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Economy of Design in Metabolic Pathways: Further Remarks on the Game of the Pentose Phosphate Cycle

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Sugar rearrangement in the pentose phosphate cycle and other related pathways were previously analysed by abstraction to a mathematical game of optimization based on certain hypotheses concerning enzymatic mechanisms of living cells (Meléndez-Hevia & Isidoro, 1985, *J. theor. Biol.* **117**, 251–263). The solution of that problem shows that the metabolic pathway, as occurs in cells, is the simplest solution of the operative problem. However, in that work, only the number of carbons in every sugar was considered. In the present paper, all structural features of the sugars and reaction mechanisms are taken into consideration, and the problem is again considered by abstraction to a mathematical model which includes all structural features of the sugars as well as all structural requirements of the enzymes in the hypotheses of the mechanisms. As in the above-mentioned paper, the hypothesis of simplicity is also imposed in order to achieve the objective (to convert six ribulose 5-phosphate into five glucose 6-phosphate) in the least number of steps (or with the least number of free intermediates), and the least number of carbons in the intermediates. It is concluded that the optimal, or simplest, solution of this problem is the same procedure as that occurring in living cells. The Calvin cycle in photosynthesis and the "L-type" of the pentose phosphate cycle are also analysed arriving at similar conclusions in both cases. These results suggest some reflections about the logic in the design of metabolic pathways, and the possible role of the hypothesis of simplicity in cell evolution.

1. Introduction

The whole set of metabolic pathways in the living cell seems to be a very complicated network, as if it were a labyrinth of enzymatic conversions among metabolites. It might seem difficult to find a rational sense in its design; however, in this complex network, there are certain features of simplicity. Let us consider two of them. (1) The many different enzymes which participate do so on the basis of very few different catalytic mechanisms; moreover, there are relatively few different amino-acid-residues participating as catalytic groups in any active site, and the use of the same coenzymes—such as pyridoxal phosphate or thiamine pyrophosphate—to catalyse different reactions is frequent. In fact, the types of reactions or mechanisms of catalysis are, essentially, very few and, on the basis of these limited resources, the whole set of metabolic transformation is carried out in the living cells. (2) There is an extensive set of metabolites common to several metabolic pathways such as pyruvate, acetyl-CoA, glucose 6-P, ribose 5′-P etc., and especially transfer pairs,

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such as NAD^+/NADH, ATP/ADP, glutamate/α-ketoglutarate, etc. All these are cross-way points among different metabolic pathways, and make the coupling among different processes possible.

However, these two emphasized features of simplicity have probably determined the complexity of metabolic pathways, since few available mechanisms of reaction can make a given synthesis or degradation very laborious, even more as if the convenience of passing through certain intermediary metabolites, common to other pathways, is taken into consideration. Thus, considering a given pathway in cellular metabolism, the above arguments lead us to the following situation: on the one hand, enzymatic mechanisms available in the living cell determine a certain complexity (in number of steps) for a given conversion of a substrate in the corresponding end product, and on the other, let us make the assumption that, for reasons of economy, this complexity must be as small as possible. The question raised then is, have the living cells observed this argument of simplicity in the design of metabolic pathways? A way to explore this subject is the study of a given metabolic pathway, defined by the substrate (S) and the product (P) involved, by means of the design of a mathematical operational model to account for the conversion of S into P, according to a set of hypotheses which describe all the enzymatic mechanisms available, and then adding the hypothesis of simplicity. This hypothesis says that the conversion of S into P must be obtained in the minimum number of steps (or through the least number of intemediates), and involving the least complexity (the least number of carbons) in such intermediates. This mathematical problem of optimization can be resolved, and then the obtained solution can be compared with the procedure carried out by the living cell. Thus, certain direct conclusions will be obtained concerning the question raised above.

In a previous paper (Meléndez-Hevia & Isidoro, 1985), the pentose phosphate cycle and some related pathways were analysed, reaching the conclusion that the "F-type" and "L-type" pentose phosphate cycles, each including its particular hypotheses, as well as the Calvin cycle in photosynthesis, such as they occur in the living cells, are the simplest solutions of every particular raised problem. However, in order to simplify these problems and their theoretical treatment, only the number of carbons of every sugar were considered, and other structural features, such as configuration of their carbons, their state of phosphorylation, and the carbonyl function (aldose or ketose) were not taken into consideration; therefore, enzymes which account for such transformations (epimerases, isomerases and phosphatases) were not included in the hypotheses of the mechanisms. In this paper, a complete view of the problems is presented, considering all structural and mechanistic features of the pathways in the non-oxidative phase (sugar interconversions), and the above question is again analysed. By using a mathematical model we demonstrate that the living cell solutions for the three above-mentioned pathways are the simplest operational solutions for these problems.

2. Mathematical Model

Let us first consider the classic, or "F-type" pentose phosphate cycle, as described by Horecker *et al.* (1954), and consider two phases in it, according to the nature of

molecular transformations, the oxidative phase, from glucose 6-phosphate to ribulose 5-phosphate (mainly involving oxidations and decarboxylation); and the non-oxidative phase, from ribulose 5-phosphate to glucose 6-phosphate (which mainly involves reactions of sugar interconversions). This second phase will be formulated here by the following mathematical model, including: (1) the above-mentioned operational aim; (2) a set of hypotheses of the mechanisms which describe all the possibilities of reaction available in the cell, related with this problem; and (3) the imposed hypothesis of simplicity, which is the real aim of our study.

(1) OBJECTIVE

To convert six ribulose 5-phosphate into five glucose 6-phosphate, by means of the permissible mechanisms, described as follows:

(2) HYPOTHESES OF THE MECHANISMS

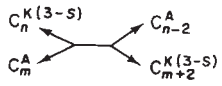
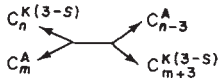
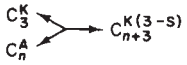
There are seven possible operations involving some structural conditions in every case. At a given moment of the operational course, any one can be used, if the structural conditions are observed, to advance toward the solution. Every one of these operations is defined as one step:

(a) *Transketolase.* A set of two carbons can be transferred from one sugar to another. The sugar which donates the carbons must always be a keto-sugar with its third carbon in S configuration, and the sugar which accepts the carbons must always be an aldo-sugar. The two carbons transferred are the first of the keto-sugar (C-1 and C-2, as a glycolaldehyde group). The two sugars which participate in this process must be phosphorylated in their last carbon only. The products of this reaction are also an aldo- and a keto-sugar, respectively, with the same structural features of the substrates, i.e., as a result of the reaction the keto-sugar is converted into an aldo-sugar (with two carbons less) and the aldo-sugar is converted into a keto-sugar (with two carbons more), the latter having its third carbon in S configuration, all these being monophosphorylated in their last carbon. It can be seen that this reaction is always reversible because of its symmetry, i.e., it being considered in either direction, both the two substrates and the two products account for the same structural features. This mechanism is summarized in Table 1.

(b) *Transaldolase.* A set of three carbons can be transferred from any sugar to another. The donor must always be a keto-sugar with its third carbon in S configuration and the fourth one in R configuration and the acceptor must always be an aldo-sugar. The carbons which are transferred are the first three of the keto-sugar (C-1, C-2 and C-3, as a dihydroxyacetone-group). The two sugars which participate in this reaction must be monophosphorylated in their last carbon. The products of this reaction are also an aldo- and a keto-sugar, respectively, with the same structural features as the substrates, as in the previous case. All other features of the preceeding case are equally of application here, except the number of carbons transferred. This mechanism is also summarized in Table 1.

TABLE 1

Scheme of the mechanism features of the reactions involving rearrangement in the number of carbons among sugars. Subscripts represent the number of carbons in every sugar. Other structural features are indicated as superscripts: A, aldo; K, keto; (3-S), third carbon in S configuration. All sugars are mono-phosphorylated in their last carbon with the exception of the product of aldolase condensation, which is biphosphorylated in its first and last carbon

Transketolase	Transaldolase	Aldolases
		

(c) *Aldolases.* As a particular case of the reactions catalysed by transaldolase (which transfers a group of dihydroxyacetone to an aldose), it is possible to condense this free sugar with any aldo-sugar to give a single keto-sugar; it must be taken into account that the third carbon of dihydroxyacetone has no chirality, therefore having no possible R or S configuration. The two substrates of this reaction (dihydroxyacetone and the aldo-sugar) must be monophosphorylated in their last carbon. The sugar product, whose number of carbons is the summation of its substrates, is a keto-sugar with its third carbon in S configuration and the fourth one in R configuration; it is biphosphorylated, in its first and last carbons. This reaction is not symmetrical, as in the preceeding two where the substrates have the same structural features as the products. Thus, its reversibility cannot be concluded only by looking at its mechanism; however, it must be taken into account that it is reversible in cellular conditions, and therefore an opposite reaction to the one described here (the breaking up of a keto-sugar into dihydroxyacetone plus the corresponding aldose) is also possible, by means of aldolase activity, provided all structural requirements described here are observed. This mechanism is also summarized in Table 1.

(d) *Epimerases.* The configuration of a given carbon of any sugar can be changed from R to S (or the contrary case) without altering the configuration of the other carbons or other structural features of the sugar. Obviously, this reaction is also reversible. This reaction can only occur on a sugar monophosphorylated in its last carbon.

(e) *Isomerases.* The carbonyl group function (aldehyde in C-1, or ketone in C-2) can be changed in any sugar without altering other strutural features of the sugar. If the reaction occurs in the direction from keto to aldo, a new chiral centre is

obviously formed on the second carbon, which always takes the R form. This reaction is reversible, eliminating the chirality (in R form) of the second carbon of the aldose, and it can only occur on a sugar monophosphorylated in its last carbon.

(f) *Phosphatases.* The phosphate groups in C-1 of a biphosphorylated keto-sugar (in its first and last carbon) can be removed, giving a keto-sugar only phosphorylated in its last carbon. This reaction does not alter other structural features of the sugar and it is irreversible under cellular conditions.

(g) *Kinases.* A phosphate group can be transferred from ATP to the C-1 of a any sugar, monophosphorylated in its last carbon, giving ADP plus the corresponding biphosphorylated sugar. This reaction does not alter other structural features of the sugar and it is also irreversible under cellular conditions. In fact, this last mechanism is not used in the way to the solution of the problem; however, it is convenient to describe it here, because it is antagonistic to (f). Thus, all possible operations described above can be realized in any direction, according to the most convenient procedure.

As has been stated, any one of the seven operations described above will be one step when it is used. There are, in addition, two more conditions which must be observed:

(h) There are no restrictions concerning the use of the different possible mechanisms of reaction, i.e., the use of any described mechanism is not obligatory and there is no limit to the number of times every possible mechanism may be used. The choice of the appropriate mechanism for every step will be made according to its convenience, in order to arrive at the solution.

(i) On the way toward the solution, there will be several intermediary products with a certain number of carbons (all of them sugars, according to the imposed mechanisms). Any of these intermediates cannot have fewer than three carbons (a structural condition for any sugar).

(3) HYPOTHESIS OF SIMPLICITY

The optimal solution must involve: (a) the smallest number of steps (enzyme reactions) and (b) the smallest number of carbons in the free intermediates during the solution process. There is no hierarchy in these two conditions; however this hierarchy is, unnecessary, as has been discussed previously (Meléndez-Hevia & Isidoro, 1985), and it will also be seen here.

3. Solution

The only reactions which allow a rearrangement of the number of the carbons of the sugars and which, therefore, account for a net progress to the solution, are those catalysed by transketolase, transaldolase and aldolases. The other reactions can only allow the action of these or give end products of the objective. Let us

consider now the steps necessary in addition to those which change the number of carbons of the sugars:

(1) Ribulose 5-phosphate, initial substrate of the pathway, can never be a substrate for the three enzymes mentioned above, since it does not account for their mechanism hypotheses; it must be isomerized or epimerized to give an appropriate substrate of any of these enzymes. Therefore *six steps*, one for every substrate, are necessary at the beginning.

(2) Glucose 6-phosphate, the end product of the pathway, can be originated (a) directly from transketolase, transaldolase or aldolase, or (b) by means of epimerase or isomerase reactions under a preceeding intermediary one. In case (b), one step which does not involve carbon exchange is obviously necessary for every glucose 6-phosphate obtained. In case (a), considering forward and reverse directions of the three reactions mentioned above (see Table 1), by means of transketolase or transaldolase mechanisms, glucose 6-phosphate can only be produced from a ketose of 8 and 9 carbons, respectively, these having their 5th and 6th carbon, respectively, in the S configuration (in addition to their third one) to account for the third carbon of glucose in such a configuration. By means of the aldolase mechanism, glucose 6-phosphate could also only be derived from a ketose with 9 carbons, having its 6th carbon in the S configuration. Thus, for any possibilities of the case (a), one additional step of epimerization under the 5th or the 6th carbon is necessary. Therefore, in whatever case, *five steps* of epimerization or isomerization, one for every end product (glucose 6-phosphate) are necessary, these being independent of the six steps mentioned in (1).

(3) With the independence of the intermediary mechanisms, the pathway is started from six monophosphorylated sugars, while the products are only five monophosforylated sugar. Therefore, at some time, a phosphate group must be released. On the other hand, neither transketolase, transaldolase nor aldolase account for it, which can only be carried out by phosphatase. Therefore, phosphatase must be used at least once, which gives us *one* step.

(4) Furthermore, always according to the hypotheses of the mechanisms, this phosphate group can only be released from a biphosphorylated ketose, which can only be produced by an aldolase reaction (obviously it cannot be originated from a kinase reaction because the phosphate group must come from ribulose 5-phosphate). Therefore, aldolase must be used at least once, in the condensation direction. However, in order to use aldolase in this direction, dihydroxyacetone phosphate is necessary as a substrate and, according to the hypotheses of the mechanisms (see Table 1), this sugar cannot come from transketolase or transaldolase reaction. In fact, dihydroxyacetone phosphate can only come from (a) aldolase by the reverse reaction, which is not a solution of the problem, because it leads us to the same situation with the disadvantage of using more steps, or (b) isomerization of glyceraldehyde 3-phosphate, which can be produced by means of transketolase or transaldolase reactions. This last possibility is thus the only solution of this particular problem and involves *one step* of isomerization on a triose phosphate.

Therefore, with the independence of reactions which involve progress in carbon distribution (transketolase, transaldolase and aldolase) the following steps are also

necessary: (a) six steps of epimerases or isomerases on the six ribulose 5-phosphate for a start; (b) five steps of epimerases or isomerases to obtain the appropriate configuration of the five end products of glucose 6-phosphate; (c) one step of phosphatase to release a phosphate group; and (d) one step of isomerization to obtain a dihydroxyacetone phosphate. This gives us 13 necessary steps which do not involve changes in the number of carbons in the sugars. On the other hand, it has been demonstrated in the previous paper that with respect to the number of carbons, only considering the transformation of six C_5 into five C_6 by means transketolase, transaldolase and aldolases (without taking into consideration the configuration of the carbons, functional groups of the sugars and phosphate groups), such a transformation cannot be made in less than seven steps; this solution is represented in Fig.1A. We can now conclude, therefore, that for the whole problem there cannot be a solution involving less than 20 (13+7) steps.

With respect to the number of carbons of the intermediate sugars, it has been demonstrated in the previous paper that a solution which contains fewer than two intermediates with seven carbons is not possible. It is, therefore, demonstrated that there cannot be a solution with fewer than two C_7 intermediates and twenty steps. However, it does not demonstrate that such solutions (a) exists, or that if it exists it must be the only one; but if such a solution does exist, there is no other solution

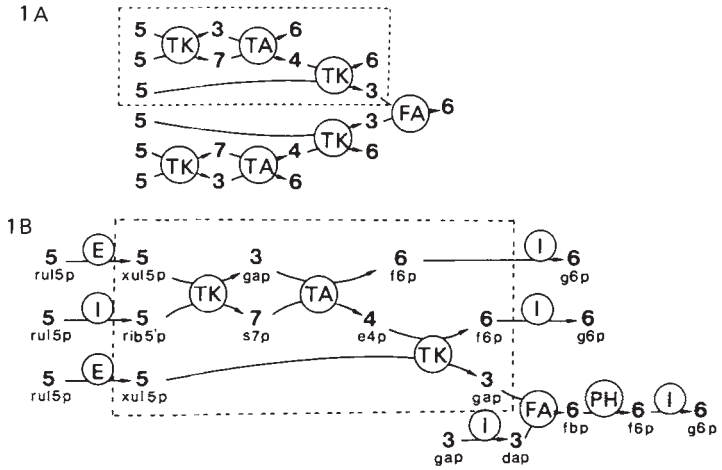


FIG. 1. Non-oxidative phase of the pentose phosphate cycle ("F-type"), as formulated by Horecker *et al.* (1954), for the rearrangement of six ribulose 5-phosphate into five glucose 6-phosphate. Panels within dashed lines include one symmetrical half of the total process, and numbers express the numbers of carbons in every sugar. (A) simplified scheme where only the number of carbons in every sugar are considered and, thus, reactions catalysed by epimerase, isomerases and phosphatase are not represented. (B) Complete scheme of each symmetrical half, including the link of the two schemes from trioses phosphate and further reactions. Enzymes represented are: E, epimerase; FA, fructose 1,6-biphosphate aldolase; I, isomerases; PH, phosphatase; TA, transaldolase; TK, transketolase. Sugars represented are: dap, dihydroxyacetone phosphate; e4p, erythrose 4-phosphate; f6p, fructose 6-phosphate; fbp, fructose 1,6-biphosphate; gap, glyceraldehyde 3-phosphate; g6p, glucose 6-phosphate; rib5'p, ribose 5'-phosphate; rul5p, ribulose 5-phosphate; s7p, sedoheptulose 7-phosphate; xul5p, xilulose 5-phosphate.

with fewer steps, or with fewer than two intermediates with seven carbons. In fact, the cell solution of the whole problem (shown in Fig. 1B) has precisely all these features which, as has been demonstrated, is an optimal or irreducible solution.

4. The Calvin Cycle in Photosynthesis

Let us consider the non-reductive phase of the Calvin Cycle in photosynthesis (only the interconversions of phosphorylated sugars), as the conversion of twelve glyceraldehyde 3-phosphate into six ribulose 5-phosphate plus one glucose 6-phosphate. For this problem, only the objective is changed with respect to the pentose phosphate pathway discussed above, but the hypotheses of the mechanisms and the simplicity remain the same as described above.

Then, by applying a similar reasoning, an equivalent model can be designed, and its solution, obtained by the same procedure as in the preceeding case, has the following steps which do not involve changes in the number of carbons of the sugars: (a) *six steps* of isomerization or epimerization to obtain the six ribulose 5-phosphate; (b) *one-step* of isomerisation or epimerization to give glucose 6-phosphate; (c) 5 *steps* of releasing a phosphate group by means of phosphates, because twelve monophosphorylated sugars are converted into seven; (d) 5 *steps* of isomerization to give dihydroxylacetone phosphate, as has been demonstrated, in the preceding section.

Thus, the new solution obtained now involved 17 minimal additional steps, which added to the nine described in the previous paper to account for the rearrangement of carbons among sugars (Fig. 2A), give us 26 steps as the minimum necessary for any complete solution. It can be seen that the procedure carried out by living cells (shown in Figure 2B) has precisely this number of steps, therefore being an optimal solution.

5. The "L-Type" of the Pentose Phosphate Cycle

The "L-type" of the pentose phosphate cycle, proposed by Williams and co-workers (see Willams & Clark, 1971; Williams *et al.*, 1978b; Williams, 1980, 1981) is a different version of the pentose phosphate pathway which these authors have described for the liver and other tissues. This pathway was also analysed in the previous paper, in light of this treatment, but only considering the number of carbons of the sugars, arriving at the same conclusion: the pathway "L-type", as proposed by Williams is the simplest solution of the problem, taking into account its specific hypotheses of the mechanisms, where transaldolase reaction is not included. Now, considering all structural features of the sugars and enzyme conditions, the present problem can again be considered, because it contains some new and interesting characteristics about metabolic organization.

The objective is now the same as for "F-type": to convert six ribulose 5-phosphate into five glucose 6-phosphate. The hypothesis of simplicity remains the same, as described above, but the hypotheses of the mechanisms are rather different. Williams

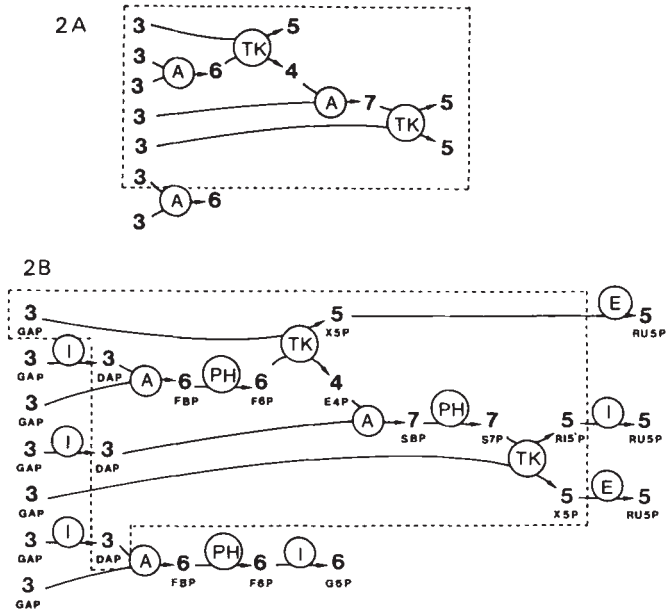


FIG. 2. Non-reductive phase of the Calvin cycle in the dark phase of photosynthesis for the rearrangement of twelve glyceraldehyde 3-phosphate into six rubolose 5-phosphate and one glucose 6-phosphate. Numbers express the numbers of carbons in every sugar; A and B parts, as well as panels within dashed lines are as in Fig. 1. Symbols for enzymes are: A, aldolases; E, epimerase; I, isomerases; PH, phosphatases; TK, transketolase. Symbols for sugars are: DAP, dihydroxyacetone phosphate; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1, 6-biphosphate; GAP, glyceraldehyde 3-phosphate; G6P, glucose 6-phosphate; R15'P, ribose 5'-phosphate; RU5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; SBP, sedoheptulose 1,7-biphosphate; X5P, xilulose 5-phosphate.

and co-workers propose for this pathway the following two important features concerning mechanisms of reaction with respect to the classical or "F-type" pathway: (1) transaldolase activity does not participate in "L-type"; (2) a phosphotransferase activity which can transfer a phosphate group between sugars is included. Therefore, the present hypotheses of the mechanisms, with respect to enzymatic reactions, are as follows: there are seven possible operations involving, in every case, some structural conditions. Every one of these operations is defined as one step: (a) transketolase, (b) aldolases, (c) epimerases, (f) isomerases, (e) phosphatases, (f) kinases, (g) phosphotransferases. All seven possible reactions, with the exception of the phosphotransferases, have been described above for "F-type", and they apply here also with the same features. Note that transaldolase is not included, and that there is a new possibility of mechanism, namely, (g) phosphotransferases. These enzymes transfer a phosphate-group from C-1 of a biphosphorylated ketose to C-1 of a monophosphorylated ketose. In fact, the D-*glycero*-D-*ido*-Octulose 1,8 biphosphate: D-*altro*-heptulose 7-phosphotransferase activity was first proposed by Williams & Clark (1971) (see also Williams *et al.*, 1978), who suggested a dual role for both phosphatase and phosphotransferase enzymes. The same group of researchers (Arora *et al.*, 1985) have reported its identification and measurement in tissues

with the "L-type" of pathway. Hypotheses (2h) and (2i), as described above, remain unchanged, as well as the hypothesis of simplicity. Thus, from all these hypotheses, a similar model can be designed for this case, and it can be demonstrated that the structure of the pathway proposed by Williams and co-workers (see Fig. 3) is an optimal solution of this problem.

Proof

(1) Since this model (L-type) differs from that discussed above (F-type) only with respect to the mechanism of carbon rearrangement (transaldolase) and with respect to the mechanism of phosphate transfer (phosphotransferase), the rest of the hypotheses remaining equal, all reasoning outlined above for "F-type" about the 13 steps necessary with independence of the mechanisms for rearranging carbon among sugars, can be applied here. However, for the present, we will only admit to demonstrating *11 steps*, because more than one isomerization of glyceraldehyde 3-phosphate will be necessary here, and all of them will be discussed below together with the phosphate-releasing step.

(2) There are only two possibilities of mechanisms for the rearrangement of carbons among sugars in this model: transketolase and aldolases. By only using transketolase, it is not possible to arrive at the solution (five hexoses) because any set of reactions starting from pentoses (with five carbons), each including the transfer

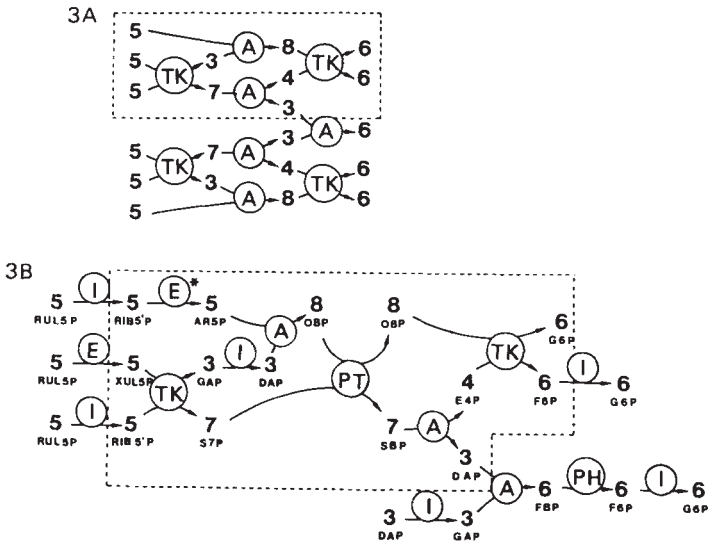


FIG. 3. Non-oxidative phase of the pentose phosphate cycle ("L-type"), as formulated by Williams & Clark (1971), for liver cells and other tissues, for the rearrangement of six ribulose 5-phosphate into five glucose 6-phosphate. Numbers express the numbers of carbons in every sugar. Parts A and B, as in Fig. 1. Symbols for enzymes are: A, aldolases; E, epimerases; I, isomerases; PH, phosphatase; PT, phosphotransferase; TK, transketolase. Symbols for sugars are: AR5P, arabinose 5-phosphate; DAP, dihydroxyacetone phosphate; E4P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FBP, fructose 1,6-biphosphate; GAP, glyceraldehyde 3-phosphate; G6P, glucose 6-phosphate; O8P, octulose 8-phosphate; OBP, octulose 1,8-biphosphate; RIB5'P, ribose 5'-phosphate; RUL5P, ribulose 5-phosphate; S7P, sedoheptulose 7-phosphate; SBP, sedoheptulose 1,7-biphosphate; XUL5P, xilulose 5-phosphate. The reaction of ribose 5'-phosphate 2'-epimerase (marked with asterisk is discussed in the last paragraph of the Discussion.

of two carbons, can only give sugars with an odd number of carbons. The use of aldolases is thus necessary, and since these enzymes extract or add three carbons (dihydroxyacetone phosphate) to a sugar, the three compounds involved in such reactions are always two sugars with an odd number of carbons (one of them always being dihydroxyacetone phosphate) and one sugar with an even number of carbons. Therefore, aldolases must be used as many times as the number of sugars of an even number of carbons found in the solution (five hexoses), and it must be observed that combined use of aldolase and transketolase does not reduce this minimum number of aldolases, because the use of transketolase does not change the parity in the number of carbons of the sugars.

Aldolases must, therefore, be used at least five times, but note that the use of these enzymes involves certain necessary additional steps in order to account for structural features of these reactions, according to the hypotheses of the mechanisms, and the objective:

(a) if such reaction occurs in the sense of condensation, two additional steps are necessary: isomerization to give dihydroxyacetone phosphate as has been demonstrated above, and the action of a phosphatase to release the phosphate group on C-1 of the end product; on the other hand, this loss of a phosphate group must further be balanced with the introduction of a phosphate group by means of a kinase, because the five end products, glucose 6-phosphate, are monophosphorylated.

(b) If the reaction of aldolase occurs in the direction of the break, a number of additional steps are also necessary: the substrate must be a ketose biphosphate and, therefore, a kinase must have worked previously in order to give it, which implies a corresponding phosphate to balance the phosphate group number in the pathway; furthermore, a product of this reaction, dihydroxyacetone phosphate, must be isomerized into glyceraldehyde 3-phosphate, if transketolase is going to work on it.

All these additional steps can, however, be simplified: according to hypotheses of the mechanisms, the pair kinase-phosphatase could be substituted by only one enzyme (phosphotransferase); the balance of phosphate groups could be achieved in this manner by the use of this enzyme between two aldolases. Since the action of five aldolases is necessary, as has been demonstrated above, at least *two steps* of a phosphate group transfer by phosphotransferases and *one step* for phosphate elimination by a phosphate are necessary, to achieve the balance of phosphate groups in the whole solution, because the conversion of six pentose phosphates into five hexose phosphates necessarily involves the release of one phosphate group. These three steps, with regard to phosphate group balance, are the minimum possible, all other ways having a great number of steps.

With respect to isomerization to triose phosphate, the number of steps could also be simplified. In fact, the participation of the five aldolase steps could be appropriately ordered for minimizing the additional steps. Phosphotransferase action implies that the first aldolase must work in the direction of condensation, followed by an aldolase in the contrary direction. This should occur twice, since phosphotransferase works twice, and then *two additional steps* are necessary to isomerize glyceraldehyde 3-phosphate into dihydroxyacetone phosphate as demonstrated above; this accounts

for two minimum additional steps with regard to the reaction of four aldolases. The fifth aldolase would be the last step of carbon transfer in the solution if such a solution were symmetrical, i.e., the step which accounts for the coupling of the two symmetrical halves of the solution (see Meléndez-Hevia & Isidoro, 1985). In such a case, it must work in the direction of condensation and its two substrates must come from two equal reactions; if these are transketolases, one additional step of isomerization is necessary to give dihydroxyacetone phosphate; if aldolases, however, the products in the most favourable case must be two trioses (equal, according to the symmetry), both dihydroxyacetone phosphate or both glyceraldehyde 3-phosphate and, consequently, one of them must be isomerized for the action of this fifth aldolase. Any other possibility leads us to the use of aldolases more times (which involves a greater number of additional steps) or to unsymmetrical solutions. However, as has been discussed in the previous paper, is symmetrical solutions exist, the simplest solution among them is simpler than any other unsymmetrical solution. Certainly the use of this fifth aldolase in another way (aldolic break, or condensation of two sugars with a different number of carbons) leads us to a greater number of additional steps. It is demonstrated, therefore, that this fifth aldolase involves, at least, *one additional step.*

Thus, the additional hypotheses of the mechanisms considered in this work, involving all the structural conditions of the reactions, impose a minimum of 17 additional steps, over the nine previously described which account for the simplest solution for carbon rearrangement (Fig. 3A). Therefore, the total conversion of six ribulose 5-phosphate into five glucose 6-phosphate cannot be achieved in less than 26 (17+9) steps. Note, however, as for the preceding cases, that up to here, we have demonstrated only that it is not possible for a solution to have fewer than 26 steps and, moreover, we cannot confirm that such a solution, if it exists, is the only one; as in the preceding cases, our demonstration refers to the minimum number of steps which any solution must have. With respect to the number of carbons in intermediate sugars, it has been demonstrated in the previous paper that a solution having fewer than two sugars of seven carbons and two of eight carbons is not possible. Obviously, if a solution exists having precisely such a number of steps and these intermediate sugars, another simpler one cannot exist. In this particlar case, for the "L-type" pentose phosphate cycle, a solution like this exists, and it is precisely the way proposed by Williams & Clark (1971) for this metabolic pathway in living cells (see Fig. 3B).

6. Discussion

It is clear that there are many ways to design the analysed pathways, according to their objectives, if only the hypotheses of the mechanisms are taken into consideration. The hypothesis of simplicity is, from our viewpoint, the most outstanding feature in this study. It seems as if living cells have resolved certain complicated—and bothersome—problems of combinatorial optimization. Thus, the following question is pertinent: why in the three cases analyzed is cell solution precisely the simplest solution of the every problem?

Cell evolution has probably not had any other possibility. In fact, all enzymes being present, it can be assumed that all possible reactions could occur (in different rates, according to every substrate concentration and affinity of enzymes). In this way, the cell behaviour would proceed by testing different choices until finding the simplest way. In order to organize a given metabolic pathway, the objective of this can be determined by the demand of a given product and the availability of a given substrate. Then, the cell utilizes the whole set of enzymes available to convert thus substrate into several possible products by testing all the enzymes. Many of these products are poor intermediates without any clear output, but others, the correct ones, continue on their way toward the solution which could be the reason why a hypothesis of simplicity has been imposed allowing cells to do without certain unecessary enzymes, which moreover facilitates the operations. Natural selection in cellular evolution has occurred on all levels, and the results described here suggest that the hypothesis of simplicity has been taken into account during the course of cellular organization.

In the three pathways considered here, erythrose 4-phosphate occurs as intermediate. This sugar, which relates these pathways with the synthesis of aromatic compounds, always appears in the simplest solution of every considered problem. In principle, the use of a pre-existent metabolite to be used in other metabolic processes is another way of cellular economy which, as we commented above, could have conditioned the evolution of metabolic pathways and, consequently, imposed a certain degree of complexity. Note, however, that this complexity seems to be as small as possible. In fact, in the three pathways studied here living cells have always observed the hypothesis of simplicity. It is not unwise to think that an equivalent theoretical treatment like the one developed here for pentose phosphate metabolism can be applied to other metabolic pathways, arriving at equivalent conclusions.

The hypothesis of simplicity, as formulated above, involves two conditions: (a) the smallest number of steps, or enzyme reactions, i.e., the smallest number of free intermediates; and (b) the smallest number of carbons in them, i.e., the simplest complexity in every intermediate. The hierarchy between these two conditions has not been considered here because, as discussed in the preceding paper and as has been observed here, there is no alternative, since the simplest solution is that which utilizes the smallest number of steps, as well as the smallest number of carbons in the intermediates. However, this problem can appear in the study of other pathways, in which case a hierarchy should be imposed with respect to these two conditions. In fact, in certain cases, these two conditions could be antagonistic, since the first one is on the basis of economy of the pathway itself, but the second one could affect other metabolic pathways. It must be considered, however, that a view of the metabolic map indicates that the greater number of cross-way metabolites are molecules (or groups) with a small number of carbons (pyruvate, acetyl, etc.). The possible hierarchy between the two conditions of the hypotheses of simplicity could be analysed in the light of further studies of the operative organization of cell metabolism.

The "L-type" of the pentose phosphate cycle has been a subject of controversy by several groups of researchers (see Katz, 1981; Landau, 1981; Wood, 1981;

Williams, 1981; Rongstad *et al.*, 1982; Landau & Wood, 1983; Williams *et al.*, 1983). As pointed out in the preceding paper, what determines the more complicated "L-type" with respect to "F-type" is the lack of transaldolase in the former, as a possible reaction included in the hypothesis of mechanisms. This enzyme has been discarded although it exists in liver (Williams *et al.*, 1978a). However, Williams *et al.* (1978b) have described the inhibition of liver transaldolase by arabinose 5-phosphate, an intermediate of this pathway (see Fig. 3B), which could explain this lack of activity.

In the Calvin cycle, however, there is a different reason for not using transaldolase. This enzyme does not appear in the optimal solution (Fig. 2). Therefore, under conditions in which the use of this enzyme was imposed, a more complicated procedure would be carried out. Transaldolase is, thus, useless for the Calvin cycle and, therefore, chloroplasts do not need it. Plant cells have transaldolase in cytoplasm, where the pentose phosphate pathway occurs (see e.g., a review in ap Rees, 1985), but it seems that this enzyme has not been found in chloroplasts (see reviews on chloroplast proteins in Ellis, 1981; Kirk *et al.*, 1978). Thus, for the problem of the Calvin cycle, transaldolase reaction could be removed from the hypothesis of the mechanisms. However, this was not necessary because it has not been used in the solution and thus, this subject relates again the hypothesis of simplicity with cellular organization during evolution.

On the other hand, as mentioned above, the design of the "L-type" of the pentose phosphate pathway (Fig. 3B) is one possibility for the simplest solution, but not the only one. In fact, the epimerization of ribose 5′-phosphate to give arabinose 5-phosphate (marked with an asterisk in Fig. 3B), necessary to give an S configuration of the third carbon of glucose 6-phosphate, could occur elsewhere in the pathway, such as, e.g., after the second transketolase. In such a case, allose 6-phosphate would have been produced. The question regarding the non-utilization of glucose 6-phosphate 3-epimerase instead of ribose 5′-phosphate 2′-epimerase is a special feature of the design of this metabolic pathway, but note that it does not involve a more complicated procedure, whereas it does give a crossway point between the pentose phosphate cycle and D-arabinose 5-phosphate metabolism, which could be seen as another apsect of the hypothesis of simplicity.

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