2?

13. The temperature and temperature effect will be constant during a simulation, so fill in "=C4" in cell C5 and "=D4" in cell D5.

14. Now this temperature effect must be incorporated into the G3P and DHAP computations. This can be done by multiplying the change per iteration by the temperature effect in column D. So, for example, cell F5 will now read:

$$= F4-D4*A4*F4 + D4*B4*G4$$

15. Likewise, cell G5 should change to:

$$= G4 - D4*B4*G4 + D4*A4*F4$$

16. Take this whole row 5, copy it, and then paste it over all the other rows from row 6 down to row 44. Now test the effect of temperature:

a) With the temperature at 25°C, run the model. It should give you the same results as before.

b) In cell C4 change the value of temperature to 4°C (common in refrigerators) and press Enter. How are the results different from the 25°C case? To check your work, the first few lines should look like this:

	A	B	C	D	E	F	G
1	G3P-DHAP Simulation withTemperature						
2							
3	G to D	D to G	Temperature	Temp Effect	Time	G3P	DHAP
4	0.1	0.00475	4	0.23325825	0	0.5	0.5
5	0.1	0.00475	4	0.23325825	1	0.48889108	0.51110892
6	0.1	0.00475	4	0.23325825	2	0.47805359	0.52194641
7	0.1	0.00475	4	0.23325825	3	0.4674809	0.5325191

Figure 8. Correct simulation for 4° C.

c) Change the temperature to 40°C and run the model again. How are these results different from the 25°C case or the 4°C case?

_____

_____

17. Answer the following questions:

   a) In this model, does temperature influence the relative concentrations of G3P and DHAP once equilibrium is attained? _____ (yes or no)

   b) What *does* temperature influence?

   _____

   _____

Standard Delta G

   ΔG is short for _____ (initials "GFEC"). In our lectures about thermodynamics, you were told about the effect of ΔG on a reaction. When ΔG is negative, the reaction is said to be _____ (starts with an S), and when ΔG is positive, the reaction is said to be _____. A quantity called $\Delta G'^\circ$ (standard ΔG) predicts the net reaction direction when starting with 1 M concentrations of both reactants and products. When starting with these equal concentrations, a reaction with a positive $\Delta G'^\circ$ would produce more _____ (reactants or products); a reaction with a negative $\Delta G'^\circ$ would produce more _____ . In other words, at equilibrium the one with negative $\Delta G'^\circ$ would have a preponderance of _____ (reactants or products). In this exercise, you will explore a model (already written) that allows you to change the $\Delta G'^\circ$ of the DHAP --> G3P reaction and observe its effect.

   Note that from now on, we will be talking about reactions with a stated direction. DHAP is the reactant here and G3P is the product. "DHAP <--> G3P" has no clear reactants and products and we cannot specify its $\Delta G'^\circ$ for that reason. However, "DHAP --> G3P" has DHAP as its reactant, G3P as its product, and its $\Delta G'^\circ$ is +1.8 at 25° C. On the other hand, the $\Delta G'^\circ$ of the G3P --> DHAP reaction at 25°Cis -1.8.

Exploring the Effect of Standard Delta G

1. Make sure you have finished all the exercises above. Then save your work on your laptop so you can refer to your simulation in the future. Go to **http://biology.clemson.edu/bpc/bp/Lab/110/handouts/Delta_G_G3P_DHAP.xlsx** and put the downloaded spreadsheet on your desktop. You may have to check your download folder in order to find it.

2. This file is similar to the first model you constructed, but:

   a) There are no reaction probabilities specified. Instead, a standard delta G ($\Delta G'^\circ$) is used to make the higher reaction probability 0.1 per iteration and the lower one some fraction of 0.1 determined by $\Delta G'^\circ$;

   b) the initial $\Delta G'^\circ$ is+1.8, the true $\Delta G'^\circ$ of the DHAP → G3P reaction at 25°C. To avoid confusion, DHAP is called the reactant and G3P is called the product;

   c) the temperature is assumed to be constant at 25°C.

3. The graph shows the results under these default conditions. Verify that the results are the same as what you observed with your model.

4. Go to cells E4 and F4 and vary the initial reaction mixtures from all G3P to all DHAP and anywhere in between. To allow the results to fit on the graph, keep the initial sum of G3P and DHAP at 1.0. Verify that the results are similar to your observations with your own model.

5. Return the initial concentrations to 0.5 and 0.5. Now, make some predictions about the effect of $\Delta G'^\circ$:

   a) The "Stan DG" on the spreadsheet shows the $\Delta G'^\circ$ of the *DHAP --> G3P reaction*. If the $\Delta G'^\circ$ is moved from +1.8 to +4.0, the effect on the reaction will be to make _____ (DHAP or G3P) more dominant in the equilibrium reaction mixture.

   b) If the $\Delta G'^\circ$ were changed to some small positive value like +0.2, the equilibrium reaction mixture would consist of

   _____.

   c) If the $\Delta G'^\circ$ were changed to exactly 0, the equilibrium reaction mixture would consist of

   _____.

   d) If the $\Delta G'^\circ$ were changed to a value like -4.0, the equilibrium reaction mixture would consist of

_____.

6. Test all these predictions. Were you right?

_____

7. Sketch the rough time course of the G3P(product) concentration under each of the following conditions. In all cases, keep the sum of DHAP and G3P at 1.0. For example, if [G3P] = 0.5, then [DHAP] = 0.5. If [G3P] = 0, then [DHAP] = 1.0. Also, ΔG'° refers to the *DHAP --> G3P reaction*. Hint: the first one is done for you, and after that you should be able to solve the rest by asking what has changed from the first one, and what effect that change will have.
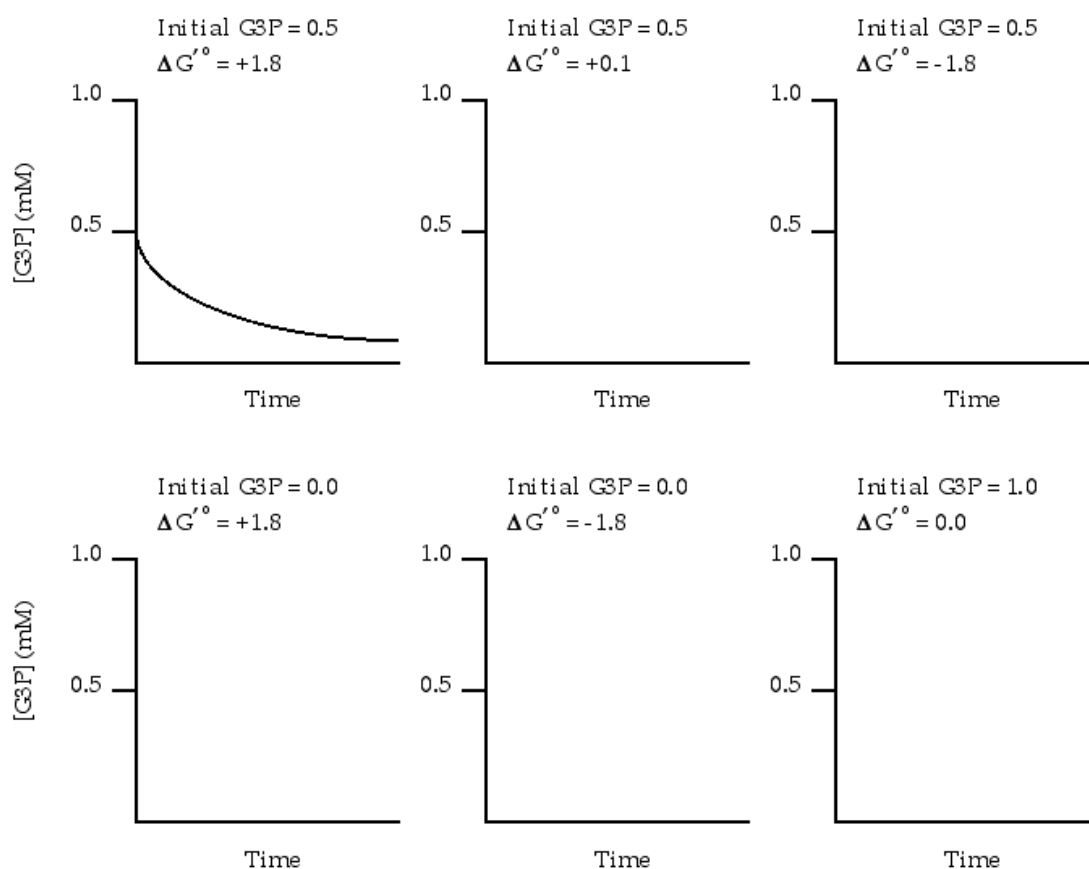
Figure 9. Predicted G3P results for several values of ΔG'°.

8. Test your predictions with the downloaded model.

9. When finished, if you want, you may save the downloaded spreadsheet onto your laptop.

Notes for the Instructor

Biology students tend to be resistant to mathematics, but Clemson freshman biology students have had no problems with this exercise. If the background has been covered and they are merely following the directions, they should be able to complete the first exercise (using the spreadsheet model that they construct) in about 20 minutes, and the exercise with the downloaded spreadsheet in about 10 minutes.

At Clemson, the exercise is preceded by a lecture discussion of thermodynamics, so students are familiar with the concept of ∆G'° before starting it. If students have the student outline ahead of time, a good introduction would be to go through the exercise and ask the class to fill in all the blanks (already done on the instructor copy, downloaded from the URL at the end of these notes), explaining concepts as needed. We insert the exercise into a respiration lab during the incubation time of yeast and mitochondrial experiments. At Clemson, students use their own laptops, and are sufficiently familiar with Excel so that following the directions is not difficult. They easily understand the concept of simulation in Excel, that Excel's "eye" sweeps from left to right on a line and then down to the left end of the next line.

The two problem areas are understanding the basic equations of the model and simple typing mistakes. The basic equations are:

$$\Delta G3P = -0.1*G3P + 0.00475*DHAP \qquad (1)$$
$$\Delta DHAP = -0.00475*DHAP + 0.1*G3P \qquad (2)$$

The students have difficulties understanding why the + and – signs are where they are in these equations. It may help to point out that a molecule (such as G3P) cannot add to its concentration, but it can decrease its concentration by converting to the other molecule. This establishes that in the ∆G3P equation, the negative coefficient must always be associated with G3P, and the negative coefficient must be paired with DHAP in the ∆DHAP equation. Also, what is added to one molecule must be subtracted from the other.

Typing errors will always be a problem, and just require checking. When the directions call for:

"Cell D5 should have:　　= D4 – A4*D4 + B4*E4
Cell E5 should have:　　= E4 – B4*E4 + A4*D4"

it would be easy to confuse As, Ds, Bs and Es, or to have a circular reference in which cell D5 makes reference to itself instead of cell D4.

A final "concept" problem is understanding the meaning of a positive standard delta G. Students may have heard in high school that a negative delta G means a reaction is spontaneous, and they may misinterpret that to mean that only reactions with negative standard delta Gs can occur. It is true that a reaction with a positive standard delta G will not form more products when started with equal concentrations of reactants and products. However, as long as continual addition of reactants or the removal of products keeps the ratio of products to reactants low, a reaction with a positive standard delta G can still convert all the reactants that reach it to products. This is illustrated by the model reaction in this exercise:
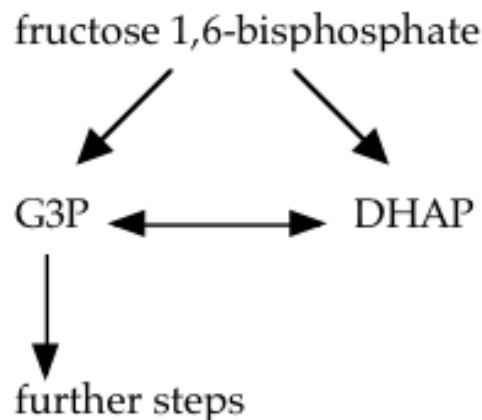

Figure 10. DHAP is continuously added, and G3P is constantly removed.

Even though the G3P ↔ DHAP reaction equilibrium favors DHAP by a factor of 21, DHAP cannot continue without converting to G3P. Glycolysis depends on the fact that almost all the DHAP will convert to G3P because DHAP is continually added and G3P is rapidly drained away into the following steps of glycolysis.

Instructor's Version of the Exercise

There is an instructor's version of this exercise with filled-in blanks and a more extensive notes to the instructor at **http://biology.clemson.edu/bpc/bp/Lab/110/handouts/Excel_Modeling_MS_Instructor.docx.**

Acknowledgements

This presentation was derived from one that I made at the 2001 ABLE meeting in Chicago. However, that one modeled using Stella, not Excel.

Literature Cited

Nelson, D. L. and M. M. Cox. 2000. *Lehninger: Principles of Biochemistry*. Third edition. Worth Publishers, New York, 1152 pages.

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