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Simulation of Directions for a Reversible Enzyme Reaction by Using LabVIEW Graphical Programming Language

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Abstract: Computer simulation of single substrate single product reversible enzyme reaction's directions using LabVIEW graphical programming software is described. The program determines and displays reaction's direction, calculates concentrations of reactant and product as they occur and allows graphical, digital and spread sheet outputs; Red blood cells Triose Phosphate Isomerase (TPI) reaction direction proceeds initially in the forward direction (G_3P to DHAP) for 46 reaction cycles; then, reaction direction oscillates. During oscillation, the rate of forward reaction (G_3P to DHAP) is slower than the backward reaction rate (DHAP to G_3P). It takes 16 reaction cycles in forward direction to match 3 reaction cycles in the reverse direction ($16_F/3_R$). the direction oscillation pattern of TPI is $((5_F/1_R)_2\,(6_F/1_R)_1)_n$, that is a twice repeat of 5 consecutive forwards followed by 1 backward direction, followed by a single repeat of 6 consecutive forwards and one backward directions and is repeated continuously. Direction oscillation pattern for TPI is k_m dependent and it is more sensitive to forward k_m (for G_3P) value. Computer simulation of reversible reactions is a new research tool that provides information not possible by the traditional means of enzyme activity measurements.

Key words: TPI, metabolism, computer simulation, LabVIEW

INTRODUCTION

The overall flow of metabolites through a metabolic pathway is termed flux; metabolic flux is controlled tightly by modulation of key enzyme activities in that pathway (Melendez-Hevia *et al*., 1992). Oscillation of metabolites in metabolism was noticed earlier using conventional and complicated methods (Betz and Chance, 1964; Hess and Boiteux, 1971; Richter and Ross, 1981; Chance and Wassermann, 1986; Shulman, 1988; Danø *et al*., 1999). Specific metabolites concentrations act on key enzymes to redirect metabolic flux to meet cell demand (Betz and Chance, 1964; Chance and Wassermann, 1986; Sugden *et al*., 1989; Jackson, 1993; Weber *et al*., 2002; McDonald and Webber, 1995; Elia, 1995; Bali and Thomas, 2001; Ainscow and Brand, 1999). The role of reversible reactions that proceed (or follow) key enzymes in a metabolic pathway needs more evaluation with regard to its role on metabolic control (Periappuram *et al*., 2000). Computer simulation showed altered behavior of glycolysis when reversibility of normally considered irreversible reactions is allowed; (Cornish-Bowden and Cardenas, 2001). Reversible reactions are common in metabolic pathways; for example, glycolysis has three reversible enzyme reactions: first, the phosphoglucoisomerase reaction that catalyses the conversion of glucose-6 phosphate and fructose 6 phosphate; second is the Triose Phosphate Isomerase (TPI) that catalyses conversion of glyceraldehyde 3-phosphate (G_3P) and Di Hydroxy Acetone Phosphate (DHAP); third is the phosphoglycerate mutase reaction that catalyses the conversion of 3 phosphoglycerate and 2-phosphoglycerate.

A number of computer simulation programs have been designed to describe aspects of enzymology and metabolic pathways non of these programs deals with directions of reversible reactions; (Mulquiney and Kuchel, 1999; Tomita *et al*., 1999; Ehlde and Zacchi, 1995; Cornish-Bowden and Hofmeyer, 1991; Mendes, 1993).

The aims of this study were to design computer program that simulate directions of the reversible enzyme reaction; to relate reaction direction to concentrations on both sides of reaction at any moment and to identify relative frequency of forward and backward directions at concentrations of metabolites in the cell.

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MATERIALS AND METHODS

Software required

Software: LabVIEW software (a trademark of National instruments Austin TX, USA) is a graphical programming language that uses icons instead of lines of text, which is used to create applications; The user interface (known as the front panel) is built from graphical codes. The block diagram contains this code connected by lines that guide the flow of information from one code to the next (Kalkman, 1995; Novoselov *et al.*, 2002; Regan and Gregory, 1995).

Reaction parameters, equations and inputs required: The rate of forward and backward reactions are calculated using Michaelis-Menten equation that accommodates competition between reactant with product for binding the active site (in this reversible reaction).

The rate of forward reaction $v_{f,(g3p>dhap)}$ is

$$V_{f,(g3p>dhap)} = \frac{V_{Max(g3p>dhap)} * \left(\frac{G3P}{K_{m,g3p}}\right)}{1+\left(\frac{DHAP}{K_{m,dhap}}\right)+\left(\frac{G3P}{K_{m,g3p}}\right)} \quad (1)$$

While the rate of the reverse reaction $v_{r,(dhap>g3p)}$ will be

$$V_{r,(dhap>g3p)} = \frac{V_{Max(dhap>g3p)} * \left(\frac{DHAP}{K_{m,dhap}}\right)}{1+\left(\frac{DHAP}{K_{m,dhap}}\right)+\left(\frac{G3P}{K_{m,g3p}}\right)} \quad (2)$$

Where,

- $v_{f,(g3p\ to\ dhap)}$, $v_{r,(dhap\ to\ g3p)}$, $V_{max\ (g3p\ to\ dhap)}$ and $V_{max\ (dhap\ to\ g3p)}$ are the initial and maximum rate for G_3P to DHAP direction and DHAP to G_3P directions, respectively;
- $K_{m,g3p}$ and $K_{m,dhap}$ are the affinity constants for G_3P in the G_3P to DHAP direction and for DHAP in the DHAP → G_3P direction, respectively.

The direction of the reaction is determined using the Gibs free energy change equation, which can be expressed as:

$$\Delta G = \Delta G^{o'} + RT * \ln\left(\frac{Pr}{Re}\right) \quad (3)$$

Where:

ΔG = Free energy change
$\Delta g^{o'}$ = Standard free energy change
R = Gas constant = 8.315 J mol^{-1} K (1.987 cal mol^{-1} K)
T = Absolute temperature in Kelvin degrees.
Pr = Product concentration
Re = Reactant concentration

The forward reaction is the reaction that initially has negative ΔG value where it is in this case the G3P to DHAP direction. The reverse reaction is the reaction that initially has positive ΔG value, in this case DHAP to G3P direction.

The following inputs are used to calculate TPI reaction rates from red blood cells (Mulquiney and Kuchel, 1999):

$$[G3P] = 5.7\ \mu M$$
$$[DHAP] = 17.5\ \mu M$$
$$K_{eq} = 20.7$$
$$K_{m,g3p} = 434\ \mu M$$
$$K_{m,dhap} = 162\ \mu M$$
$$V_{max\ (g3p>dhap)} = 16.6\ \mu M\ S^{-1}$$
$$V_{max\ (dhap>g3p)} = 1.76\ \mu M\ S^{-1}$$

Note that

$V_{max\ (g3p\ to\ dhap)} = K_{cat,\ f} *$ (enzyme concentration)
$V_{max\ (dhap\ to\ g3p)} = K_{cat,\ r} *$(enzyme concentration)

The enzyme concentration was reduced by a factor of 10^{-3}.

Program flow: The program can be seen in a flow chart as shown in Fig. 1 while Fig. 2 shows the front panel of the whole programme. It can be broadly explained as follows:

- Upon pressing the start button, the enzyme reaction takes place in cycles by using the While Loop technique and incorporating the Shift registers method in LabView.
- Each cycle of the Shifted registered While Loop is composed of several steps known as sequence structures; where eight sequence structures are used in this design.
- Each sequence structure performs a set of procedures or steps.
- The clear history chart is applied for each time the program is executed.

The program can be divided into main sub-tasks as follows:

Task 1: Calculating the ΔG value: This is the 1st sequence of the While Loop as shown in Fig. 3, where the value of gs (Standard Free energy change) and g (Free energy change) are calculated as follows:

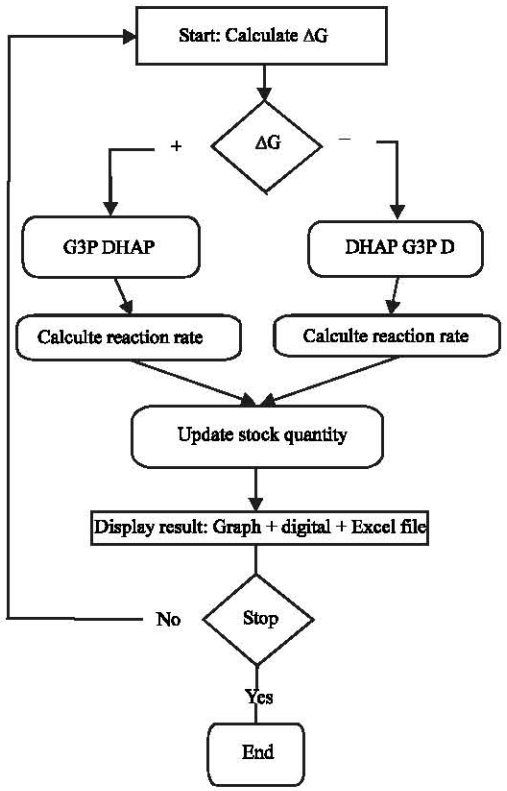


Fig. 1: Flow diagram of the programme

$$g_s = -8.314 * T_m * \ln(K_{eq}) \quad (4)$$

$$g = g_s + 8.314 * T_m * \ln\left(\frac{dhapi}{g3pi}\right) \quad (5)$$

where:

T_m = The absolute temperature in Kelvin degrees and it can be determined by the user from the program front panel.

K_{eq} = The (equilibrium constant) and it can be determined by the user from the program front panel.

dhapi = DHAP initial concentration and it can be determined by the user from the program front panel.

g3pi = G3P initial concentration and it can be determined by the user from the program front panel.

Task 2: Determination of reaction direction: This task is executed in 2nd sequence of the While Loop as shown in Fig. 4, where the reaction direction will be determined by using the free energy change equation. The equation is organized in specific procedures named formula node. This task has a case structure either to be TRUE or FALSE which is determined by the value of ΔG from Task 1 where the TRUE case is selected if $\Delta G \geq 0$ and the FALSE is selected if $\Delta G > 0$. The whole task can be expressed as follows:

- Calculates the free energy change ΔG from inputs described in Task 1.
- The output is directed to a selector (less or equal to zero selector) that evaluates the sign of the of ΔG value.
- The selector directs the ΔG negative value to the proper set of programming steps organized as case structures.
  - The TRUE case structure responds if the selector finds $\Delta G \geq 0$ In this case reaction will proceed in the direction G3P → DHAP and uses the rate Eq. 1 that describe G3P as the reactant.
  - The other case structure is (FALSE), which responds if the selector finds $\Delta G < 0$. In this case reaction will proceed in the direction DHAP → G3P and uses the rate equations that describe DHAP as the reactant.

Task 3: Determining and indicating the direction of the reaction loop: This task is executed in 3rd sequence of the While Loop, where a case structure has been designed indicate and display the direction of the reaction loop reaction as shown in Fig. 5. The TRUE case structure indicates the direction G3P → DHAP, while the FALSE case structure calculates indicates the direction DHAP → G3P.

There are two direction controls appear in the front panel, each control has two directions. The first direction control has either G3P → DHAP direction, which indicates reaction is in progress, or G3P → X that indicates reaction is not in progress in the specified direction. The second direction control has either DHAP → G3P which indicates reaction is in progress, or DHAP → X indicates reaction is not in progress in the specified direction.

Task 4: Preparation for updating stock quantities: The amount of change in reactant produced by each reaction cycle is determined by the rate of reaction, this quantity should be added to the available quantity of product before the current reaction cycle begins and should be subtracted from the original reactant stock. Tasks 2, 3 and 4 are performed in sequence structure number 0.

Task 5: Updating stock quantities in the memory of the program: This task is executed in 4th sequence where the

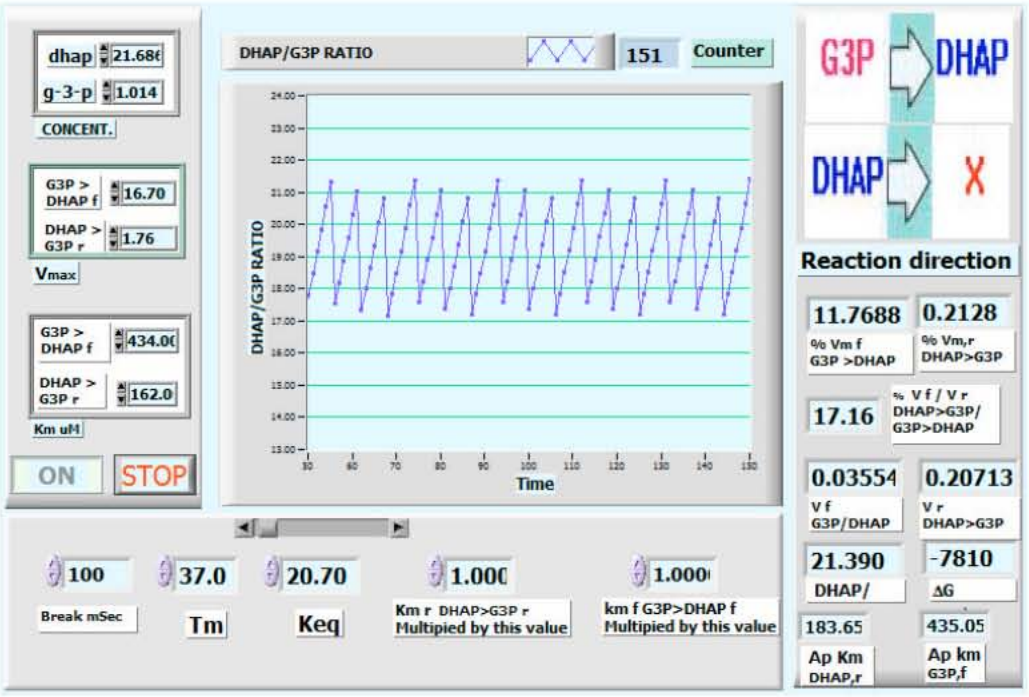


Fig. 2: Front panel of the programme

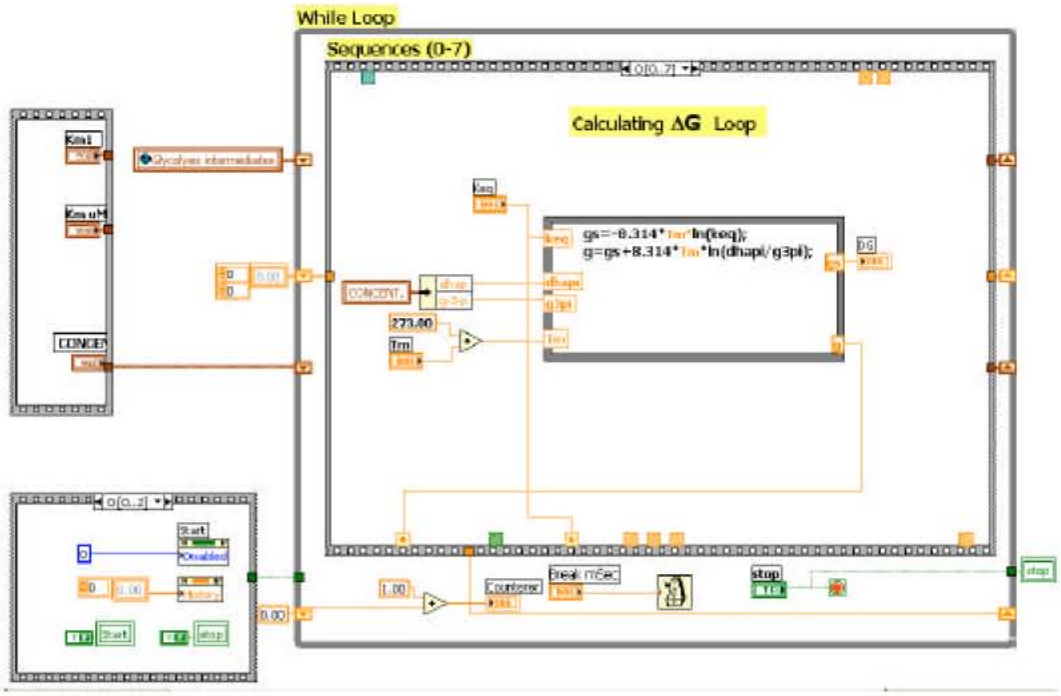


Fig. 3: Calculating ΔG

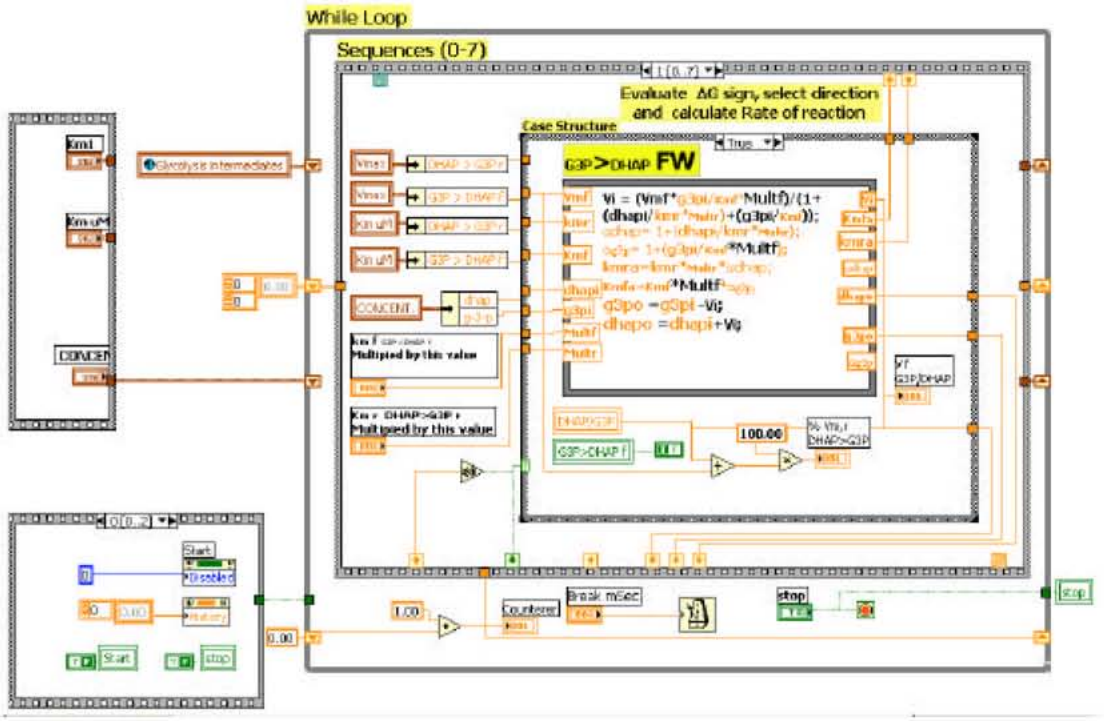


Fig. 4: Evaluating ΔG sign, select direction and calculate rate reaction

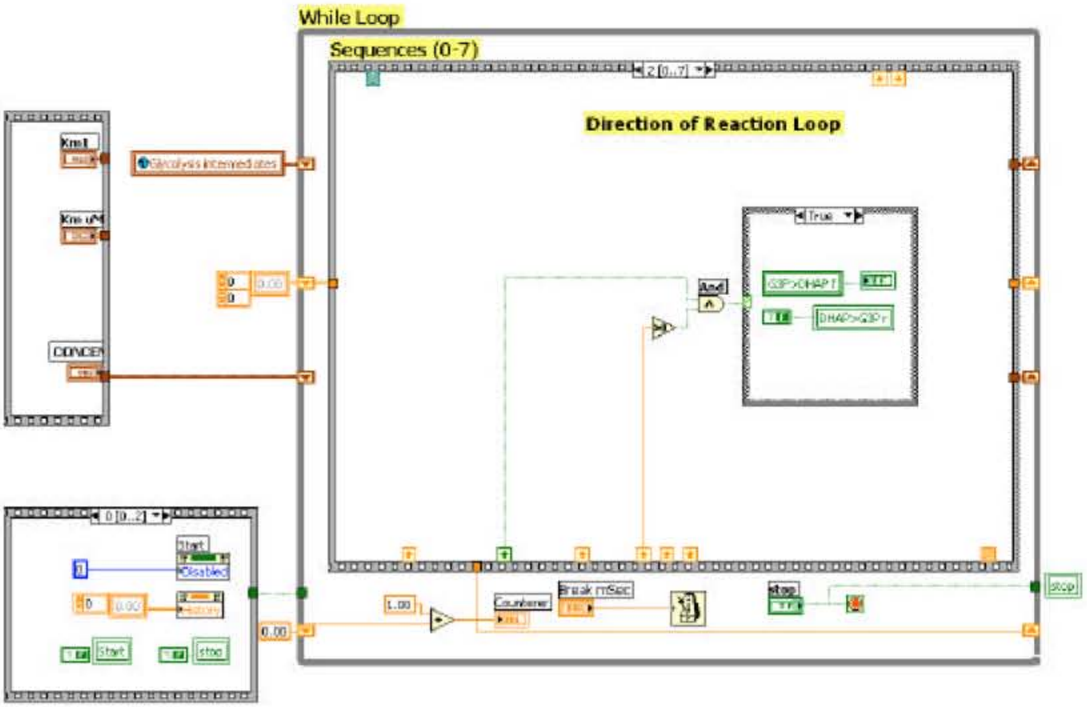


Fig. 5: Selecting and indicating the direction of G3P and DHAP

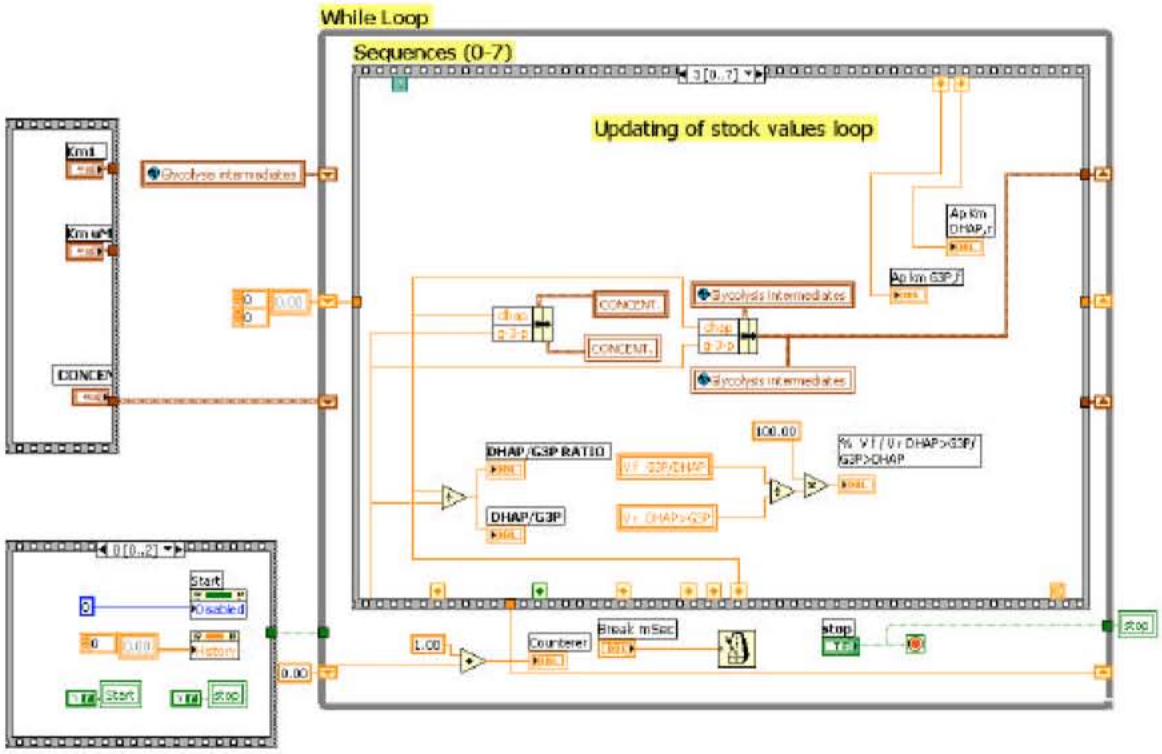


Fig. 6: Updating the local and global variables

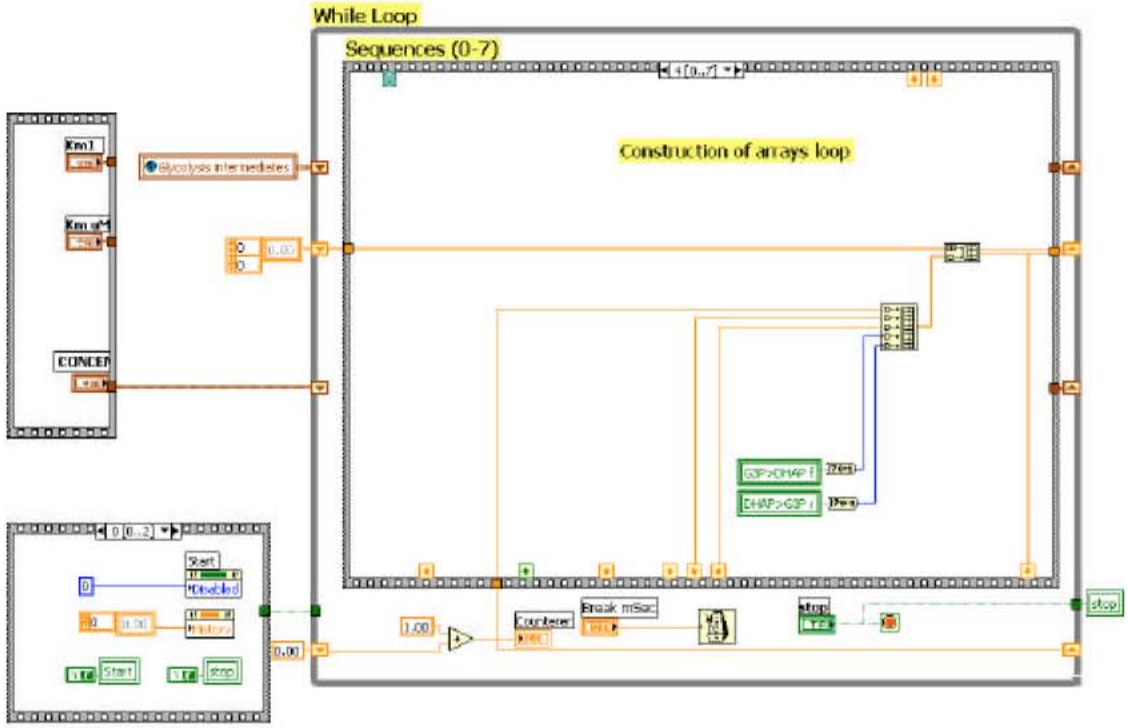


Fig. 7: Construction of loop results

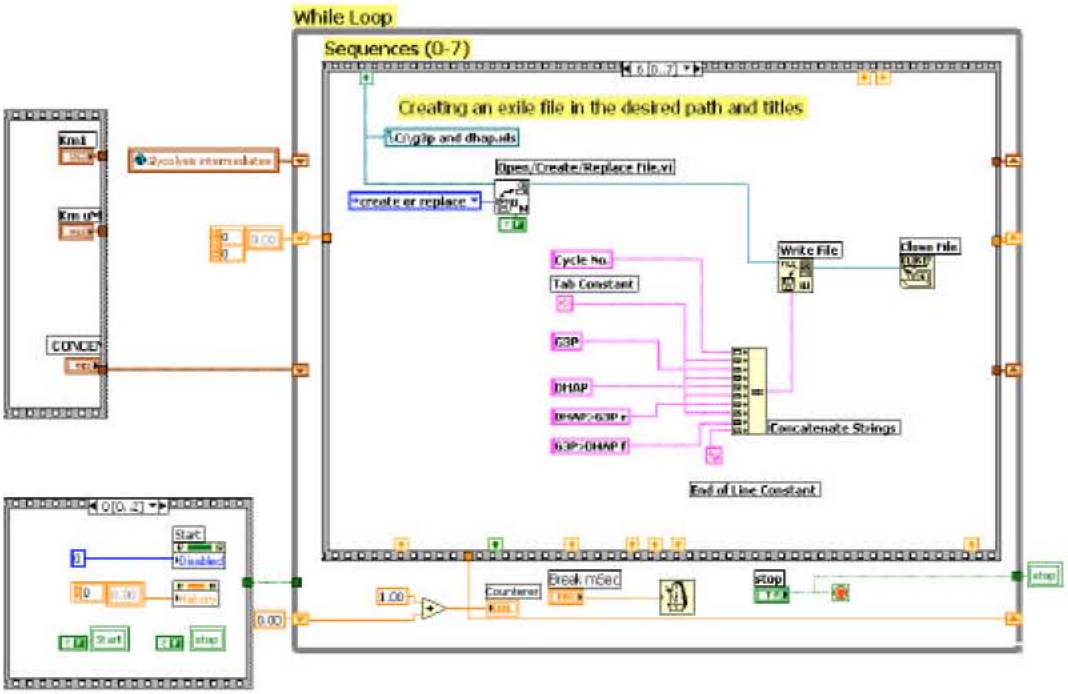
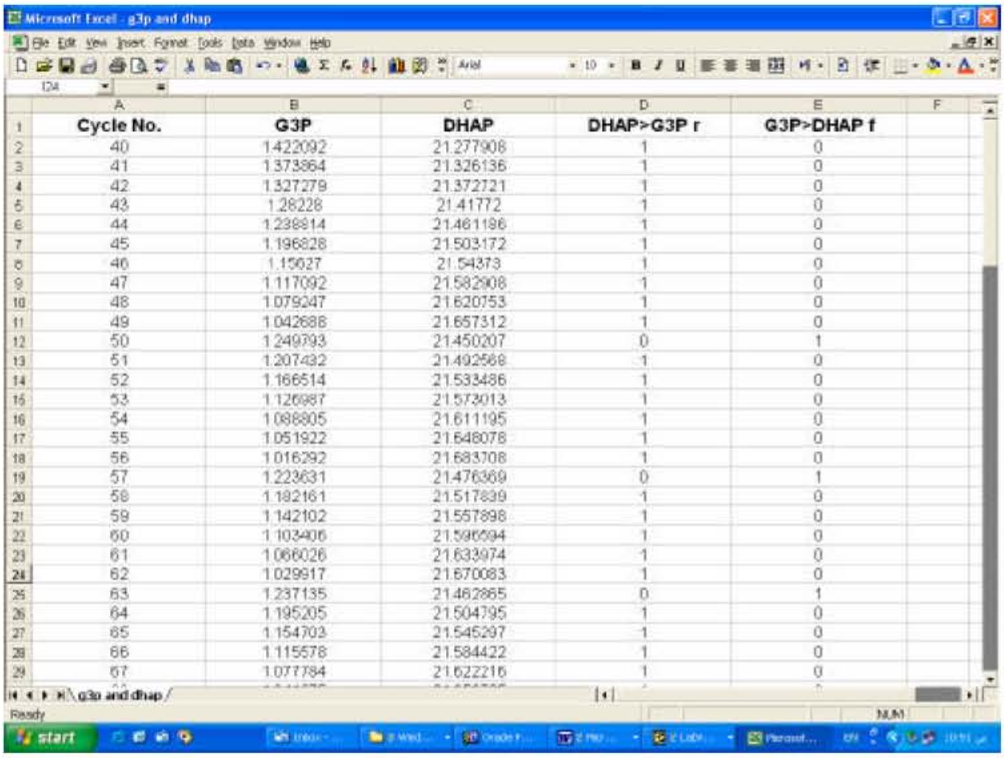


Fig. 8: Creating an excel file and transferring results into it



Microsoft Excel - g3p and dhap

	A	B	C	D	E
1	Cycle No.	G3P	DHAP	DHAP>G3P r	G3P>DHAP f
2	40	1.422092	21.277908	1	0
3	41	1.373864	21.326136	1	0
4	42	1.327279	21.372721	1	0
5	43	1.28228	21.41772	1	0
6	44	1.238814	21.461186	1	0
7	45	1.196828	21.503172	1	0
8	46	1.15627	21.54373	1	0
9	47	1.117092	21.582908	1	0
10	48	1.079247	21.620753	1	0
11	49	1.042688	21.657312	1	0
12	50	1.249793	21.450207	0	1
13	51	1.207432	21.492568	1	0
14	52	1.166514	21.533486	1	0
15	53	1.126987	21.573013	1	0
16	54	1.088805	21.611195	1	0
17	55	1.051922	21.648078	1	0
18	56	1.016292	21.683708	1	0
19	57	1.223631	21.476369	0	1
20	58	1.182161	21.517839	1	0
21	59	1.142102	21.557898	1	0
22	60	1.103406	21.596594	1	0
23	61	1.066026	21.633974	1	0
24	62	1.029917	21.670083	1	0
25	63	1.237135	21.462865	0	1
26	64	1.195205	21.504795	1	0
27	65	1.154703	21.545297	1	0
28	66	1.115578	21.584422	1	0
29	67	1.077784	21.622216	1	0

Fig. 9: Print out of an excel file

new values of reactant and product concentrations are updated in the memory of the program as shown in Fig. 6. Two sets of special memory variables are used: the local and the global variables; the local variable can communicate with other local variable in different sequence structures and provide data to that location; the global variable can communicate with other global variables located in different structures but in the same program; (control glycolysis is a local variable and glycolysis intermediates is a global variable).

Any of the variables mentioned can be a single reactant or it can be a group of reactants grouped in clusters, as is the case here.

The data generated in sequence structure 0 is passed to sequence structure 1 were group of orders contained in this icon named (convert cluster) will be used to update indicators and controls of the front panel from the updated values in the local and global variables.

Task 6: Displaying results in the front panel: The data is displayed in the front panel in the form of a graph or as a digital counter. % V_{max}, G3P, DHAP and Total are digital indicators that show the updated values; moreover the data is produced in the front panel as a real time graphical display that shows the DHAP concentration in waveform graph, that is the change in concentration of DHAP with time. The graph is very flexible and allows different X and Y-axis forms to be displayed.

It is relevant to mention that all values stored in the memory variables in the block diagram area can be displayed in the front panel.

Task 7: Constructing results and storing data in excel file: These tasks are executed in sequence 4, 6 and 7 where the results will be accumulated into a result array as shown in Fig. 7. The results will be transferred and stored into an Excel file with a specified location as shown in Fig. 8. Print out of the results are shown in Fig. 9.

Task 8: Repeat tasks 1 through 7: While loop described earlier in the block diagram contains the sequence structures mentioned earlier keep repeating orders until stop button is pressed.

RESULTS

A single substrate single product bi-directional (reversible) enzymatic reaction is simulated using LabVIEW graphical programming language; Reaction direction is determined initially as DHAP → G3P until the automatic selection switch direction receives a change in the sign of ΔG, then reaction proceeds in opposite direction; eventually reaction proceeds back and forth in oscillation fashion as concentration of DHAP and G3P change and cause ΔG to change from positive value to zero and finally to negative value.

Figure 2 represents the front panel of the program that provides a user interface to introduce reaction parameters and at the same time display information about reaction events such as DHAP and G_3P concentrations or DHAP/G_3P ratio, reaction direction, % V_{max}, k_m, apparent k_m of forward and reverse reaction; moreover LabVIEW allow introduction of speed control to increases rate of reaction or a time break in μ seconds that slows reaction as required.

TPI reaction directions and substrates concentration during oscillation: TPI reaction direction under conditions specified in the reaction parameters above, proceeds initially in the forward direction (G3P to DHAP) for 46 reaction cycles; from reaction cycle 46 onward reaction oscillate (changes directions back and forth). The rate of forward reaction (G3P to DHAP) is slower than the backward reaction during oscillation. It takes 16 reaction cycles in forward direction to match 3 reaction cycles in the reverse direction ($16_F/3_R$). As DHAP and G_3P concentrations are changing and competing each other for the same active site the forward k_m (for G_3P) and reverse k_m (for DHAP) are continuously changing to a new (apparent) k_m values during oscillation.

Directions oscillation pattern describes frequency of forward and reverse directions. Under conditions specified in reaction parameters above, directions oscillation pattern is $((5_F/1_R)_2\ (6_F/1_R)_1)_n$; that is a twice repeat of 5 consecutive forwards followed by 1 backward direction, followed by a single repeat of 6 consecutive forwards and one backward directions. This directions oscillation pattern is repeated continuously. Directions oscillation pattern is forward and reverse k_m dependent; moreover, it is more sensitive to forward k_m (for G3P) value. Figure 2 represents oscillation pattern of using forward K_m(for G3P) of 434 μM and reverse K_m(for DHAP) of 162 μM.

DISCUSSION

Computer simulation of directions of reversible reactions is a new research tool that provides information not possible by the traditional means of measurements:

First, the program allows prediction of reaction's direction at any concentration of metabolites, such information is displayed, stored or printed.

Second, the program allows evaluating the effect of changing forward k_m, backward k_m, on the direction of the

reaction. Competitive inhibitors (currently used as drugs for key enzymes) may be used to modify k_m values of reversible enzymes and hence modify direction of reactions and find new drug targets.

Third, the program if extended to simulate complete pathway or all-cellular reactions can modify flux of metabolism. This is extremely important in finding new regulator signals for reversible enzymes that allow manipulation of metabolism in the desired direction to produce the desired effect, or to limit the flux in unwanted direction; moreover the program allows predicting the effect of substituting one enzyme for another from different tissues, organs or species as a first step to control cellular events such as growth and cellular specialization during cell development, an extremely important issue in stem cells research and cloning.

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