Is phosphofructokinase the rate-limiting step of glycolysis?
Lisardo Boscá and Carlos Corredor

The quantitative aspects of the regulation of glycolytic flux are still not well established. Even though convincing evidence that phosphofructokinase (PFK) participates in the regulation of the flux has been provided only for erythrocytes (albeit hexokinase seems to be more important in this respect), it is accepted that PFK is the main rate-controlling enzyme of glycolysis in different tissues. Some measure of participation in this control has also been suggested for both hexokinase and pyruvate kinase. The main reasons for this generalized belief are that PFK catalyses a reaction very far from equilibrium and that it exhibits a complex and sophisticated regulatory behaviour that reflects its ability to integrate many different signals from different pathways. However, as first expressed by Kacser and Burns, no single enzyme is likely to be responsible for the regulation of the metabolic flux through any pathway.

'Rate limiting' or 'rate controlling' enzymes mean different things to different people. For our own purposes, however, let us define rate-limiting enzyme in terms of a pathway such as Fig. 1, in which for each step the reaction velocity could be equal to, greater or lower than \( v \). In the case shown, enzymes A, C and D are not control enzymes, since having activities greater than \( v \), neither substrates nor products would accumulate. On the other hand, enzyme B can be rate-limiting, but only under certain conditions. When the activity of B is less than or equal to \( v \), B is the rate-limiting enzyme, since substrates would accumulate and only a certain amount of product would appear. This amount, being less than the capabilities of the enzymes down the pathway to utilize it, would not limit the flux through any of them. However, when the activity of B is greater than \( v \), any one of the other enzymes may assume the rate-limiting role.

Which one of the other enzymes becomes rate-limiting depends on the kinetic properties of each relative to the others under the conditions considered. Kacser and Burns and Heinrich and Rapoport have independently introduced the concept of a 'sensitivity coefficient', \( Z \), defined as the relation between the rate of fractional change of the flux (\( \Delta F/F \)) and the fractional change of activity of the enzyme causing the change (\( \Delta E/E \)):

\[
Z = \frac{\Delta F/F}{\Delta E/E}
\]

Under steady-state conditions, the sum of the sensitivity coefficients of all enzymes in a pathway is unity. The relative participation of any given enzyme in the control of the pathway will depend on the magnitude of its \( Z \), becoming greater as \( Z \) approaches 1. Thus, \( Z \) for the rate-limiting enzyme will be close to 1. However, the nature of the summation theorem above implies that under a given set of conditions no one enzyme needs to be rate-limiting, but rather each one will contribute to the control of the rate of flux. Groen et al. have shown that in the resting state the control of respiration is exerted at the level of inner membrane passive permeability to protons, while in intermediate and active states the control step cannot be pinpointed, and seems to be distributed among different intervening steps.

In the example initially proposed, B is a regulatable enzyme, since its activity can change from less to more than \( v \), and this change must depend on effectors that may be present under any given circumstances. Following Kacser and Burns, we must say that the value of \( Z \) for this enzyme must also change, approaching 1 when the activity is equal to or less than \( v \), and becoming much smaller when it is higher than \( v \). It follows that when \( Z \) approaches 1, enzyme B would be the rate-limiting enzyme of the pathway. However, under any other circumstance, either one - or none - of the other enzymes would assume the rate-limiting role, depending on their own particular values of \( Z \).

As may be apparent from the above discussion, \( Z \) for any given enzyme depends on the intrinsic factors that determine the enzyme activity and on the effectors that may alter that activity. It follows, that when an enzyme occurs in different isozymic forms whose characteristics may vary from tissue to tissue and from organism to organism, it is to be expected that \( Z \) for each isozyme will also vary according to its intrinsic characteristics. In this case, it may be predicted that just as in the same cell the rate-limiting step may change from one to another enzyme(s) depending on the set of conditions, the enzyme that controls the flux in one organism, or even in a tissue of the same organism, may not control it in another tissue or organism.

The phosphofructokinases
Phosphofructokinase is an interesting example of these ideas. In Table I we have gathered published data on steady-state concentrations of several glycolytic intermediates in several tissues and organisms. On the basis of these data it is possible to classify cells into groups with respect to fructose 1,6-bisphosphate (FBP) concentration and especially to the ratio fructose 6-phosphate (F6P)/FBP. (1) those with a low (\( \mu \)M) content of FBP and a F6P/FPB ratio higher than 1, and (2) those with a high (mM) concentration of FBP and a F6P/FPB ratio lower than 0.1.

Normal mammalian cells would fall into group 1. In these cells the substrate...
concentration is higher than the total product concentration. Considering that the $K_m$ for the next enzyme in the sequence, aldolase, is of the order of $5 - 10 \mu\text{m}$ and that the free FBP is of the order of $10\%$ of the total FBP, it is clear that PFK product does not accumulate under steady-state conditions. On the other hand, cells in group 2 show a clear accumulation of PFK product, three orders of magnitude above the $K_m$ for aldolase. This implies that PFK cannot be the rate-limiting step of glycolysis in this type of cell, and control is exerted further down the pathway.

Physiological role of the change in rate-limiting step

It has been shown that the regulatory properties of the different isozymes of PFK are similar for most tissues and organisms studied, differing only in the degree of response to ATP inhibition and thus, to inhibition by other effectors. Therefore, it is surprising to find that this highly multimodulated enzyme appears not to be rate-limiting under glucose utilizing, steady-state conditions in cells of group 2.

Nevertheless, this is in accord with findings that in ascites tumour cells the concentration of the positive effector of PFK, fructose, 2,6-bisphosphate, is in the range for maximal PFK activation, and FBP concentration is in the millimolar range (L. Boscá, unpublished results). This lack of regulation at the PFK level may be related to the capacity of the tumour to metabolize large amounts of glucose to lactate, concomitant with a relatively normal oxygen consumption, indicative of utilization of other metabolic fuels.

The physiological role of this shift in the rate-limiting step from PFK is not apparent. However, one may suggest that it has to do with the very large glycolytic fluxes, found in cells such as tumours or yeasts, that excrete the final glycolytic product to the medium. One may also suggest that this shift might occur in the same cell, as a means for coping with maximal energy requirements.

Platelets, for example, have low glycolytic rates under resting conditions. However, once they are made to adhere and agglutinate, a high glycolytic rate from glycogen is triggered to meet the energy requirements imposed by the new emergency state. Under these conditions, factors such as an increase in positive effectors would activate the PFK, over and beyond the ability of the pathway to utilize the PKF product, FBP. Thus, the enzyme which was rate-limiting under resting conditions ceases in that function when the cells need the maximum possible amounts of energy; in so doing, it allows maximal glucose utilization, i.e. it plays its role in up-regulating the flux.

Final remarks

We have raised the question as to whether PFK is the rate-limiting enzyme of glycolysis. An exploration of the published data seems to support this idea in the case of the isozymes present in several normal mammalian tissues. However, the same is not true for the isozymes of ascites tumour cells, mammalian spermatids and yeast, where the rate-limiting step of glycolysis is to be found further down the pathway. What the implications are, if any, of the large amounts of FBP which accumulate in these cells, is not known at present. It is also not known what enzyme or step becomes rate-limiting in these cells, or what factors regulate it. It seems though, of medical and economic importance to explore these questions further.

We would like to suggest that the situation discussed above is not limited to glycolysis, but that it may be a more general consequence of the phenomenon of isozymic diversification. The existence of different isozymes in different types of cells would allow for the occurrence of different means of control of the metabolic flux from organism to organism, and even from tissue to tissue, that would result in different metabolic rates under the same set of conditions.

Acknowledgements

The authors wish to thank Professor A. Sols.

References


Table I. The concentrations of glycolytic intermediates in some cell types

<table>
<thead>
<tr>
<th></th>
<th>G6P</th>
<th>F6P</th>
<th>FBP (mol)*</th>
<th>G1-3-P</th>
<th>DHAP</th>
<th>F6P/FBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat liver</td>
<td>0.55</td>
<td>0.15</td>
<td>0.05</td>
<td>0.11</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Rat skeletal muscle</td>
<td>0.84</td>
<td>0.21</td>
<td>0.06</td>
<td>0.32</td>
<td>3.5</td>
<td>11</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>0.06</td>
<td>0.03</td>
<td>0.01</td>
<td>0.01</td>
<td>0.03</td>
<td>12</td>
</tr>
<tr>
<td>Platelets</td>
<td>0.05</td>
<td>0.015</td>
<td>0.014</td>
<td>0.003</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ascites tumour cells</td>
<td>0.73</td>
<td>0.26</td>
<td>3.6</td>
<td>0.23</td>
<td>1.7</td>
<td>13</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>0.90</td>
<td>0.30</td>
<td>4.5</td>
<td>0.52</td>
<td>1.1</td>
<td>15</td>
</tr>
<tr>
<td>Rat spermatids</td>
<td>0.02</td>
<td>0.02</td>
<td>4.7</td>
<td>1.5b</td>
<td>0.07</td>
<td>13</td>
</tr>
</tbody>
</table>

*Where the original reports are given in units other than millimolar, conversion factors were used assuming that: (1) water available to the cell is of the order of 60% of the wet wt, (2) 10^6 cells = 1 mg wet wt, (3) 10^10 platelets = 375 mg wet wt.

b Represents the sum of both triose phosphates.