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Shift in rat liver glycolysis control from fed to starved conditions

Flux control coefficients of glucokinase and phosphofructokinase

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The distribution of flux control coefficients of a glycolytic system in starved rat liver has been determined. The flux control coefficient profile in starved conditions is compared with normal fed conditions showing that in the former phosphofructokinase enhances more than 2-fold its flux control coefficient while glucokinase decreases slightly. The results also show that the starved system has a more complex structure probably because of the greater influence on the flux of the reverse substrate cycles and the synthesis and degradation fructose 2,6-bisphosphate reactions.

Liver glycolysis; Starvation; Glucokinase; Phosphofructokinase; Metabolic control; Flux control coefficient

1. INTRODUCTION

In spite of the fact that liver is mainly a gluconeogenic tissue a great deal of work has been done on the regulation of glycolysis in this tissue [1]. This interest is justified because the mammalian liver is a highly metabolically versatile tissue, having to cope with a wide range of different metabolic conditions such as starvation or regeneration.

Phosphofructokinase (PFK) has been traditionally pointed to as one of the major regulation points of glycolysis. Since the discovery of fructose 2,6-bisphosphate [2] as a strong stimulator of this enzyme, and later of phosphofructokinase II as the enzyme that synthesizes it [3], the ideas about the role of PFK (now PFK I) on the regulation of the pathway changed considerably [1,4].

On the other hand in the last five years the so-called flux control theory, first presented by Kacser and Burns [5] and Heinrich and Rapoport [6] and subsequently developed by these and other authors ([7–10]; see [11] for a review) has been extensively used to describe quantitatively the control of flux of metabolic systems [12–17]. This theory is based on the definition of several coefficients, one of them being the flux control coefficient (FCC) which possesses an important significance. The coefficient is defined as

$$Ce_i^J = \delta J/\delta e_i * e_i/J$$

where J is the flux through a metabolic pathway, and e_i the enzyme activity of any one of the enzymes of the system. Its magnitude represents the fractional change in the flux provoked by an infinitesimal fractional change in the e_i activity. This value expresses the relative importance of e_i in controlling the flux through the whole system. An important property of the FCCs of a system is the summation property [5,6] which tells us that in a given metabolic pathway the summation of all of them must add up to the unity.

$$\sum_{i=1}^{n} Ce_i = 1$$

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In this way this property gives us an indication of what is the relative importance of every enzyme in controlling the flux through a pathway.

Our aim in this work is to study the changes of the glycolytic FCC ratio GK/PFK from normal to starved rat liver. We will use for this purpose a method described by ourselves elsewhere [18]. This procedure has been applied to other metabolic pathways [19] and permits us to determine the value of the ratio in any metabolic system of whatever complexity by shortening it with auxiliary enzymes. Finally the summation property will provide us with a means to gain insight into the differences between the two systems analyzed.

2. MATERIALS AND METHODS

2.1. *Animals and diets*

Female Wistar albino rats, fed on a standard laboratory diet (65% carbohydrate, 11% fat, 24% protein) ad libitum were used as a control source for fed liver extracts. The starved rats were kept without food for 72 h before experiments. All animals were housed at 20–22°C with light from 7:00 to 19:00 h and were killed at between 90 and 100 days of age at 10:00 h.

2.2. *Experimental system*

The experimental model was previously described [18] where glucose is converted into glycerol 3-phosphate by means of the activity of a soluble extract from rat liver. The last three steps of the pathway were accelerated by adding commercial aldolase, triose phosphate isomerase and glycerol 3-phosphate dehydrogenase as auxiliary enzymes. Reactions were carried out at 35°C and the flux was measured by recording NADH decay at 340 nm in a Hitachi 100-60 spectrophotometer. Total volume of the reaction mixtures was 2 ml containing 100 μl of diluted liver extract to give 1.5 mg of protein/ml, 5 mM glucose, 1 mM ATP, 0.28 mM NADH, 2.5 mM phosphocreatine, 15 μM fructose 2,6-bisphosphate (except in the starved conditions as discussed below), 5 units of creatine kinase/ml, 1 unit of fructose 1,6-bisphosphate aldolase/ml, 5 units of triose-phosphate isomerase/ml, and 3 units of glycerol 3-phosphate dehydrogenase/ml, all diluted in 50 mM Hepes/10 mM sodium phosphate buffer, pH 7.4, containing 100 mM KCl, 10 mM $MgCl_2$ and 1 mg/ml of trypsin inhibitor (type II). Titration experiments were realized in order to determine FCC according to the method previously described [18]. All reagents and enzymes were obtained from Sigma Chemical Company, St. Louis, MO, USA.

2.3. *Enzyme and protein assays*

Hexokinase (GK) assays were carried out as described by Pilkis [20] with 1 mM ATP. The PFK assay was carried out in accordance with published methods [21] with 1 mM ATP. Velocity of the reaction was continuously monitored by recording changes in absorbance at 340 nm with a Hitachi 100-60 spectrophotometer (Hitachi, Tokyo, Japan). Temperature was stabilized at 35°C with a thermocirculator (Churchill Instruments Co., Perivale, Middx, England). Fructose biphosphate aldolase, triose phosphate isomerase, glycerol 3-phosphate dehydrogenase and the commercial enzymes used in titration experiments (hexokinase and PFK) were assayed as indicated in [22] and their K_m values determined in the same conditions as that of the experimental system. FCC were determined using the expression

$$Ce_i^J = (Q_1 - J)/Q_1 \quad (1)$$

where J is the actual flux through the system and Q_1 an experimental value obtained by titration experiments [18].

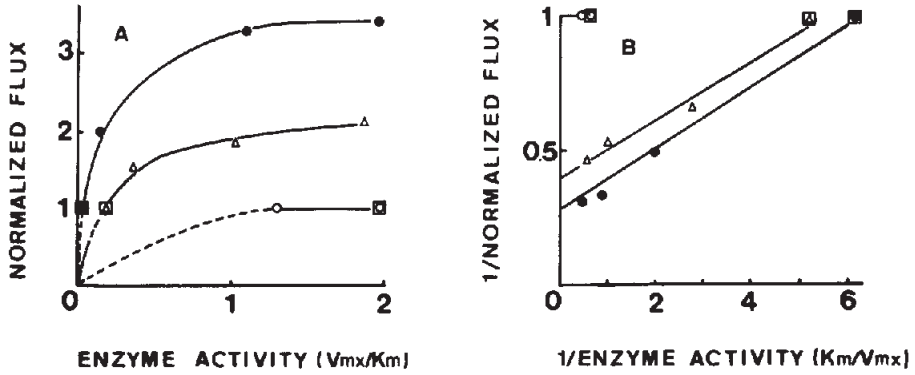

Fig.1. Titration of the starved extract with enzymes. (A) Metabolic system was titrated with hexokinase (●), phosphoglucose isomerase (○) and PFK (△) under the experimental conditions described in section 2. Flux of the metabolic pathway without added enzymes is expressed as 1 on the ordinate axis, and the points marked in every curve correspond to the flux with the enzyme present in liver extract only. Addition of hexokinase or PFK to the system enhances the flux, showing substantial values for their FCC, while addition of phosphoglucose isomerase does not affect the pathway flux. Broken lines, arbitrarily traced, show zones of the curves without experimental data, corresponding to enzyme activities lower than the ones present in the extract. Values of hexokinase activity are multiplied by 100 in the graph for graphical convenience. (B) Double-reciprocal plots give the Q_1 values which have been used in eqn 1 at the intercepts with the ordinate. In this plot reciprocal values of hexokinase activity have been multiplied by 0.01.

Table 1

	Enzyme activities		Flux control coefficients	
	Fed	Starved	Fed	Starved
Glucokinase	0.07 ± 0.002	0.015 ± 0.004	0.77 ± 0.025	0.72 ± 0.006
Phosphofructokinase	0.05 ± 0.015	0.019 ± 0.001	0.24 ± 0.01	0.59 ± 0.05
Ratio GK/PFK	1.4 ± 0.01	0.78 ± 0.03	3.2 ± 0.005	1.2 ± 0.015

The values of fed conditions are taken from [18]. Fluxes are 0.02 ± 0.002 and 0.003 ± 10^{-4} μmol of glycerol 3-phosphate/min per protein mg for fed and starved conditions, respectively. Enzyme activities are expressed as μmol of reaction product produced/min per protein mg. Results are given as the mean ± SD for three experiments

Protein concentration was assayed in a Hepes free sample by the method of Lowry et al. [23] with bovine serum albumin (Sigma) as standard.

3. RESULTS

In fig.1, the curves obtained from titration experiments with hexokinase and PFK on the starved system are represented. The response of the system to enhancement of enzymes was hyperbolic as previously described [18] giving the FCC set shown in table 1. Table 1 also shows the activities of GK and PFK and the value of glycolytic fluxes both in the fed and starved rat liver. As we can see in the starved conditions the enzyme activities decrease to 21.42% (GK) and 38% (PFK) from the values in the fed conditions. These changes are followed by a diminution in the flux through the pathway to 15% of the initial value. All these changes are in good agreement with the more gluconeogenic character of the pathway in starved conditions. The rearrangement of FCC changes enhances the control of the PFK catalysed step. The consequence is then that, although in two different metabolic situations the GK step is mainly responsible for the control, the ratio between the values of this coefficient and that corresponding to the PFK is quite different. Because of this big difference we can conclude that in starved conditions the relative importance of the PFK in the control of the pathway flux is higher than in normal fed conditions.

4. DISCUSSION

When starvation is imposed, the level in blood glucagon increases promoting the activation (by covalent phosphorylation) of fructose-2,6-bisphosphatase and the deactivation (also by covalent phosphorylation) of PFK II [4]. These mechanisms promote the depletion in the concentration of fructose 2,6-bisphosphate and subsequently, the fall in the actual PFK I activity in the medium. In fact, the addition of fructose 2,6-bisphosphate to starved assays does not modify the system response. This effect, together with the observed diminution in its maximal activity, can explain the increased value of the observed FCC of this enzyme. On the other hand, the decreased activity of GK is not enough to alter significantly the value of its FCC.

There is another important difference between the fed and the starved situation reflected by the summation property. Provided that in both cases the phosphoglucose isomerases have a null FCC, in the first one, summation of FCC is 1 showing that the pathway is 'closed'. But in the starved case summation of the analysed coefficients is greater than 1. That means that the system has some steps with negative values of FCC: the structure of the pathway must be more complex here. Which are these steps? Two different sets of reactions could be responsible for this. First the reverse reaction in either of the two substrate cycles (glucose-6-phosphatase and fructose-1,6-bisphosphatase) and second, the enzyme which promotes the degradation of the PFK stimulator fructose 2,6-bisphosphate. Through this last effector the activity of this enzyme can influence the FCC profile of the system.

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