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STOICHIOMETRIC PROPERTIES OF THE NON OXIDATIVE PHASE OF THE PENTOSE PHOSPHATE CYCLE

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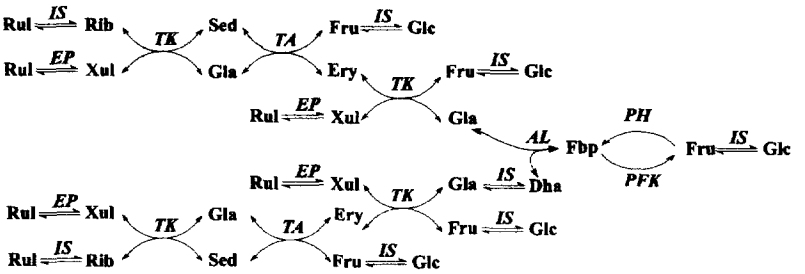
1. INTRODUCTION

Many metabolic pathways can be viewed as a linear sequence of chemical reactions each of them catalysed by a particular enzyme. In these routes, either the enzymes or the intermediates are not shared, and the study of both the stoichiometric properties and the flux distribution through the pathway can be easily carried out. On the contrary, in branched pathways the flux distribution can depend on both the system parameters and external constraints. A third level of complexity appears in routes in which, though they are not branched (there are only one initial and final product), some intermediates and enzymes are involved in more than one reaction. In these cases, the system behaviour also depends strongly on both the internal and external constraints.

The non oxidative phase of the Pentose Phosphate Cycle (PPC) may be considered as a paradigm of this third kind of pathways. This route has been defined as the conversion between 6-carbon sugars and 5-carbon sugars. Likely, as a consequence of this complexity, its function within the metabolism is not well specified. Usually, the role of this pathway is considered as a way to oxidate hexoses to yield both CO_2 and NADPH, the later being needed to the synthesis of fat acids in adipose tissue. However, in other tissues its function can be different as has been well described [1]. Thus, in a general meaning, this pathway ought to be placed within the more broad problem of the interconversion between monosaccharides. Notice that this conversion has (and might have in the origin of life) a fundamental role in the cell metabolism, since this set of reactions brings about the link between the energetic metabolism (hexoses) and the informative material (pentoses) of the cell.

Figure 1a shows the classical representation of the non oxidative phase of the PPC. This design can be found in the adipose tissue and it is known as the F-cycle [1]. Nevertheless, other different reaction schemes, appearing in other tissues and organism, have been observed [1,2,3]. As an example, figure 1b depictes the L-cycle that was firstly described by Williams in 1971 in liver [2]. As can be seen, it presents important differences with respect to the F-cycle.

(a)



(b)

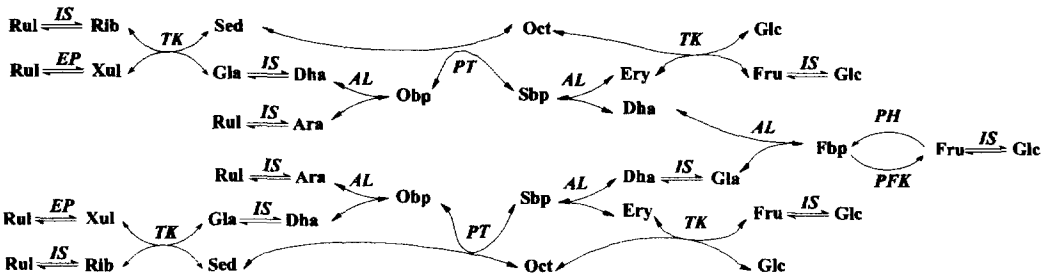


Fig. 1. Schemes which show the classical representation of two different designs (the F-cycle (a) and the L-cycle (b)) for the non-oxidative phase of the pentose phosphate pathway. In these schemes the enzymes that change the number of carbons of the sugars (TK, transketolase ; TA, transaldolase ; AL, aldolase), as well as the auxiliary enzymes that allow subsequent reactions (IS, isomerase ; EP, epimerase ; PH, phosphatase ; PT, phosphotransferase ; PFK, 6-phosphofructokinase) are depected. Abbreviations of sugars : Ara, arabinose 5-P ; Dha, dihydroxyacetona phosphate ; Ery, erytrose 4-P ; Fbp, fructose 1,6-bis-phosphate ; Fru, fructose 6-P ; Gla, glyceraldehyde 3-P ; Glc, glucose 6-P ; Obp, octulose 1,8-bis-phosphate; Oct, octulose 8-P ; Rib, ribose 5'-P ; Rul, ribulose 5'-P, Sed, sedoheptulose 7-P ; Xul, xylulose 5-P.

All these designs share several aspects that are worthy to be pointed out. Firstly, they can be classified within the group of specially complex pathways above discussed. For instance, in the F-cycle, the same enzyme (denoted as TK) acts on two different reactions, and the intermediate Gla also appears as a substrate and/or product in several reactions. Similar situations can be found in other designs of this interconversion. Secondly, the same kind of essential enzymes are present in these pathways. These enzymes are the transketolase (TK), the transaldolase (TA) and the aldolase (AL). Their mechanism is always the same; the TK catalyses the transfer of a 2-carbon fragment from a sugar to another, the TA produces a transference of a 3-carbon fragment and the AL induces the condensation or decondensation of sugars, a triose being always involved. Schematically, these reactions can be described as follows:

$$C_n + C_{m-2} \overset{TK}{\rightleftharpoons} C_m + C_{n-2}$$

$$C_n + C_{m-3} \overset{TA}{\rightleftharpoons} C_m + C_{n-3}$$

$$C_3 + C_n \overset{AL}{\rightleftharpoons} C_{n+3}$$

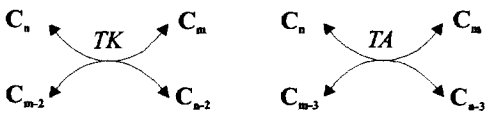
Here, the subindex refers to the number of carbons of the sugars.

Although the enzymatic mechanisms are similar in every pathway, their specificities are different. Whereas in the F-cycle AL acts exclusively on C6, both C7 and C8 are also substrates of this enzyme in the L-cycle. Moreover, in the later design, C8-sugar can be a donor of a 2-carbon fragment by means of TK, reactions that does not appear in the F-cycle. Notice also that the TA is noy involved in the L-cycle.

Together with these essential enzymes, other auxiliary enzymes are involved in each pathway: isomerases, epimerases, phosphotranferases, etc. Although the dynamic and the possibilities of carrying out the conversion depends also on these enzymatic activities, the stoichiometric organisation of the pathway are not affected by them, since their action do not change the number of carbons of sugars, and therefore they will not be considered in this work.

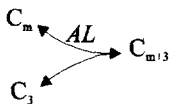
2 WAY TO REPRESENT THE GLOBAL REACTION MECHANISMS OF THESE COMPLEX PATHWAYS

Usually, the enzymatic reactions that appear in these pathways are implicitly described as bisubstrate reactions which would take place through the ternary complex C_m-Enz-C_n, (Enz being any essential enzyme). For instance:



(2.1)

for the TK and TA, and

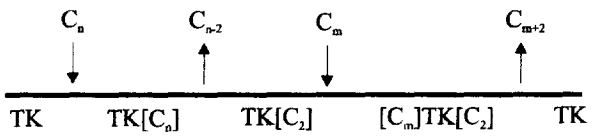


(2.2)

for the AL. It could be thought that, for example, the mechanism of TK would occurs through the following sequence of steps

$$TK + C_n + C_{m-2} \rightleftharpoons TK[C_nC_{m-2}] \rightleftharpoons TK[C_{n-2}C_m] \rightleftharpoons TK + C_{n-2} + C_m \tag{2.3}$$

However, the actual mechanism is better described by a Ping-Pong reaction [4], as follows:



(2.4)

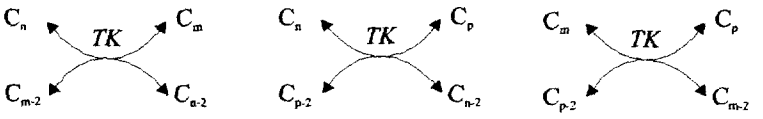
Notice that C_n and C_m are independent substrates, then any conversion between them is possible (provided a set of structural constraints are accomplished). Thus, the reactions of transference by means of this enzyme must be modelled as the coupling between two hemireactions through a common intermediate ($TK[C_2]$):

$$\begin{aligned} TK + C_n &\rightleftharpoons TK[C_2] + C_{n-2} \\ TK + C_m &\rightleftharpoons TK[C_2] + C_{m-2} \end{aligned} \tag{2.5}$$

One of the hemireactions will progress only if the other one progresses in the opposite direction. In this context, enzyme specificity refers to the kind of sugar that can be associated to the free enzyme. Then, if the enzyme can act on sugars of n, m and p carbons, the following reactions could take place:

$$\begin{aligned} TK + C_n &\rightleftharpoons TK[C_2] + C_{n-2} \\ TK + C_m &\rightleftharpoons TK[C_2] + C_{m-2} \\ TK + C_p &\rightleftharpoons TK[C_2] + C_{p-2} \end{aligned} \tag{2.6}$$

and as a consequence, the following coupling must be considered:



(2.7)

In other words, if the two first couplings exit, then the third one also should occur.

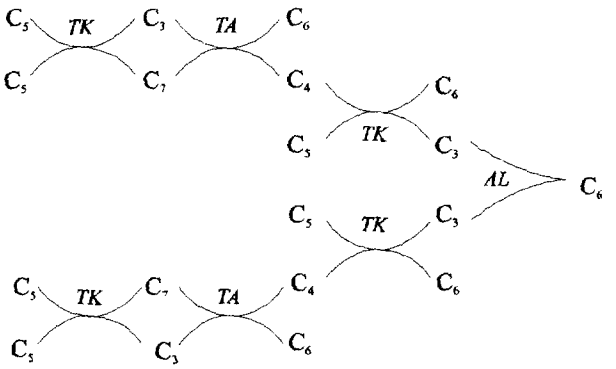


Fig. 2. A simplify representation for the F-cycle of the non-oxidative phase of the pentose phosphate pathway.

A similar reasoning applies to both TA and AL. Therefore, despite of the F-cycle is usually described in a simplified way as in figure 2, actually it should be modelled by the following hemireactions:

$$\begin{aligned} TK + C_5 &\rightleftharpoons TK[C_2] + C_3 \\ TK + C_6 &\rightleftharpoons TK[C_2] + C_4 \\ TK + C_7 &\rightleftharpoons TK[C_2] + C_5 \\ TA + C_6 &\rightleftharpoons TA[C_3] + C_3 \\ TA + C_7 &\rightleftharpoons TA[C_3] + C_4 \\ AL + C_6 &\rightleftharpoons AL[C_3] + C_3 \\ AL + C_3 &\rightleftharpoons AL[C_3] \end{aligned} \tag{2.8}$$

As can be derived from these considerations, the pathway organization, even its ability to carry out a particular conversion, depends on the specificity of the enzymes involved in this pathway. Under absolute non-specificity conditions, the following hemireactions must be taken into account besides the previously written (2.8):

$$\begin{aligned} TK + C_8 &\rightleftharpoons TK[C_2] + C_5 \\ TA + C_8 &\rightleftharpoons TA[C_3] + C_5 \\ AL + C_7 &\rightleftharpoons AL[C_3] + C_4 \\ AL + C_8 &\rightleftharpoons AL[C_3] + C_5 \end{aligned} \tag{2.9}$$

Under stationary conditions, these hemireactions could be in principle coupled each other in several ways, depending on both the boundary constraints and the system parameters (kinetic constants, enzyme concentration, etc.). As it was stated above, these couplings occur through the intermediates TK[C2], TA[C3] and AL[C3]. At this regime, the relative rates of the hemireactions take particular values according to the stoichiometric constraints.

The classical way of representing schematically the reaction mechanisms (arrows between intermediates) is based on two chemical properties: sequence of reactions, and the stoichiometry. The complex routes here considered are characterized precisely because they are not sequential and have not a defined stoichiometry. This feature makes that a new kind of representation is necessary for these kind of pathways. A better way of representing the system would be as a reaction network, in which the intermediates are the nodes, connected each other by lines or transformations. Figure 3 shows schematically the corresponding chemical reaction network for the interconversion C6/C5.

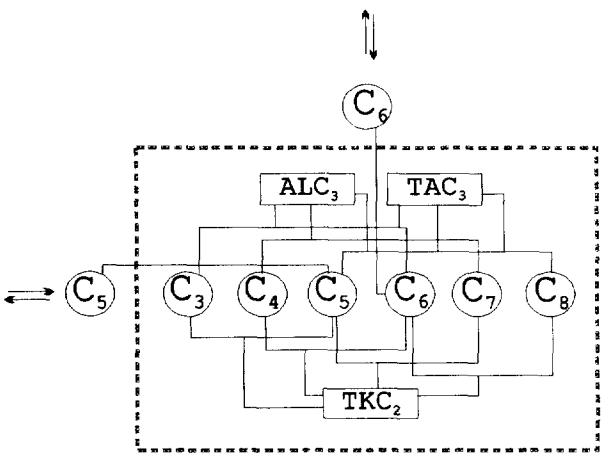


Fig. 2. Schematic representation of the interconversion between 5-carbons sugars and 6-carbons sugars as a reaction network.

The flux distribution at the steady state has to be compatible with both stoichiometric (indeed, a consequence of mass action law) and boundary constraints. In principle, many flux distributions compatible with both restrictions can be found .

Let us consider again the L-cycle described by Williams. Usually, this pathway has been represented as in figure 1b [2]. Assuming that the mechanism of the enzymes involved in the route is that described in (2.4),

this scheme would be unsuitable at steady state. The reason is that AL breaks C_7-sugars and forms C_8 sugars in the same compartment, and this would imply that the hemireaction AL+C3 $\rightleftharpoons$ ALC3 should occur in both directions simultaneously. However, this contradictory result can be avoided if the couplings of the figure 4 are performed.

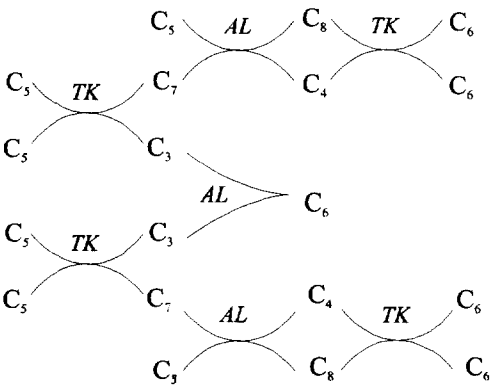


Fig. 4. A schematic representation for the L-type of the non-oxidative phase of the pentose phosphate pathway. In this scheme AL acts like TA transferring three carbons between 5-carbons sugars and 7-carbons sugars.

Even so, in this pathway those activities involve in the non-reductive phase of the Calvin cycle are present. Then, according to Principle of Activity [5], Calvin cycle would be also working, and then it should be also superimposed into the scheme shown in figure 4. Thus, it seems clear that the organization of this pathway is more complex that it could be thought.

3. ENZYMATIC ACTIVITIES AND STRUCTURAL ORGANIZATION OF THE PATHWAY

Given a particular set of enzymatic activities that make possible the stoichiometric conversion in the non-oxidative phase of the pentose phosphate pathway, a main questions arises: How many different dynamic organizations can the system adopt?. In other words, how many possible flux distributions compatible with a stoichiometric interconversion (with a net flux through the pathway) are possible?.

As it was already said, the possible substrates for the TK are the sugars C5, C6, C7 and C8, whereas the TA and AL only can works on C6, C7 and C8. For a particular system, these enzymes can be totally non-specific, or on the contrary, have catalytic capacity on particular substrates. In fact, the set of activities of each enzymes defines the structural properties of the pathway. Since each of the reactions described in (2.8) and (2.9) can be carried out only if the corresponding enzyme has that activity, there are 2^{10} different activity combinations. These sets of enzymatic activities can be written in a vectorial form, each vector element being 1 or 0 whether the corresponding enzyme has got or not respectively this particular activity. So, for instance, the activity vector

$$\begin{matrix} & \text{TK} & \text{TA} & \text{AL} \\ \mathbf{a}_1=([1,1,1,0], & [1,1,0], & [1,0,0]) \end{matrix} \tag{3.1}$$

means that the TK has activity on sugars C5, C6 and C7, but not on C8-sugars; the TA can act on C6 and C7, and the AL act only on C6-sugars.

However, not all the activity vectors correspond to a stoichiometric interconversion. By using a stoichiometric network analysis [6,7], it is not difficult to find the correspondence between the activity vectors and the set of flux distributions through the pathway. In fact, the temporal evolution of the concentration of the intermediate **x** can be expressed as the product of the stoichiometric matrix and the rate vector **v** (vector whose components are the rate of every reactions involved in the route):

$$\mathbf{x} = \mathbf{N}\,\mathbf{v} \tag{3.2}$$

N being the stoichiometric matrix, whose rows are related to the metabolites whereas the columns represent the reactions in which each of them are involved. The expression of this matrix can be computed from the reaction scheme (2.8) and (2.9)[9].

An equation similar to (3.2) can be derived for each activity vector. The stationary solutions can be easily found, by solving the algebraic linear system

$$\mathbf{N}\,\mathbf{v} = 0 \tag{3.3}$$

Perhaps, the major result of this analysis is that only 51 activity vectors are feasible for the stoichiometric transformation between sugars of five carbons and sugars of six carbons. Physical considerations as the specificity hypothesis, that is if a enzyme acts on sugar of m carbons and m+2 carbons then it must act on sugars of m+1 as well [8], or the independence from initial conditions, reduce even more this number to 21. Therefore, the stoichiometric interconversion C_5/C_6 can only be carried out by a few activity vectors.

In addition, the subset of activity vectors that allow a net flux through the pathway at the steady state (stoichiometrically feasible) can be classified in two groups: (a) those related to a fixed velocity vector such as the activity vector (3.1), and (b) those related with infinite rate vectors. The first ones have a determinate flux distribution, whereas the latter have a non-determinate flux distribution through the different reactions of the pathway.

4. WHAT IS THE PHYSICAL MEANING OF A DETERMINATE SOLUTION?

It is not difficult to find the physical meaning of a given determinate flux distribution. The elements of the rate vector have particular values in relation to the net flux of the conversion, and these do not depend on either the external constraints (boundary conditions) or the system parameters (kinetic constants, enzyme concentrations, etc.). As an example, the following velocity vector is the unique solution for the activity vector (3.1).

$$\mathbf{v}_1=([6,-5],[4,-2,-2,0],[-2,2,0],[-1,0,0,1])^T \tag{4.1}$$

which means that, for example, reaction $TK+C_5 \rightleftharpoons TK[C_2]+C_3$, at the steady state, is working with a rate four times greater than the net flux of conversion. Figure 5 shows the flux distribution through the enzymatic network for the activity vector $\mathbf{a}_1$corresponding with enzymatic activities of the F-cycle.

The other six activity vectors that imply determinate solutions are

$$\mathbf{a}_2= ([1,1,1,0],[0,0,0],[1,1,0]) \tag{4.2}$$

$$\mathbf{a}_3= ([1,1,1,1],[0,1,1],[1,0,0]) \tag{4.3}$$

$$\mathbf{a}_4= ([1,1,1,1],[1,1,0],[0,0,1]) \tag{4.4}$$

$$\mathbf{a}_5= ([1,1,1,1],[0,1,1],[0,1,0]) \tag{4.5}$$

$$\mathbf{a}_6 = ([1,1,1,1],[0,1,1],[0,0,1]) \tag{4.6}$$

$$\mathbf{a}_7 = ([1,1,1,1],[0,0,0],[0,1,1]) \tag{4.7}$$

being the corresponding rate vectors

$$\mathbf{v}_2 = ([6,-5],[4,-2,-2,0],[0,0,0],[-3,2,0,1])^T \tag{4.9}$$

$$\mathbf{v}_3 = ([6,-5],[2,-2,-2,2],[0,2,-2],[-1,0,0,1])^T \tag{4.10}$$

$$\mathbf{v}_4 = ([6,-5],[3,-2,-2,1],[-2,2.0],[0,0,-1,1])^T \tag{4.11}$$

$$\mathbf{v}_5 = ([6,-5],[1,-2,-2,3],[0,2,-2],[0,-1,0,1])^T \tag{4.12}$$

$$\mathbf{v}_6 = ([6,-5],[1,-2,-1,2],[0,2,-2],[0,0,-1,1])^T \tag{4.13}$$

$$\mathbf{v}_6 = ([6,-5],[1,-2,-2,3],[0,0,0],[0,2,-3,1])^T \tag{4.14}$$

It is worthy to notice that the activity vectors $\mathbf{a}_2$ correspond to the enzyme activities described for the Calvin cycle. That is, with these enzymatic activities the interconversion C6/C5 can be carried out, and with an unique relative flux distribution.

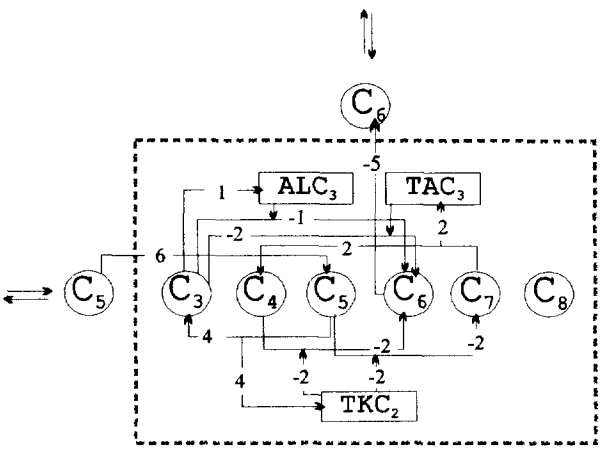


Fig. 5. F-cycle represented as a reaction network. The lines of figure 2 now have adopted a particular direction and a particular value in relation to the total flux of the pathway. The intermediate C_8 is not connected with any other intermediate since no enzyme has got activity on it

5. WHAT IS THE MEANING OF AN INDETERMINATE SOLUTION?

Among the 21 activity vectors that make possible a stoichiometrically feasible conversion, 14 are related to indeterminate solutions for the rate vectors. As an example, let us consider the activity vector

$$([1,1,1,1],[0,0,0],[1,1,1]) \tag{5.1}$$

that correspond with the enzymatic activities of the L-type-Williams cycle [2]. The rate vector, solution of (5.1) is

$$\mathbf{v}=([6,-5],[4+\lambda_2, -2, -2, -\lambda_2],[0, 0, 0],[-\lambda_2-3, 2, \lambda_2, 1])^T \quad (5.2)$$

that, as can be seen, implies infinite solutions (obtained for particular values of the real parameter λ_2). Similar velocity vectors can be found for the other 13 activity vectors.

In fact, this solution space has a vectorial structure, and any vector that belongs to it can be expressed as a lineal combination of a basis (a complete generator of the space). It is well known that the choice of this basis is arbitrary. Nevertheless, it seems reasonable to choose those vectors that are the simplest. By the simplest vector(s), we mean a vector with the highest number of null entries. In this particular example, this basis could be formed by the following four vectors :

$$\mathbf{b}_1=([6,-5],[4, -2, -2, 0],[0, 0, 0], [-3, 2, 0, 1])^T \quad (5.3)$$

$$\mathbf{b}_2=([0, 0],[1, 0, 0, -1],[-1, 0, 1], [0, 0, 0, 0])^T \quad (5.4)$$

$$\mathbf{b}_3=([0, 0],[1, 0, 0, -1],[0, 0, 0], [-1, 0, 1, 0])^T \quad (5.5)$$

$$\mathbf{b}_4=([0, 0],[0, 0, 0, 0],[1, -1, 0], [-1, 1, 0, 0])^T \quad (5.6)$$

Then, any solution can be written as a linear combination of the four vectors of the basis, i.e.

$$v_i = \lambda_1 b_1 + \lambda_2 b_2 + \lambda_3 b_3 + \lambda_4 b_4, \lambda_i \in \Re \quad (5.7)$$

The physical meaning of the basis vectors is clear. They can be viewed as specific conversions in a similar way as that in figure 5. Note that, among them, there are one that allow the conversion C6/C5, while other are futile cycles that, taken separately, do not produce a net flux through the pathway. The main conclusion to be remarked is that whereas the relation to the net flux of particular reactions rates is fixed, independently of any internal or external constraint, for other reactions their contribution can change as a function of these constraints. First results demonstrate that this kind of organization can have a large influence on the general behaviour of the pathway, mainly on dynamic aspects and on the adaptation to external variations (work in progress).

6. CONCLUDING REMARKS

The previous reasoning has pointed out some questions that it is worthy to deal with in a certain detail. Previously, it has been considered that simplicity of metabolic pathways might be the result of optimising some target function during evolution [5]. Moreover, it has been stated that the simplest route is that involving the lowest number of reactions. Melendez-Hevia *et al.* [8] have shown that the net flux through a linear pathway increases as the number of enzymatic reactions decreases. In the kind of pathways which we are studying in the present work some intermediates are involved in more than one reaction, and as a consequence the concept of simplicity might be redefined as a network property. The relation between the number of nodes and connections is a commonly used measure of complexity in chemical networks. Then, the lower this ratio, the higher the complexity of the network. Then, a system with a higher number of intermediates (nodes) than other, not necessarily must be more complex. An alternative to this definition, valid for this kind of metabolic systems, is that simplicity can be measured by the number of null entries of the activity vectors.

Although a simpler metabolism posses a number of advantages from a evolutionary point of view[8], complexity, as appears in this kind of systems, can be used to present a high versatility and therefore to respond better to external changes. Complex pathways can be adapted optimally to get different cellular goals. So, in principle, it can be said that those activity vectors that give rise to determinate solutions are related to simpler networks than those whose corresponding solutions are indeterminate.

We suggest that present-day metabolism is brought about in agreement with these criteria. Then, most of the actual situations correspond with activity vectors related to indeterminate solutions, that means multiple flux distributions depending on both internal and external constraints. This fact could explain that only under particular experimental conditions well known pathways, as the L-cycle of PPC, can be observed. In other cases, when the cell needs a high specification in the flux regime, the system tends to remove some enzymatic activities. Such a case occurs in the non reductive phase of the Calvin cycle. The presence of a TA activity would provoke a indeterminate solution, and possibly with negative consequences for the net flux conversion. Light inhibition of this activity solves the matter.

As a final comment we would like to say that obviously the indetermination of the flux distribution is a consequence of the non-specificity of the enzymes. Is this non-specificity an intrinsic property of this kind of metabolic systems or, on the contrary, is the result of an evolutive permissiveness in order to obtain more versatile metabolic pathways?. Some clues to answer this question, within the more general context of monosaccharide interconversion, can be found in a recent article submitted [8].

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REFERENCE

1. WOOD, T. *The pentose phosphate pathway*, Academic Press, Orlando (1985).
2. WILLIANS, J. F., ARORA, K. K., & LONGENECKER, J. P., The pentose pathway: a random harvest. *Int. J. Biochem.* **19**, 749-817 (1987).
3. SUSSKIND, B. M., WARREN, L. G. & REEVES, R. A pathway for the interconversion of hexose and pentose in the parasitic amoeba Entamoeba histolytica. *Biochem. J.* **204**, 191-196 (1982).
4. DATTA, A. G & RACKER, E., Mechanism of action of transketolase. *J. Biol. Chem.* **236**, 617-623. (1961)
5. MELENDEZ-HEVIA, E., The game of the pentose phosphate cycle : A mathematical approach to study the optimization in design of metabolic pathways during evolution. *Biomed. Biochim. Acta.* **90**, 903-916 (1990).
6. REDER, C., Metabolic Control Theory : A Structural Approach. *J. Theor. Biol.* **135**, 175-201. (1988)
7. CLARKE, B. L. Stochiometric Network Analysis. *Cell Biophysics.* **12**, 237-253. (1988)
8. MELENDEZ-HEVIA, E, WADDELL, T. G., MONTERO, F., Optimization of Metabolism : The evolution of metabolic pathways toward simplicity through the game of the pentose phosphate cycle. *J. Theor. Biol.* **166**, 201-220. (1994)
9. NUÑO, J. C., SANCHEZ-VALDENEBRO, I., PEREZ-IRATXETA, C., MELÉNDEZ-HEVIA, E., MONTERO, F., Network organization of cell metabolism : monosaccharide interconversion. *Submitted.*