Analysis of Metabolic Control

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- Detailed studies of metabolic regulation were not feasible until the basic chemical steps in a pathway had been clarified and the responsible enzymes characterized.
- By the middle of the twentieth century, all 10 enzymes of the glycolytic pathway had been purified and characterized. In the next 50 years much was learned about the regulation of these enzymes by intracellular and extracellular signals, through the kinds of allosteric and covalent mechanisms described in previous sessions. The conventional wisdom was that in a linear pathway such as glycolysis, catalysis by one enzyme must be the slowest and must therefore determine the *rate of metabolite flow, or flux*, through the whole pathway. For glycolysis, PFK-1 was considered the rate-limiting enzyme, because it was known to be closely regulated by fructose 2,6-bisphosphate and other allosteric effectors.

Analysis of Metabolic Control

- With the advent of genetic engineering technology, it became possible to test this "single rate-determining step" hypothesis by increasing the concentration of the enzyme that catalyzes the "rate-limiting step" in a pathway and determining whether flux through the pathway increases proportionally. Most often it does not; the simple explanation **(a single rate determining step) is wrong.**

- It has now become clear that, in most pathways, the control of flux is distributed among several enzymes, and the extent to which each contributes to the control varies with metabolic circumstances—the supply of the starting material (say, glucose), the supply of oxygen, the need for other products derived from intermediates in the pathway (say, glucose 6-phosphate for the pentose phosphate pathway in cells synthesizing large amounts of nucleotides), the effects of metabolites with regulatory roles, and the hormonal status of the organism (such as the levels of insulin and glucagon), among other factors.

Analysis of Metabolic Control

❑***Why are we interested in what limits the flux through a pathway***?

To understand the action of hormones or drugs, or the pathology that results from a failure of metabolic regulation, we must know where control is exercised. If researchers wish to develop a drug that stimulates or inhibits a pathway, the logical target is the enzyme that has the greatest impact on the flux through that pathway. And the bioengineering of a microorganism to overproduce a product of commercial value requires a knowledge of what limits the flux of metabolites toward that product.

Analysis of Metabolic Control

The Contribution of Each Enzyme to Flux through a Pathway Is Experimentally Measurable

- There are several ways to determine experimentally how a change in the activity of one enzyme in a pathway affects metabolite flux through that pathway.
- Consider the experimental results shown in Figure 15-9.

When a sample of rat liver was homogenized to release all soluble enzymes, the extract carried out the glycolytic conversion of glucose to fructose 1,6-bisphosphate at a measurable rate. (This experiment, for simplicity, focused on just the first part of the glycolytic pathway.) When increasing amounts of purified hexokinase IV (glucokinase) were added to the extract, the rate of glycolysis progressively increased. The addition of purified PFK-1 to the extract also increased the rate of glycolysis, but not as dramatically as did hexokinase. Addition of purified phosphohexose isomerase was without effect. These results suggest that hexokinase and PFK-1 both contribute to setting the flux through the pathway (hexokinase more than PFK-1), and that phosphohexose isomerase does not.

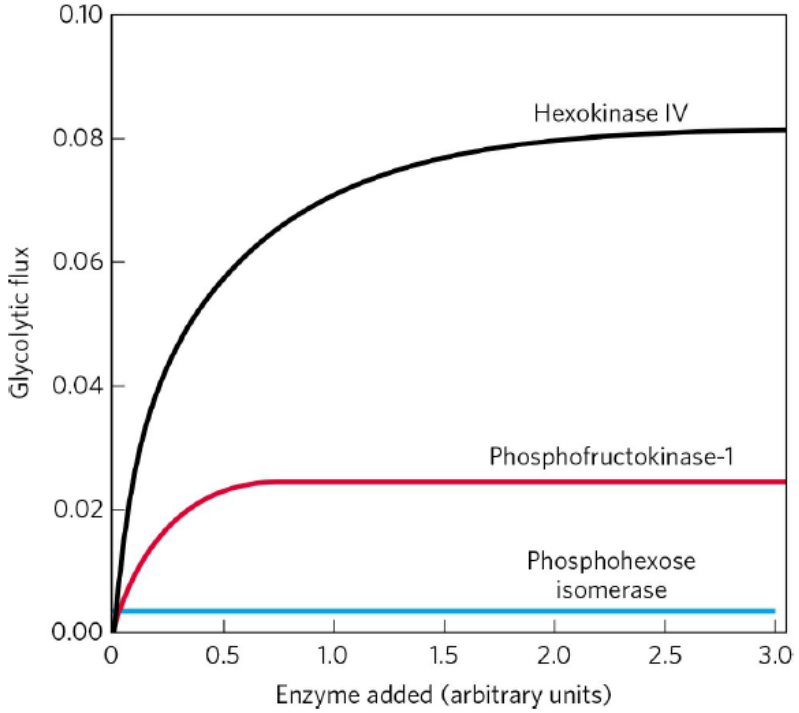
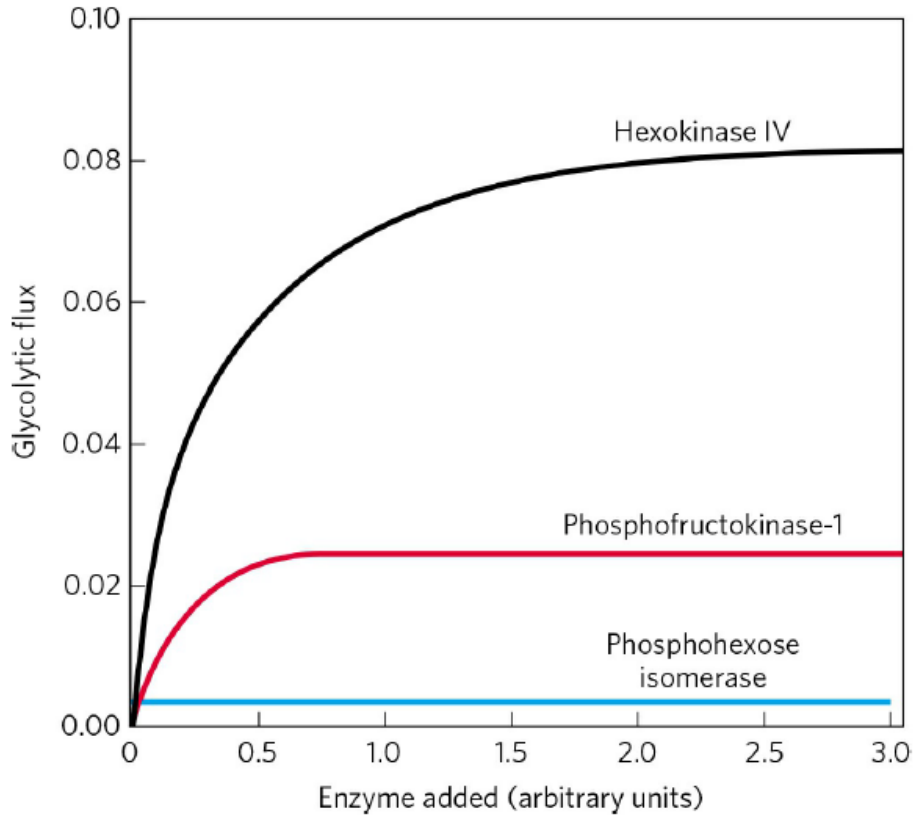


FIGURE 15-9 Dependence of glycolytic flux in a rat liver homogenate on added enzymes. Purified enzymes in the amounts shown on the *x* axis were added to an extract of liver carrying out glycolysis in vitro. The flux through the pathway is shown on the *y* axis.
[Source: Data from N. V. Torres et al., *Biochem. J.* 234:169, 1986.]

Analysis of Metabolic Control

The Contribution of Each Enzyme to Flux through a Pathway Is Experimentally Measurable

- Similar experiments can be done on intact cells or organisms, ***using specific inhibitors or activators to change the activity of one enzyme*** while observing the effect on flux through the pathway.
- ***The amount of an enzyme can also be altered genetically***; bioengineering can produce a cell that makes extra copies of the enzyme under investigation or has a version of the enzyme that is less active than the normal enzyme. Increasing the concentration of an enzyme genetically sometimes has significant effects on flux; sometimes it has no effect.



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Analysis of Metabolic Control

The Contribution of Each Enzyme to Flux through a Pathway Is Experimentally Measurable

- ***Three critical parameters***, which together describe the responsiveness of a pathway to changes in metabolic circumstances, lie at the center of metabolic control analysis. We turn now to a qualitative description of these parameters and their meaning in the context of a living cell.

1. *The Flux Control Coefficient (**C**)* Quantifies the Effect of a Change in Enzyme Activity on Metabolite Flux through a Pathway
2. *The Elasticity Coefficient (ε)* Is Related to an Enzyme's Responsiveness to Changes in Metabolite or Regulator Concentrations
3. *The Response Coefficient (R)* Expresses the Effect of an Outside Controller on Flux through a Pathway

The Flux Control Coefficient Quantifies the Effect of a Change in Enzyme Activity on Metabolite Flux through a Pathway

- Quantitative data on metabolic flux, obtained as described in Figure 15-9, can be used to calculate a ***flux control coefficient, C***, for each enzyme in a pathway. This coefficient expresses *the relative contribution of each enzyme* to setting the rate at which metabolites flow through the pathway—that is, *the flux, J.*

- ***C*** can have any value from 0.0 (for an enzyme with no impact on the flux) to 1.0 (for an enzyme that wholly determines the flux). An enzyme can also have a negative flux control coefficient. In a branched pathway, an enzyme in one branch, by drawing intermediates away from the other branch, can have a negative impact on the flux through that other branch (Fig. 15-10).

- ***C*** is not a constant, and it is not intrinsic to a single enzyme; it is a function of the whole system of enzymes, and its value depends on the concentrations of substrates and effectors.

- When real data from the experiment on glycolysis in a rat liver extract (Fig. 15-9) were subjected to this kind of analysis, investigators found *flux control coefficients* (for enzymes at the concentrations found in the extract) of *0.79 for hexokinase*, *0.21 for PFK-1*, and *0.0 for phosphohexose isomerase*. It is not just fortuitous that these values add up to 1.0; we can show that for any complete pathway, the sum of the flux control coefficients must equal unity.

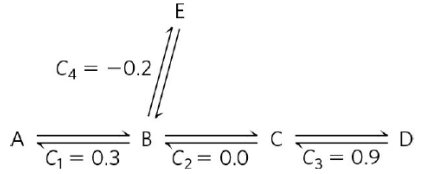

FIGURE 15-10 Flux control coefficient, C, in a branched metabolic pathway. In this simple pathway, the intermediate B has two alternative fates. To the extent that reaction B → E draws B away from the pathway A → D, it controls that pathway, which will result in a *negative* flux control coefficient for the enzyme that catalyzes step B → E. Note that the sum of all four coefficients equals 1.0, as it must for any defined system of enzymes.

The Elasticity Coefficient Is Related to an Enzyme's Responsiveness to Changes in Metabolite or Regulator Concentrations

- A second parameter, ***the elasticity coefficient, ε***, expresses quantitatively the responsiveness of a single enzyme to changes in the concentration of a metabolite or regulator; it is a function of the enzyme's intrinsic kinetic properties.
- For example, an enzyme with typical Michaelis-Menten kinetics shows a hyperbolic response to increasing substrate concentration (Fig. 15-11). At low concentrations of substrate (say, 0.1 Km), each increment in substrate concentration results in a comparable increase in enzymatic activity, yielding an ε near 1.0. At relatively high substrate concentrations (say, 10 Km), increasing the substrate concentration has little effect on the reaction rate, because the enzyme is already saturated with substrate. The elasticity in this case approaches zero.
- For allosteric enzymes that show positive cooperativity, ε may exceed 1.0, but it cannot exceed the Hill coefficient, which is typically between 1.0 and 4.0.

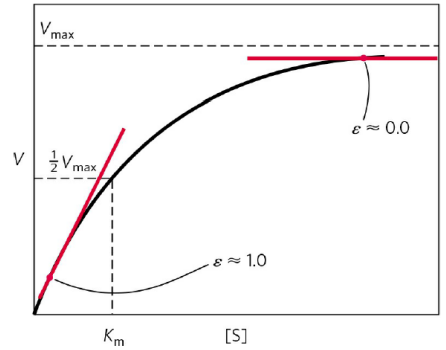

FIGURE 15-11 Elasticity coefficient, ε, of an enzyme with typical Michaelis-Menten kinetics. At substrate concentrations far below the K_m, each increase in [S] produces a correspondingly large increase in the reaction velocity, V. For this region of the curve, the enzyme has an ε of about 1.0. At [S] $\gg K_m$, increasing [S] has little effect on V; ε here is close to 0.0.

The Response Coefficient Expresses the Effect of an Outside Controller on Flux through a Pathway

- We can also derive a quantitative expression for *the relative impact of an outside factor (such as a hormone or growth factor),* which is neither a metabolite nor an enzyme in the pathway, on the flux through the pathway.

- The experiment would measure the flux through the pathway (glycolysis, in this case) at various levels of the parameter P (the insulin concentration, for example) to obtain ***the response coefficient, R***, which expresses the change in pathway flux when P ([insulin]) changes.

- The three coefficients C, ε, and R are related in a simple way: the responsiveness (R) of a pathway to an outside factor that affects a certain enzyme is a function of (1) how sensitive the pathway is to changes in the activity of that enzyme (the flux control coefficient, C) and (2) how sensitive that specific enzyme is to changes in the outside controlling factor (the elasticity, ε): $R = C \cdot \varepsilon$

- Each enzyme in the pathway can be examined in this way, and the effects of any of several outside factors on flux through the pathway can be separately determined. Thus, in principle, we can predict how the flux of substrate through a series of enzymatic steps will change when there is a change in one or more controlling factors external to the pathway.

Metabolic Control Analysis Has Been Applied to Carbohydrate Metabolism, with Surprising Results

- Metabolic control analysis provides a framework within which we can think quantitatively about regulation, interpret the significance of the regulatory properties of each enzyme in a pathway, identify the steps that most affect the flux through the pathway, and distinguish between regulatory mechanisms that act to maintain metabolite concentrations and control mechanisms that actually alter the flux through the pathway.

- ***Analysis of the glycolytic pathway in yeast***, for example, has revealed an unexpectedly low flux control coefficient for PFK-1, which, as we have noted, has been viewed as the main point of flux control—the "rate-determining step"—in glycolysis. Experimentally raising the level of PFK-1 fivefold led to a change in flux through glycolysis of less than 10%, suggesting that the real role of PFK-1 regulation is not to control flux through glycolysis but to mediate metabolite homeostasis—to prevent large changes in metabolite concentrations when the flux through glycolysis increases in response to elevated blood glucose or insulin. Recall that the study of glycolysis in a liver extract (Fig. 15-9) also yielded a flux control coefficient that contradicted the conventional wisdom; it showed that hexokinase, not PFK-1, is most influential in setting the flux through glycolysis. We must note here that a liver extract is far from equivalent to a hepatocyte; the ideal way to study flux control is by manipulating one enzyme at a time in the living cell. This is feasible in many cases.

Metabolic Control Analysis Has Been Applied to Carbohydrate Metabolism, with Surprising Results

- Investigators have used nuclear magnetic resonance (NMR) as a noninvasive means to determine the concentrations of glycogen and metabolites in the five-step pathway from glucose in the blood to glycogen in myocytes of rat and human muscle. They found that the flux control coefficient for glycogen synthase was smaller than that for either the glucose transporter (GLUT4) or hexokinase. This finding contradicts the conventional wisdom that glycogen synthase is the locus of flux control and suggests that the importance of the phosphorylation/dephosphorylation of glycogen synthase is related instead to the maintenance of metabolite homeostasis—that is, regulation, not control. Two metabolites in this pathway, glucose and glucose 6-phosphate, are key intermediates in other pathways, including glycolysis, the pentose phosphate pathway, and the synthesis of glucosamine. Metabolic control analysis suggests that when the blood glucose level rises, insulin acts in muscle to (**1**) increase glucose transport into cells by conveying GLUT4 to the plasma membrane, (**2**) induce the synthesis of hexokinase, and (**3**) activate glycogen synthase by covalent alteration. The first two effects of insulin increase glucose flux through the pathway (control), and the third serves to adapt the activity of glycogen synthase so that metabolite levels (glucose 6-phosphate, for example) will not change dramatically with the increased flux (regulation).

Metabolic Control Analysis Has Been Applied to Carbohydrate Metabolism, with Surprising Results

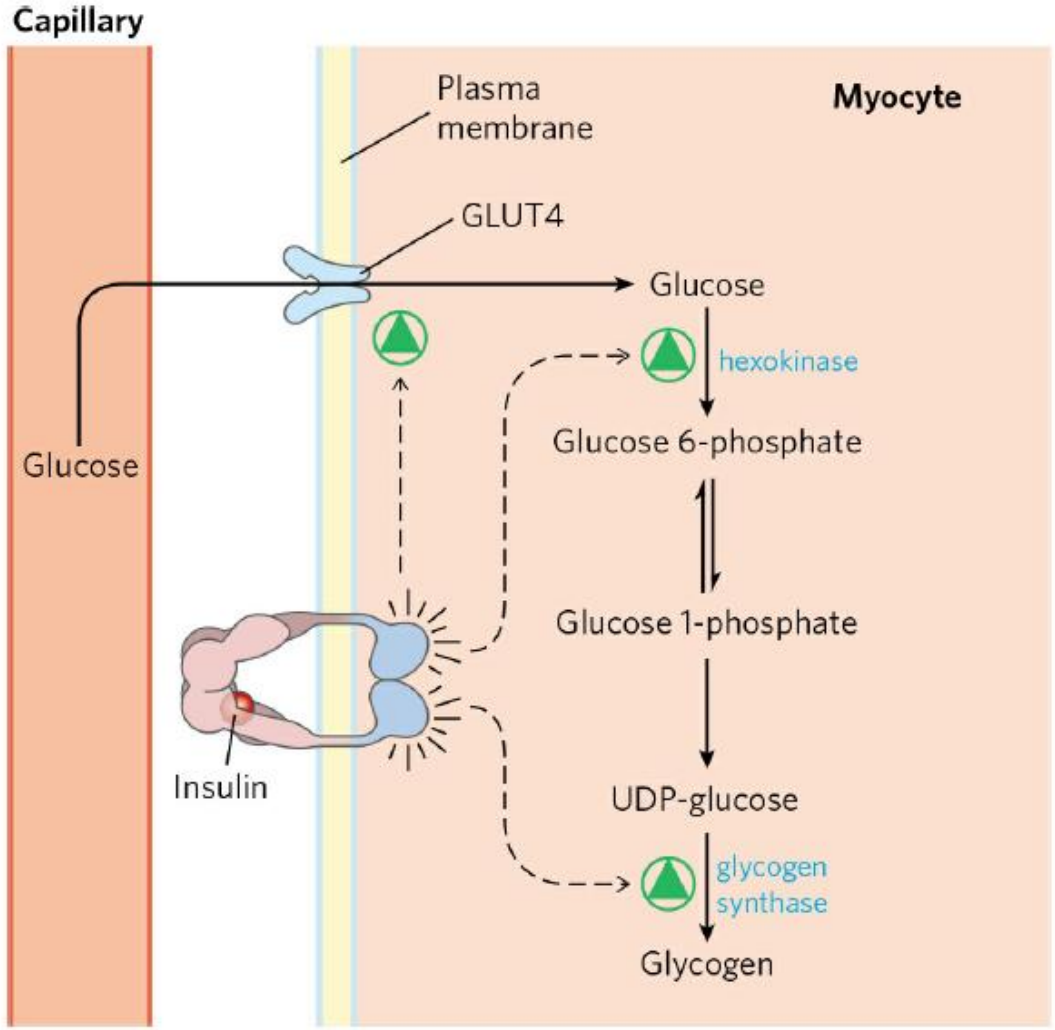

FIGURE 15-12 Control of glycogen synthesis from blood glucose in muscle. Insulin affects three of the five steps in this pathway, but it is the effects on transport and hexokinase activity, not the change in glycogen synthase activity, that increase the flux toward glycogen.