ethanol and acetaldehyde). Each subunit of this dimeric enzyme binds one NAD^+ and two Zn^{2+} ions, although only one of these ions participates directly in catalysis. There is significant amino acid sequence similarity between YADH and LADH, so it is quite likely that both enzymes have the same general mechanism.

C. *Energetics of Fermentation*

Thermodynamics permits us to dissect the process of fermentation into its component parts and to account for the free energy changes that occur. This enables us to calculate the efficiency with which the free energy of degradation of glucose is utilized in the synthesis of ATP. The overall reaction of homolactic fermentation is

$$\text{Glucose} \longrightarrow 2 \text{ lactate} + 2H^+$$
$$\Delta G^{\circ\prime} = -196 \text{ kJ} \cdot \text{mol}^{-1} \text{ of glucose}$$

($\Delta G^{\circ\prime}$ is calculated from the data in Table 3-4 using Eqs. [3.19] and [3.21] adapted for $2H^+$ ions.) For alcoholic fermentation, the overall reaction is

$$\text{Glucose} \longrightarrow 2CO_2 + 2 \text{ ethanol}$$
$$\Delta G^{\circ\prime} = -235 \text{ kJ} \cdot \text{mol}^{-1} \text{ of glucose}$$

Each of these reactions is coupled to the net formation of two ATPs, which requires $\Delta G^{\circ\prime} = +61 \text{ kJ} \cdot \text{mol}^{-1}$ of glucose consumed (Table 16-3). Dividing the $\Delta G^{\circ\prime}$ of ATP formation by that of lactate formation indicates that homolactic fermentation is 31% "efficient"; that is, 31% of the free energy released by this process under standard biochemical conditions is sequestered in the form of ATP. The rest is dissipated as heat, thereby making the process irreversible. Likewise, alcoholic fermentation is 26% efficient under biochemical standard state conditions. Actually, *under physiological conditions, where the concentrations of reactants and products differ from those of the standard state, these reactions have free energy efficiencies of >50%.*

a. Glycolysis Is Used for Rapid ATP Production

Anaerobic fermentation utilizes glucose in a profligate manner compared to oxidative phosphorylation: Fermentation results in the production of 2 ATPs per glucose, whereas oxidative phosphorylation yields 32 ATPs per glucose (Chapter 22). This accounts for Pasteur's observation that yeast consumes far more sugar when growing anaerobically than when growing aerobically (the **Pasteur effect;** Section 22-4C). However, *the rate of ATP production by anaerobic glycolysis can be up to 100 times faster than that of oxidative phosphorylation. Consequently, when tissues such as muscle are rapidly consuming ATP, they regenerate it almost entirely by anaerobic glycolysis.* (Homolactic fermentation does not really "waste" glucose since the lactate so produced is aerobically reconverted to glucose by the liver; Section 23-1C).

Skeletal muscles consist of both **slow-twitch** (Type I) and **fast-twitch** (Type II) **fibers.** Fast-twitch fibers, so called because they predominate in muscles capable of short bursts of rapid activity, are nearly devoid of mitochondria, so that they must obtain nearly all of their ATP through anaerobic glycolysis, for which they have a particularly large capacity. Muscles designed to contract slowly and steadily, in contrast, are enriched in slow-twitch fibers that are rich in mitochondria and obtain most of their ATP through oxidative phosphorylation. (Fast- and slow-twitch fibers were originally known as white and red fibers, respectively, because otherwise pale colored muscle tissue, when enriched with mitochondria, takes on the red color characteristic of their heme-containing cytochromes. However, fiber color has been shown to be an imperfect indicator of muscle physiology.)

In a familiar example, the flight muscles of migratory birds such as ducks and geese, which need a continuous energy supply, are rich in slow-twitch fibers and therefore such birds have dark breast meat. In contrast, the flight muscles of less ambitious fliers, such as chickens and turkeys, which are used only for short bursts (often to escape danger), consist mainly of fast-twitch fibers that form white meat. In humans, the muscles of sprinters are relatively rich in fast-twitch fibers, whereas distance runners have a greater proportion of slow-twitch fibers (although their muscles have the same color). World class distance runners have a remarkably high capacity to generate ATP aerobically. This was demonstrated by the noninvasive ^{31}P NMR monitoring of the ATP, P_i, phosphocreatine, and pH levels in their exercising but untrained forearm muscles. These observations suggest that the muscles of these athletes are better endowed genetically for endurance exercise than those of "normal" individuals.

4 METABOLIC REGULATION AND CONTROL

Living organisms, as we saw in Section 16-6, are thermodynamically open systems that tend to maintain a steady state rather than reaching equilibrium (death for living things). *Thus the **flux** (rate of flow) of intermediates through a metabolic pathway is constant; that is, the rates of synthesis and breakdown of each pathway intermediate maintain it at a constant concentration.* Such a state, it will be recalled, is one of maximum thermodynamic efficiency (Section 16-6Ba). *Regulation of the steady state **(homeostasis)** must be maintained in the face of changes in flux through the pathway in response to changes in demand.*

The terms metabolic control and metabolic regulation are often used interchangeably. However, for our purposes we shall give them different definitions: **Metabolic regulation** is the process by which the steady-state flow of metabolites through a pathway is maintained, whereas **metabolic control** is the influence exerted on the enzymes of a pathway in response to an external signal in order to alter the flux of metabolites.

A. *Homeostasis and Metabolic Control*

There are two reasons why metabolic flow must be controlled:

I. To provide products at the rate they are needed, that is, to balance supply with demand.

II. To maintain the steady-state concentrations of the intermediates in a pathway within a narrow range (homeostasis).

Organisms maintain homeostasis for several reasons:

1. In an open system, such as metabolism, the steady state is the state of maximum thermodynamic efficiency (Section 16-6Ba).

2. Many intermediates participate in more than one pathway, so that changing their concentrations may disturb a delicate balance.

3. The rate at which a pathway can respond to a control signal slows if large changes in intermediate concentrations are involved.

4. Large changes in intermediate concentrations may have deleterious effects on cellular osmotic properties.

The concentrations of intermediates and the level of metabolic flux at which a pathway is maintained vary with the needs of the organism through a highly responsive system of precise controls. Such pathways are analogous to rivers that have been dammed to provide a means of generating electricity. Although water is continually flowing in and out of the lake formed by the dam, a relatively constant water level is maintained. The rate of water outflow from the lake is precisely controlled at the dam and is varied in response to the need for electrical power. In this section, we examine the mechanisms by which metabolic pathways in general, and the glycolytic pathway in particular, are controlled in response to biological energy needs.

B. *Metabolic Flux*

Since a metabolic pathway is a series of enzyme-catalyzed reactions, it is easiest to describe the flux of metabolites through the pathway by considering its reaction steps individually. The flux of metabolites, J, through each reaction step is the rate of the forward reaction, v_f, less that of the reverse reaction, v_r:

$$J = v_f - v_r \qquad [17.1]$$

At equilibrium, by definition, there is no flux ($J = 0$), although v_f and v_r may be quite large. At the other extreme, in reactions that are far from equilibrium, $v_f >> v_r$, so that the flux is essentially equal to the rate of the forward reaction, $J \approx v_f$. *The flux throughout a steady-state pathway is constant and is set (generated) by the pathway's rate-determining step (or steps). Consequently, control of flux through a metabolic pathway requires: (1) that the flux through this* ***flux-generating step*** *vary in response to the organism's metabolic requirements and (2) that this change in flux be communicated throughout the pathway to maintain a steady state.*

The classic description of metabolic control and regulation is that every metabolic pathway has a rate-limiting step and is regulated by controlling the rate of this pivotal enzyme. These so-called regulatory enzymes are almost invariably allosteric enzymes subject to feedback inhibition (Section 13-4) and are often also controlled by covalent modification (which we discuss in Section 18-3).

Several questions arise. Are these regulatory enzymes really rate limiting for the pathway? Is there really only one step in the pathway that is rate limiting, or might there be a number of enzymes contributing to the regulation of the pathway? Does controlling these enzymes really control the flux of metabolites through the pathway or is the function of feedback inhibition really to maintain a steady state? These are complicated questions with complicated answers.

C. *Metabolic Control Analysis*

While it has been common practice to assume that every metabolic pathway has a rate-limiting step, experiments suggest that the situation becomes more complex when these pathways are combined in a living organism. Hence, it is important to develop methods to quantitatively analyze metabolic systems in order to establish mechanisms of control and regulation. **Metabolic control analysis,** developed by Henrik Kacser and Jim Burns and independently by Reinhart Heinrich and Tom Rapoport, provides a framework for considering these problems. It is a way of quantitatively describing the behavior of metabolic systems in response to various perturbations.

a. The Flux Control Coefficient Measures the Sensitivity of the Flux to the Change in Enzyme Concentration

Metabolic control analysis makes no *a priori* assumption that only one step is rate limiting. Instead, it defines a **flux control coefficient,** C^J (where J is an index, not an exponent), to measure the sensitivity of flux to a change in enzyme concentration. The flux control coefficient is defined as the fractional change in flux, J, with respect to the fractional change in enzyme concentration, [E]:

$$C^J = \frac{\partial J/J}{\partial [\mathrm{E}]/[\mathrm{E}]} = \frac{\partial \ln J}{\partial \ln [\mathrm{E}]} \approx \frac{\Delta J/J}{\Delta [\mathrm{E}]/[\mathrm{E}]} \qquad [17.2]$$

(recall that $\partial x/x = \partial \ln x$).

The flux control coefficient is the analog of the kinetic order of a reaction. If a reaction is first order in substrate concentration, [S], then doubling [S] doubles the rate of the reaction, whereas if the reaction is zero order in [S] (e.g., in a saturated enzymatic reaction), then the reaction rate is insensitive to the value of [S]. Similarly, if the flux control coefficient of an enzyme is 1, then doubling the concentration of the enzyme, [E], doubles the flux through the pathway and if it is zero, the flux is insensitive to the value of [E]. Of course, the flux control coefficient may have some intermediate value between 0 and 1. For example, if a 10% increase in the enzyme concentration increases the flux by only 7.5%, the flux control coefficient would be $0.075/0.10 = 0.75$.

The flux through a metabolic system is generally controlled by more than one enzyme. Consequently, the flux

control coefficient for each of the participating enzymes is the fraction of the total control on the pathway exerted by that enzyme. Thus, *the sum of all the flux control coefficients involved in controlling a pathway must equal 1.* This is the **additivity theorem** of metabolic control. It indicates that the flux control coefficient of a particular enzyme in a system depends, in part, on the flux control coefficients of the other enzymes in the system, that is, an enzyme's flux control coefficient is a property of the system as a whole. Indeed, *flux control in a metabolic system is shared among all the enzymes in the system,* although most of their flux control coefficients are likely to be small.

b. Recombinant DNA Technology Has Been Used to Measure Flux Control Coefficients

The flux control coefficient is a variable that has been experimentally determined *in vivo* for many enzymes that had previously been assumed to catalyze the rate-determining steps for their pathways. For example, the citric acid cycle enzyme **citrate synthase** (Sections 21-1A and 21-3A) catalyzes an irreversible reaction ($\Delta G^{\circ\prime} = -31.5$ kJ · mol^{-1}) and has therefore long been assumed to be one of the enzymes regulating the flow through the citric acid cycle (Section 21-4). Daniel Koshland determined how the activity of citrate synthase affected the flux through the citric acid cycle via genetic engineering techniques that permitted him to control the concentration of this enzyme *in vivo*. He constructed a plasmid (Fig. 17-31) that contained the gene for citrate synthase under the control of (directly downstream from) a modified ***lac* promoter,** together with the ***lacI*** gene, which encodes the ***lac* repressor** (in the absence of inducer, the *lac* repressor binds to the *lac* promoter and thereby prevents the transcription by RNA polymerase of the genes it controls; Section 5-4Aa), and the ***amp*^{R}** gene, which confers resistance to the antibiotic **ampicillin.** This plasmid was introduced into a mutant *E. coli* that lacked a gene for citrate synthase and was ampicillin-sensitive. These *E. coli* were grown in the presence of ampicillin (which killed any cells that had not taken up the plasmid) and of varying amounts of **isopropylthiogalactoside (IPTG),** a nonmetabolizable inducer of the *lac* operon (Section 31-1Aa).

Using this system, the citrate synthase concentration was measured as a function of [IPTG] and the growth rate of the *E. coli* was determined as a function of [IPTG] when glucose and/or acetate were the sole carbon sources. When acetate was the sole carbon source, the *E. coli* obtained most of their metabolic energy via the citric acid cycle and their growth varied proportionately with [citrate synthase]. The flux control coefficient of the enzyme in this case approached its maximum value of 1, that is, the flux through the citric acid cycle was almost entirely controlled by the activity of citrate synthase. However, when glucose was also available, the *E. coli* grew rapidly, even when [citrate synthase] was low, and were unaffected by changes in its value. Here, the flux control coefficient was near zero, indicating that the flux through the citric acid cycle was reduced to the point that even low concentrations of citrate synthase were in catalytic excess (evidently, when glucose is present, the citric acid cycle has a secondary role in energy production and biosynthesis in *E. coli*).

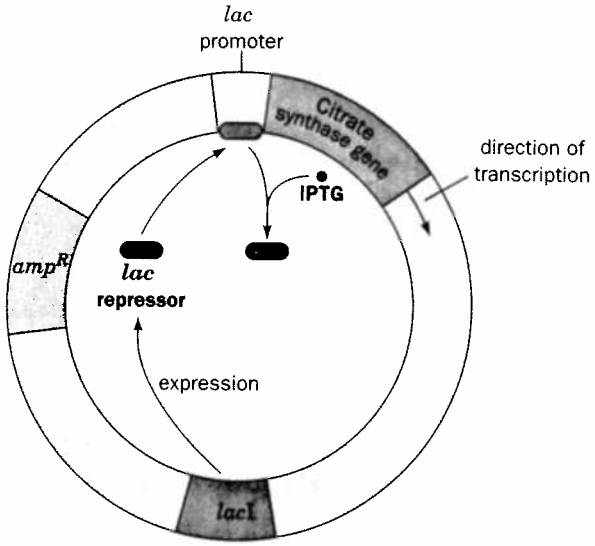


Figure 17-31 **Schematic diagram of the plasmid constructed to control the amount of citrate synthase produced by *E. coli*.** The *lacI* gene encodes the *lac* repressor, which binds to the *lac* promoter. This prevents the transcription of its immediately downstream gene, which encodes citrate synthase. On binding IPTG, the *lac* repressor releases the *lac* promoter, thereby permitting the expression of citrate synthase. Consequently, the concentration of the nonmetabolizable IPTG controls the level of expression of citrate synthase. The *amp*^{R} gene encodes a protein that provides resistance to the antibiotic ampicillin. Hence, in the presence of ampicillin, only those otherwise ampicillin-sensitive *E. coli* that have taken up the plasmid will survive.

c. The Rates of Enzymatic Reactions Respond to Changes in Flux

Let us consider how a constant flux is maintained throughout a metabolic pathway by analyzing the response of an enzyme-catalyzed reaction to a change in the flux of the reaction preceding it. In the following steady-state pathway:

$$S \xrightarrow[\text{rate-determining step(s)}]{J} A \underset{v_r}{\overset{v_f}{\rightleftharpoons}} B \xrightarrow{J} P$$

the flux, J, through the reaction A $\rightleftharpoons$ B, which must be identical to the flux through the rate-determining step(s), is expressed by Eq. [17.1] ($J = v_f - v_r$). If the flux of the rate-determining step increases by the amount ΔJ, the increase must be communicated to the next reaction step in the pathway by an increase in v_f (Δv_f) in order to re-establish the steady state. Qualitatively, we can see that this occurs because an increase in J causes an increase in [A], which in turn causes an increase in v_f. The amount of increase in [A] (Δ[A]) that causes v_f to increase the appropriate amount (Δv_f) is determined as follows:

$$\Delta J = \Delta v_f \qquad [17.3]$$

Dividing Eq. [17.3] by J, multiplying the right side by v_f/v_f, and substituting in Eq. [17.1] yields

$$\frac{\Delta J}{J} = \frac{\Delta v_f}{v_f}\frac{v_f}{J} = \frac{\Delta v_f}{v_f}\frac{v_f}{(v_f - v_r)} \qquad [17.4]$$

which relates $\Delta J/J$, the fractional change in flux through the rate-determining step(s), to $\Delta v_f/v_f$, the fractional change in v_f, the forward rate of the next reaction in the pathway.

In Section 14-2A, we discussed the relationship between substrate concentration and the rate of an enzymatic reaction as expressed by the Michaelis–Menten equation:

$$v_f = \frac{V^f_{\max}[A]}{K_M + [A]} \qquad [14.24]$$

In the simplest and physiologically most common situation, $[A] << K_M$, so that

$$v_f = \frac{V^f_{\max}[A]}{K_M} \qquad [17.5]$$

and

$$\Delta v_f = \frac{V^f_{\max}\Delta[A]}{K_M} \qquad [17.6]$$

Hence,

$$\frac{\Delta v_f}{v_f} = \frac{\Delta[A]}{[A]} \qquad [17.7]$$

that is, the fractional change in forward reaction rate is equal to the fractional change in substrate concentration. Then, by substituting Eq. [17.7] into Eq. [17.4], we find that

$$\frac{\Delta J}{J} = \frac{\Delta[A]}{[A]}\frac{v_f}{(v_f - v_r)} \qquad [17.8]$$

This equation relates the fractional change in flux through a metabolic pathway's rate-determining step(s) to the fractional change in substrate concentration necessary to communicate that change to the following reaction steps. *The quantity $v_f/(v_f - v_r)$ is a measure of the sensitivity of a reaction's fractional change in flux to its fractional change in substrate concentration.* This quantity is also a measure of the reversibility of the reaction, that is, how close it is to equilibrium:

1. In an irreversible reaction, v_r approaches 0 (relative to v_f) and therefore $v_f/(v_f - v_r)$ approaches 1. The reaction therefore requires a nearly equal fractional increase in its substrate concentration in order to respond to a fractional increase in flux.

2. As a reaction approaches equilibrium, v_r approaches v_f and hence $v_f/(v_f - v_r)$ approaches infinity. The reaction's response to a fractional increase in flux therefore requires a much smaller fractional increase in its substrate concentration.

Consequently, *the ability of a reaction to communicate a change in flux increases as the reaction approaches equilibrium.* A series of sequential reactions that are all near equilibrium therefore have the same flux and maintain concentrations of intermediates in a steady state (homeostasis).

d. The Elasticity Coefficient Measures the Sensitivity of an Enzymatic Reaction to the Change in Substrate Concentration

The ratio $v_f/(v_f - v_r)$, which measures the sensitivity of an enzymatic reaction rate to the change in substrate concentration, is called, in metabolic control analysis, the **elasticity coefficient,** ε. It is the fractional change in the net rate of an enzyme reaction, v, with respect to the fractional change in the substrate concentration, [A]:

$$\varepsilon = \frac{\partial v/v}{\partial[A]/[A]} = \frac{\partial \ln v}{\partial \ln[A]} \approx \frac{v_f}{v_f - v_r} \qquad [17.9]$$

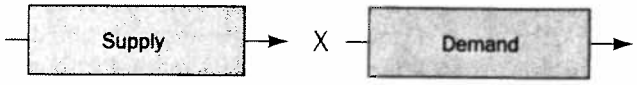
(When studying an individual enzymatic reaction, so that the fractional change in the net rate through the reaction, $\Delta v/v$, corresponds to the fractional change in the flux, $\Delta J/J$, and $[A] << K_M$, this equation is simply a rearrangement of Eq. [17.8].) The value of the elasticity coefficient depends on the kinetic characteristics of the enzyme and how close to equilibrium the enzyme is functioning. As mentioned above, if an enzyme is functioning far from equilibrium ($v_f >> v_r$), changing the substrate concentration will have a small effect on the net rate of the enzyme reaction (ε will be close to 1). However, if the enzyme is functioning very close to equilibrium so that both the forward and reverse reaction rates are much faster than the overall net rate, ε approaches infinity and it takes only a tiny change in substrate concentration to adjust to a new flux. Such large elasticity coefficients are therefore associated with maintaining homeostasis.

D. *Supply–Demand Analysis*

Early studies on control of metabolic pathways focused on individual pathways, ignoring their overall physiological functions. Control was always assumed to reside within the pathway. However, often when enzymes thought to be "rate controlling" in an individual pathway were overproduced in living organisms using genetic engineering techniques, increases in enzyme concentrations of as much as 10-fold had no effect on the flux through the specific pathway studied. The flux control coefficients of the overproduced enzymes were near zero in the *in vivo* system; they were already present in metabolic excess. The flux must somehow have been controlled from outside of the pathway. This is because, as we now realize, it is impossible to separate a pathway from the process(es) that utilizes the product(s) of that pathway (i.e., the living organisms must have reduced the activities of these enzymes in keeping with their metabolic requirements, that is, they maintained homeostasis).

Degradation pathways are inextricably linked to the biosynthetic pathways that utilize their products (Fig. 16-2). This is a **supply–demand process** and both supply and

demand are involved in the two metabolic control challenges: flux control and homeostasis. Jan-Hendrik Hofmeyr and Athel Cornish-Bowden have used metabolic control analysis to explore such a system, lumping all of the reactions of the supply pathway(s) together into one block and all of the reactions of the demand pathway(s) into a second block.



Here X is the intermediate that is produced by the supply block for use by the demand block. For the supply block, X is a product and a feedback inhibitor, so that as the concentration of X increases, the rate of flow through the supply block decreases. For the demand block, X is a substrate, so that as the concentration of X increases, the rate of flow through the demand block increases until it becomes saturated. When the flux through the supply block is equal to the flux through the demand block, the concentration of X is in a steady state, the point at which its rate of production is equal to its rate of utilization. This rate defines the actual flux through the supply–demand system and the steady-state concentration of X.

a. The Steady-State Concentration of Intermediates Responds to Changes in Supply and/or Demand

The response of the steady-state concentration of the intermediate, X, to any small change in the rate of the supply or demand block depends entirely on the elasticity coefficients of the two blocks at the steady state. Imagine that the activity of the demand block increases. This would result in a decrease of [X] and a concomitant increase in the flux through the supply block as feedback inhibition is decreased. The shift would continue until the rates through the supply and demand blocks equalize, shifting the system to a new steady state with a lower [X] and higher overall flux. Alternatively, if the activity of the supply block increases, producing a higher value of [X], the demand block would respond by increasing its rate to re-establish a new steady state at this higher [X]. *The higher the elasticity coefficient of the responding block, the smaller the change that [X] must make in order to re-establish a steady state.*

The question remains, where is the control? Is it in the supply block or in the demand block? The answer is that it is in the block for which the elasticity coefficient is lowest. Since it is the change in [X] that causes the readjustment of the steady state and the change in flux, the block for which the largest change in [X] is produced for a given change in rate is the controlling block. The change in flux for a given change in the rate in a particular block is its flux control coefficient, so the *control lies in the block that has the highest flux control coefficient and the lowest elasticity coefficient.* For example, if the supply block has a very high elasticity coefficient and the demand block has a very low elasticity coefficient, increased demand need cause very little decrease in [X] to result in a change in supply rate to reach a new steady state. However, because of the low elasticity coefficient of the demand block, there will have to be a much larger increase in [X] due to an increase in supply to cause the rate of the demand block to increase enough to reach a new steady state. Consequently, increasing the activity of the demand block would have a much larger effect on the flux than increasing the activity of the supply block. Thus, for this case, the flux is much more sensitive to changes in demand than to changes in supply, that is, the flux control coefficient of the demand block is much greater than that of the supply block.

There is a reciprocal relationship between the flux control coefficient and the elasticity coefficient. The larger the flux control coefficient, the lower the elasticity coefficient, and vice versa. The ratio of the elasticity coefficients of the supply and demand blocks determines the distribution of flux control between supply and demand. *When the ratio of the supply elasticity coefficient to the demand elasticity coefficient is greater than 1, as in our example, flux control lies in the demand portion of the pathway, and vice versa.*

b. The Elasticity Coefficient Describes the Regulation of Steady-State Intermediate Concentrations

In addition to controlling flux through the supply–demand system, the steady-state concentrations of the intermediates are also regulated. We have seen that the larger the elasticity coefficient of a given block, the smaller the change in [X] that is needed to re-establish a steady state and change the flux. Keeping the change in [X] as small as possible while changing the flux and maintaining a steady state is very important. *The larger the elasticity coefficient, the more sensitive the regulation of homeostasis.*

Flux control requires a high flux control coefficient, which requires a low elasticity coefficient. Regulation of homeostasis requires a high elasticity coefficient, which requires a low flux control coefficient. A large difference in the elasticity coefficients of the supply and demand blocks therefore leads to the exclusive control of flux by one or the other of the blocks. *The functions of flux and concentration control are mutually exclusive. If the demand block controls the flux, the function of the supply block is to regulate homeostasis.*

c. Feedback Inhibition Is Required for Homeostasis, Not Flux Control

When the demand block is exerting flux control, an increase in demand results in a decrease in the concentration of X, thereby decreasing feedback inhibition of the supply block. Feedback inhibition might therefore appear to be an essential part of the control process. In fact, this is not the case. Feedback inhibition is not part of the control system but part of the homeostasis system. It determines the range of [X] at which there is a steady state. In the absence of feedback inhibition, the supply block will be insensitive to [X] for most of that concentration range but will become sensitive to [X] near equilibrium, where the demand block could then control the flux. However, this would require

such high concentrations of X and the other metabolites in the supply pathway as to be osmotically dangerous. Feedback inhibition maintains homeostasis at physiologically reasonable metabolite concentrations.

E. *Mechanisms of Flux Control*

a. Flux through a Pathway Is Controlled at Its Rate-Determining Step(s)

The metabolic flux through an entire pathway is determined by controlling its rate-determining step(s), which by definition is much slower than the following reaction step(s). The product(s) of the rate-determining step(s) is therefore removed before it can equilibrate with the reactant, so that the rate-determining step(s) functions far from equilibrium and has a large negative free energy change. In an analogous manner, the flow of a river can only be controlled at a dam, which creates a difference in water levels between its upstream and downstream sides; this is a situation that also has a large negative free energy change, in this case resulting from the hydrostatic pressure head. Yet, as we have just seen, the elasticity coefficient, ε, of a nonequilibrium reaction ($v_f >> v_r$) is close to 1; that is, its substrate concentration must double (in the absence of other controlling effects) in order to double the reaction flux rate (Eq. [17.8]). However, some pathway fluxes vary by factors that are much greater than can be explained by changes in substrate concentrations. For example, glycolytic fluxes are known to vary by factors of 100 or more, whereas variations of substrate concentrations over such a large range are unknown. Consequently, although changes in substrate concentration can communicate a change in flux at the rate-determining step(s) to the other (near equilibrium; $v_f \approx v_r$) reaction steps of the pathway, there must be other mechanisms that control the flux of the rate-determining step(s).

The flux through a rate-determining step(s) of a pathway may be altered by several mechanisms:

1. Allosteric control: Many enzymes are allosterically controlled (Section 13-4) by effectors that are often substrates, products, or coenzymes in the pathway but not necessarily of the enzyme in question (feedback regulation). One such enzyme is PFK, an important glycolytic regulatory enzyme (Section 17-4Fb).

2. Covalent modification (enzymatic interconversion): Many enzymes that regulate pathway fluxes have specific sites that may be enzymatically phosphorylated and dephosphorylated at specific Ser, Thr, and/or Tyr residues or covalently modified in some other way. Such enzymatic modification processes, which are themselves subject to control, greatly alter the activities of the modified enzymes. This flux control mechanism is discussed in Section 18-3.

3. Substrate cycles: If v_f and v_r in Eq. [17.8] represent the rates of two opposing nonequilibrium reactions that are catalyzed by different enzymes, v_f and v_r may be independently varied. The flux through such a substrate cycle, as we shall see in the next section, is more sensitive to the concentrations of allosteric effectors than is the flux through a single unopposed nonequilibrium reaction.

4. Genetic control: Enzyme concentrations, and hence enzyme activities, may be altered by protein synthesis in response to metabolic needs. Genetic control of enzyme concentrations is a major concern of Part V of this textbook.

Mechanisms 1 to 3 can respond rapidly (within seconds or minutes) to external stimuli and are therefore classified as "short-term" control mechanisms. Mechanism 4 responds more slowly to changing conditions (within hours or days in higher organisms) and is therefore referred to as a "long-term" control mechanism.

F. *Regulation of Glycolysis in Muscle*

Elucidation of the flux regulation mechanisms of a given pathway involves the determination of the pathway's regulatory enzymes involved in the rate-determining steps together with the identification of the modulators of these enzymes and their mechanism(s) of modulation. A hypothesis may then be formulated that can be tested *in vivo*. A common procedure for establishing regulatory mechanisms involves three steps.

1. Identification of the rate-determining step(s) of the pathway. One way to do so is to measure the *in vivo* ΔG's of all the reactions in the pathway to determine how close to equilibrium they function. Those that operate far from equilibrium are potential control points; the enzymes catalyzing them may be regulated by one or more of the mechanisms listed above. Another way of establishing the rate-determining step(s) of a pathway is to measure the effect of a known inhibitor on a specific reaction step and on the flux through the pathway as a whole. The ratio of the fractional change in the activity of the inhibited enzyme to the fractional change in the total flux (the flux control coefficient) will vary between 0 and 1. The closer the ratio is to 1, the more involved that enzyme is in the regulation of the total flux through the pathway.

2. *In vitro* identification of allosteric modifiers of the enzymes catalyzing the rate-determining reactions. The mechanisms by which these compounds act are determined from their effects on the enzyme's kinetics. From this information, a model of the allosteric mechanisms for regulating the pathway may be formulated.

3. Measurement of the *in vivo* levels of the proposed regulators under various conditions to establish whether these concentration changes are consistent with the proposed regulation mechanism.

a. Free Energy Changes in the Reactions of Glycolysis

Let us examine the thermodynamics of glycolysis with an eye toward understanding its regulatory mechanisms. This must be done separately for each type of tissue in question because glycolysis is regulated in different tissues

Table 17-1 **$\Delta G^{\circ\prime}$ and ΔG of the Reactions of Glycolysis in Heart Muscle[a]**

Reaction	Enzyme	$\Delta G^{\circ\prime}$ (kJ · mol^{-1})	ΔG (kJ · mol^{-1})
1	HK	−20.9	−27.2
2	PGI	+2.2	−1.4
3	PFK	−17.2	−25.9
4	Aldolase	+22.8	−5.9
5	TIM	+7.9	~0
6 + 7	GAPDH + PGK	−16.7	−1.1
8	PGM	+4.7	−0.6
9	Enolase	−3.2	−2.4
10	PK	−23.0	−13.9

[a]Calculated from data in Newsholme, E.A. and Start, C., *Regulation in Metabolism, p.* 97, Wiley (1973).

in different ways. We shall confine ourselves to muscle tissue. First we establish the pathway's possible regulation points through the identification of its nonequilibrium reactions. Table 17-1 lists the standard free energy change ($\Delta G^{\circ\prime}$) and the actual physiological free energy change (ΔG) associated with each reaction in the pathway. It is important to realize that the free energy changes associated with the reactions under standard conditions may differ dramatically from those in effect under physiological conditions. For example, the $\Delta G^{\circ\prime}$ for aldolase is +22.8 kJ · mol^{-1}, whereas under physiological conditions in heart muscle it is close to zero, indicating that the *in vivo* activity of aldolase is sufficient to equilibrate its substrates and products. The same is true of the GAPDH + PGK reaction series. Nevertheless, in a steady-state pathway, all the reactions must have $\Delta G < 0$. This is because if $\Delta G > 0$ for any reaction, its flux would be in the reverse direction.

In the glycolytic pathway, only three reactions, those catalyzed by hexokinase (HK), phosphofructokinase (PFK), and pyruvate kinase (PK), function with large negative free energy changes in heart muscle under physiological conditions (Table 17-1). These nonequilibrium reactions of glycolysis are the candidates for the flux-control points. The other glycolytic reactions function near equilibrium: Their forward and reverse rates are much faster than the actual flux through the pathway (although their forward rates must be at least slightly greater than their reverse rates). Consequently, these near-equilibrium reactions are very sensitive to changes in the concentration of pathway intermediates (they have high elasticity coefficients) and thereby rapidly communicate any changes in flux generated at the rate-determining step(s) throughout the rest of the pathway, ensuring the maintenance of a steady state (homeostasis).

b. Phosphofructokinase Is a Major Target for Regulating the Flux of Glycolysis in Muscle

In vitro kinetic studies of HK, PFK, and PK indicate that each is controlled by a variety of compounds, some of which are listed in Table 17-2. Yet, when the G6P source for glycolysis is glycogen, rather than glucose, as is often the case in skeletal muscle (Section 18-1), the hexokinase reaction is not required. *PFK, an elaborately controlled enzyme functioning far from equilibrium, evidently is the major target for regulating glycolysis in muscle under most conditions.*

PFK (Fig. 17-32*a*) is a tetrameric enzyme with two conformational states, R and T, that are in equilibrium. ATP is both a substrate and an allosteric inhibitor of PFK. Each subunit has two binding sites for ATP, a substrate site and an inhibitor site. The substrate site binds ATP equally well in either conformation, but the inhibitor site binds ATP almost exclusively in the T state. The other substrate of PFK, F6P, preferentially binds to the R state. Consequently, at high concentrations, ATP acts as a heterotropic allosteric inhibitor of PFK by binding to the T state, thereby shifting the T $\rightleftharpoons$ R equilibrium in favor of the T state and thus decreasing PFK's affinity for F6P (this is similar to the action of 2,3-BPG in decreasing the affinity of hemoglobin for O_2; Section 10-2F). In graphical terms, at high concentrations of

Table 17-2 **Some Effectors of the Nonequilibrium Enzymes of Glycolysis**

Enzyme	Inhibitors	Activators[a]
HK	G6P	—
PFK	ATP, citrate, PEP	ADP, AMP, cAMP, FBP, F2,6P, F6P, NH_4^+, P_i
PK (muscle)	ATP	AMP, PEP, FBP

[a]The activators for PFK are better described as deinhibitors of ATP because they reverse the effect of inhibitory concentrations of ATP.

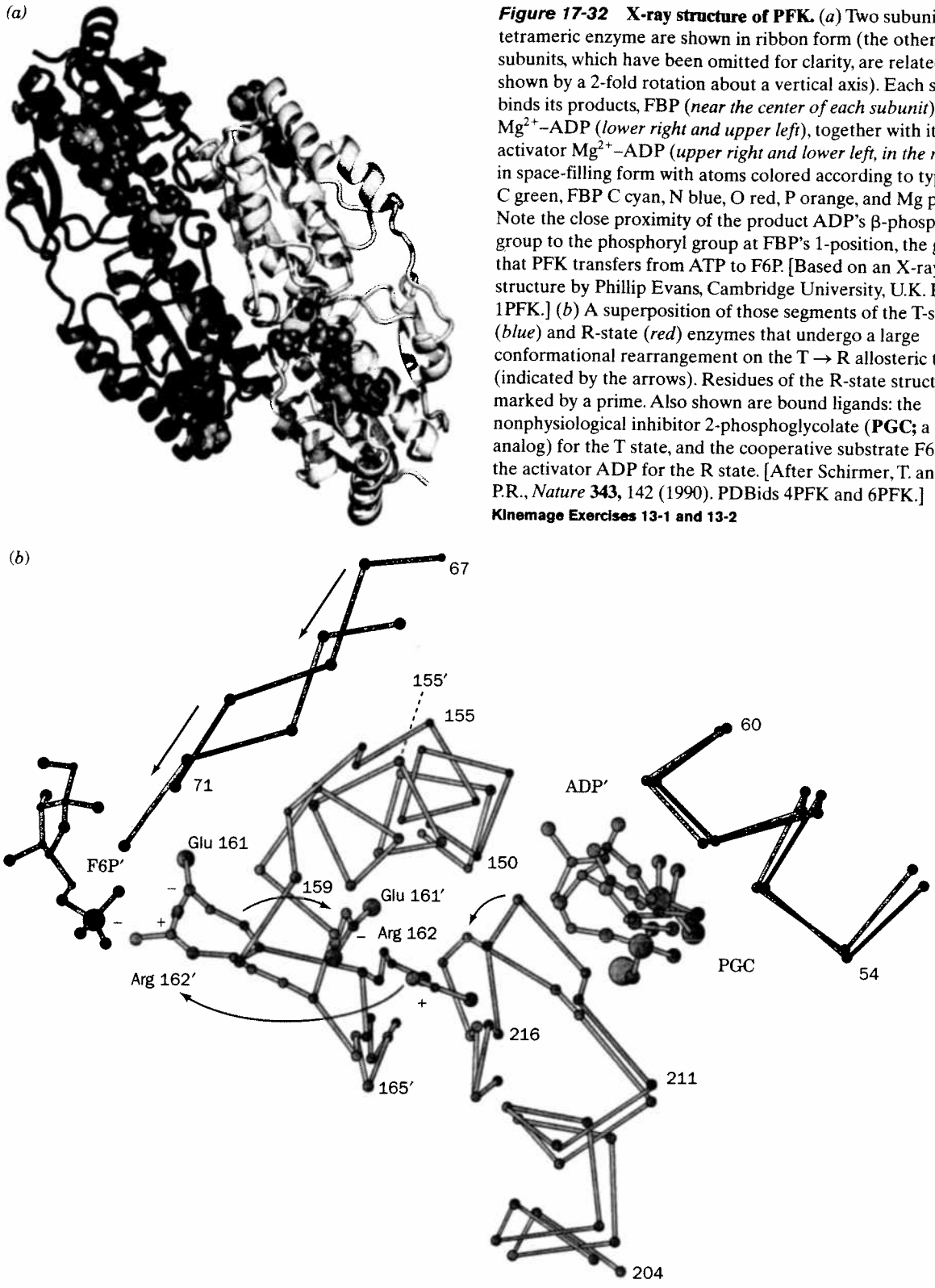


Figure 17-32 **X-ray structure of PFK.** (*a*) Two subunits of the tetrameric enzyme are shown in ribbon form (the other two subunits, which have been omitted for clarity, are related to those shown by a 2-fold rotation about a vertical axis). Each subunit binds its products, FBP (*near the center of each subunit*) and Mg^{2+}–ADP (*lower right and upper left*), together with its activator Mg^{2+}–ADP (*upper right and lower left, in the rear*), all in space-filling form with atoms colored according to type (ADP C green, FBP C cyan, N blue, O red, P orange, and Mg purple). Note the close proximity of the product ADP's β-phosphate group to the phosphoryl group at FBP's 1-position, the group that PFK transfers from ATP to F6P. [Based on an X-ray structure by Phillip Evans, Cambridge University, U.K. PDBid 1PFK.] (*b*) A superposition of those segments of the T-state (*blue*) and R-state (*red*) enzymes that undergo a large conformational rearrangement on the T → R allosteric transition (indicated by the arrows). Residues of the R-state structure are marked by a prime. Also shown are bound ligands: the nonphysiological inhibitor 2-phosphoglycolate (**PGC**; a PEP analog) for the T state, and the cooperative substrate F6P and the activator ADP for the R state. [After Schirmer, T. and Evans, P.R., *Nature* **343,** 142 (1990). PDBids 4PFK and 6PFK.] **See Kinemage Exercises 13-1 and 13-2**

ATP, the hyperbolic (noncooperative) curve of PFK activity versus [F6P] is converted to the sigmoidal (cooperative) curve characteristic of allosteric enzymes (Fig. 17-33; cooperative and noncooperative processes are discussed in Section 10-1Ba). For example, when [F6P] = 0.5 m*M* (the dashed line in Fig. 17-33), the enzyme is nearly maximally active, but in the presence of 1 m*M* ATP, the activity drops to 15% of its original level (a nearly 7-fold decrease).

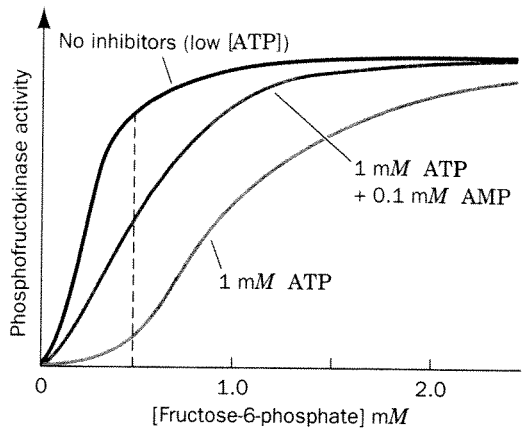


Figure 17-33 **PFK activity versus F6P concentration.** The various conditions are: blue, no inhibitors (low, noninhibitory [ATP]); green, 1 m*M* ATP (inhibitory); and red, 1 m*M* ATP + 0.1 m*M* AMP. [After data from Mansour, T.E. and Ahlfors, C.E., *J. Biol. Chem.* **243,** 2523–2533 (1968).] **See the Animated Figures.**

[Actually, the most potent allosteric effector of PFK is **fructose-2,6-bisphosphate (F2,6P).** We discuss the role of F2,6P in regulating PFK activity when we study the mechanism by which the liver maintains blood glucose concentrations (Section 18-3Fc).]

c. Structural Basis for PFK's Allosteric Change in F6P Affinity

The X-ray structures of PFK from several organisms have been determined for both the R and the T states by Phillip Evans. The R state of PFK is homotropically stabilized by the binding of its substrate fructose-6-phosphate (F6P). In the R state of *Bacillus stearothermophilus* PFK, the side chain of Arg 162 forms a salt bridge with the phosphoryl group of an F6P bound in an active site of another subunit (Fig. 17-32*b*). However, Arg 162 is located at the end of a helical turn that unwinds on transition to the T state. The positively charged side chain of Arg 162 thereby swings away and is replaced by the negatively charged side chain of Glu 161. As a consequence, the doubly negative phosphoryl group of F6P has a greatly diminished affinity for the T-state enzyme. The unwinding of this helical turn, which is obligatory for the R → T transition, is prevented by the binding of the activator ADP to its effector site on PFK in the R state, and facilitated by the binding of ATP to this effector site in the T state. Evidently, the same conformational shift is responsible for both the homotropic and the heterotropic allosteric effects in PFK.

d. AMP Overcomes the ATP Inhibition of PFK

Direct allosteric control of PFK by ATP may superficially appear to be the means by which glycolytic flux is regulated. After all, when [ATP] is high as a result of low metabolic demand, PFK is inhibited and flux through the pathway is low; conversely, when [ATP] is low, flux through the pathway is high and ATP is synthesized to replenish the pool. Consideration of the physiological variation in ATP concentration, however, indicates that the situation must be more complex. The metabolic flux through glycolysis may vary by 100-fold or more, depending on the metabolic demand for ATP. However, measurements of [ATP] *in vivo* at various levels of metabolic activity indicate that [ATP] varies <10% between rest and vigorous exertion. Yet *there is no known allosteric mechanism that can account for a 100-fold change in flux of a nonequilibrium reaction with only 10% change in effector concentration.* Thus, some other mechanism, or mechanisms, must be responsible for controlling glycolytic flux.

The inhibition of PFK by ATP is relieved by AMP. This results from AMP's preferential binding to the R state of PFK. If a PFK solution containing 1 m*M* ATP and 0.5 m*M* F6P is brought to 0.1 m*M* in AMP, the activity of PFK rises from 10 to 50% of its maximal activity, a 5-fold increase (Fig. 17-33).

[ATP] decreases by only 10% in going from a resting state to one of vigorous activity because it is buffered by the action of two enzymes: creatine kinase (Section 16-4Cd) and, of particular importance to this discussion, **adenylate kinase (AK;** also known as **myokinase**). Adenylate kinase catalyzes the reaction

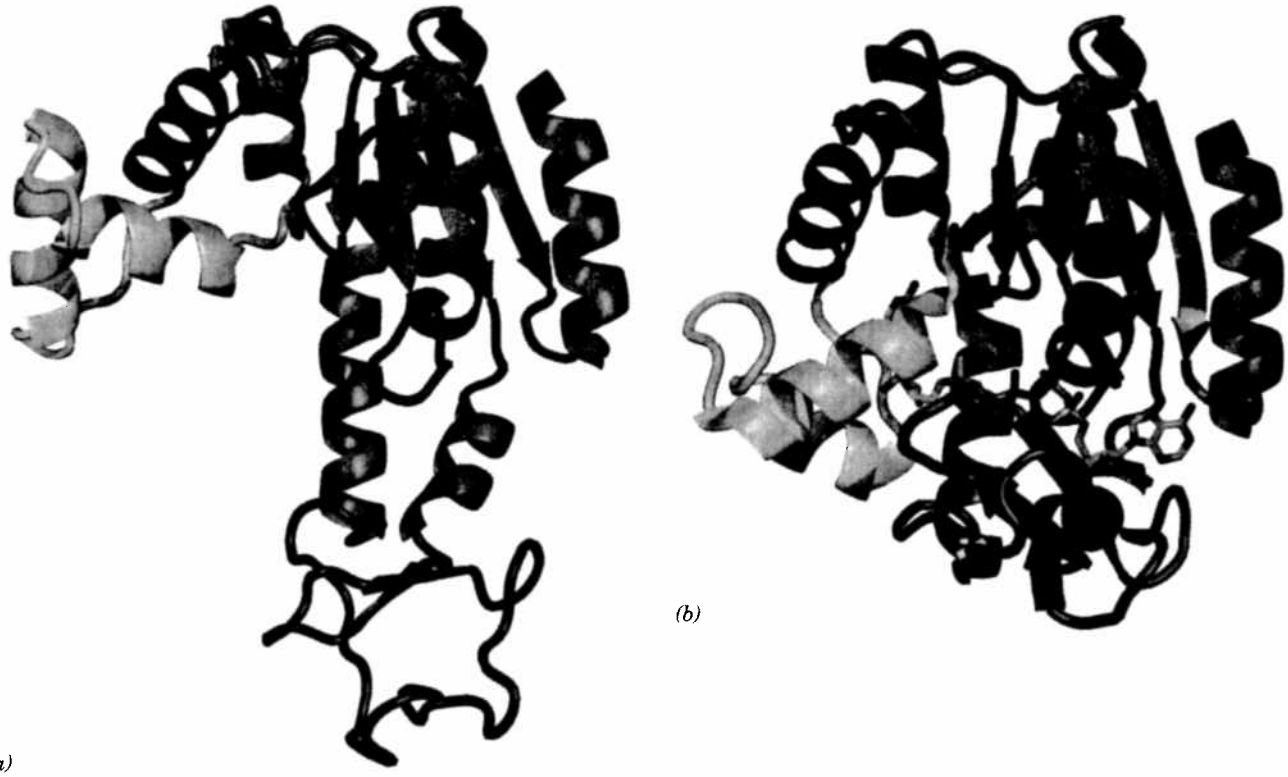
$$2\text{ADP} \rightleftharpoons \text{ATP} + \text{AMP} \quad K = \frac{[\text{ATP}][\text{AMP}]}{[\text{ADP}]^2} = 0.44$$

which rapidly equilibrates the ADP resulting from ATP hydrolysis in muscle contraction with ATP and AMP.

In muscle, [ATP] is ~50 times [AMP] and ~10 times [ADP], so that, *as a result of the adenylate kinase reaction, a 10% decrease in [ATP] will cause over a 4-fold increase in [AMP]* (see Problem 12 in this chapter). Consequently, a metabolic signal consisting of a decrease in [ATP] too small to relieve PFK inhibition is amplified significantly by the adenylate kinase reaction, which increases [AMP] by an amount sufficient to produce a much larger increase in PFK activity.

e. Adenylate Kinase's Internal Motions Act as an Energetic Counterweight to Balance Substrate Binding

Adenylate kinase, like other kinases, must be specific to prevent undesirable phosphoryl-transfer reactions such as hydrolysis. However, once the reaction has occurred, the tightly bound products must be rapidly released to maintain the enzyme's catalytic efficiency. With kinases such as hexokinase and phosphoglycerate kinase, this process is accomplished by the closing of "jaws" on the bound substrates that open when product is formed (Figs. 17-5 and 17-15), a process that is presumably driven by the exergonic free energy change of the reaction the enzyme catalyzes. However, since the AK reaction is energetically neutral (it replaces one phosphodiester bond with another), AK specificity is accomplished by a somewhat different means. Comparison of the X-ray structures, determined



(a) *(b)*

Figure 17-34 **Conformational changes in *E. coli* adenylate kinase (AK) on binding substrate.** (*a*) The unliganded enzyme. (*b*) The enzyme with the bound substrate analog Ap_5A. The Ap_5A is drawn in stick form colored according to atom type (C green, N blue, O red, and P yellow). The protein's cyan and blue domains undergo extensive conformational changes on ligand binding, whereas the remainder of the protein (*magenta*), whose orientation is the same in Parts *a* and *b*, largely maintains its conformation. Compare these structures to that of porcine AK (Fig. 8-54*b*). [Based on X-ray structures by Georg Schulz, Institut für Organische Chemie und Biochemie, Freiburg, Germany. PDBids (*a*) 4AKE and (*b*) 1AKE.]

by Georg Schulz, of unliganded AK with AK in complex with the inhibitory bisubstrate analog **Ap_5A** (two ADPs connected by a fifth phosphate) indicates that two ~30-residue domains of AK close over the Ap_5A, thereby tightly binding it and excluding water (Fig. 17-34). This comparison also suggests how AK avoids falling into the energy well of tight-binding substrates and products. On binding substrate, a portion of the protein remote from the active site increases its chain mobility and thereby "absorbs" some of the free energy of substrate binding (recall that an X-ray structure determination reveals atomic mobilities as well as positions; Section 9-4). This region "resolidifies" on product release. This mechanism, Schulz has hypothesized, acts as an "energetic counterweight" that permits facile product release and hence maintains a high reaction rate.

f. Substrate Cycling Can Increase Flux Sensitivity

Even though a mechanism exists for amplifying the effect of a small change in [ATP] by producing a larger change in [AMP], a 4-fold increase in [AMP] would allosterically increase the activity of PFK by only ~10-fold, an amount insufficient to account for the observed 100-fold increase in glycolytic flux. Small changes in effector concentration (and therefore v_f) can only cause relatively large changes in the flux through a reaction ($v_f - v_r$) if the reaction is functioning close to equilibrium. The reason for this high sensitivity is that for such reactions, the term $v_f/(v_f - v_r)$ in Eq. [17.8] (the elasticity coefficient) is large, that is, the reverse reaction contributes significantly to the value of the net flux. This is not the case for the PFK reaction.

Such equilibrium-like conditions may be imposed on a nonequilibrium reaction if a second enzyme catalyzes the regeneration of substrate from product in a thermodynamically favorable manner. Then v_r is no longer negligible compared to v_f. This situation requires that the forward process (formation of FBP from F6P) and reverse process (breakdown of FBP to F6P) be accomplished by different reactions since the laws of thermodynamics would otherwise be violated. In the following paragraphs, we discuss the nature of such **substrate cycles.**

Under physiological conditions, the reaction catalyzed by PFK:

Fructose-6-phosphate + ATP ⟶ fructose-1,6-bisphosphate + ADP

is highly exergonic ($\Delta G = -25.9$ kJ · mol^{-1}, Table 17-1). Consequently, the back reaction has a negligible rate compared to the forward reaction. **Fructose-1,6-bisphosphatase (FBPase),** however, which is present in many mammalian tissues (and which is an essential enzyme in gluconeogenesis; Section 23-1), catalyzes the exergonic hydrolysis of FBP ($\Delta G = -8.6$ kJ · mol^{-1}):

$$\text{Fructose-1,6-bisphosphate} + H_2O \longrightarrow \text{fructose-6-phosphate} + P_i$$

Note that the combined reactions catalyzed by PFK and FBPase result in net ATP hydrolysis:

$$ATP + H_2O \rightleftharpoons ADP + P_i$$

Such a set of opposing reactions is known as a substrate cycle because it cycles a substrate to an intermediate and back again. When this set of reactions was discovered, it was referred to as a **futile cycle** since its net result seemed to be the useless consumption of ATP. In fact, when it was found that the PFK activators AMP and F2,6P allosterically inhibit FBPase, it was suggested that only one of these enzymes was functional in a cell under any given set of conditions. It was subsequently demonstrated, however, that both enzymes often function simultaneously at significant rates.

g. Substrate Cycling Can Account for Glycolytic Flux Variation

Eric Newsholme proposed that substrate cycles are not at all "futile" but, rather, have a regulatory function. The *in vivo* activities of enzymes and concentrations of metabolites are extremely difficult to measure, so that their values are rarely known accurately. However, let us make the physiologically reasonable assumption that a 4-fold increase in [AMP], resulting from the adenylate kinase reaction, causes PFK activity (v_f) to increase from 10 to 90% of its maximum and FBPase activity (v_r) to decrease from 90 to 10% of its maximum. The maximum activity of muscle PFK is known from *in vitro* studies to be ~10-fold greater than that of muscle FBPase. Hence, if we assign full activity of PFK to be 100 arbitrary units, then full activity of FBPase is 10 such units. The flux through the PFK reaction in glycolysis under conditions of low [AMP] is

$$J_{low} = v_f(low) - v_r(low) = 10 - 9 = 1$$

where v_f is catalyzed by PFK and v_r by FBPase. The flux under conditions of high [AMP] is

$$J_{high} = v_f(high) - v_r(high) = 90 - 1 = 89$$

Substrate cycling could therefore amplify the effect of changes in [AMP] on the net rate of phosphorylation of F6P. Without the substrate cycle, a 4-fold increase in [AMP] increases the net flux by about 9-fold, whereas with the cycle the same increase in [AMP] causes a $J_{high}/J_{low} = 89/1 \approx 90$-fold increase in net flux. Consequently, under the above assumptions, *a 10% change in [ATP] could stimulate a 90-fold change in flux through the glycolytic pathway by a combination of the adenylate kinase reaction and substrate cycles.*

h. Physiological Impact of Substrate Cycling

Substrate cycling, if it has a regulatory function, does not increase the maximum flux through a pathway. On the contrary, it functions to decrease its minimum flux. In a sense, the substrate is put into a "holding pattern." In the case described above, *the cycling of substrate is the energetic "price" that a muscle must pay to be able to change rapidly from a resting state, in which substrate cycling is maximal, to one of sustained high activity.* However, the rate of substrate cycling may itself be under hormonal or nervous control so as to increase the sensitivity of the metabolic system under conditions when high activity (fight or flight) is anticipated (we address the involvement of hormones in metabolic regulation in Sections 18-3E and 18-3F).

In some tissues, substrate cycles function to produce heat. For example, many insects require a thoracic temperature of 30°C to be able to fly. Yet bumblebees are capable of flight at ambient temperatures as low as 10°C. Bumblebee flight muscle FBPase has a maximal activity similar to that of its PFK (10-fold greater than our example for mammalian muscle); furthermore, unlike all other known muscle FBPases, it is not inhibited by AMP. This permits the FBPase and PFK of bumblebee flight muscle to be highly active simultaneously so as to generate heat. Since the maximal rate of FBP cycling possible in bumblebee flight muscle generates only 10 to 15% of the required heat, however, other mechanisms of thermogenesis must also be operative. Nevertheless, FBP cycling is probably significant because, unlike bumblebees, honeybees, which have no FBPase activity in their flight muscles, cannot fly when the temperature is low.

i. Substrate Cycling, Thermogenesis, and Obesity

Many animals, including adult humans, generate some of their body heat, particularly when it is cold, through substrate cycling in muscle and liver, one mechanism of a process known as **nonshivering thermogenesis** (the muscle contractions of shivering or any other movement also produce heat; another mechanism of nonshivering thermogenesis is described in Section 22-3Da). Substrate cycling is stimulated by thyroid hormones (which stimulate metabolism in most tissues; Section 19-1D), as is indicated, for example, by the observation that rats lacking a functioning thyroid gland do not survive at 5°C. Chronically obese individuals tend to have lower than normal metabolic rates, which is probably due, in part, to a reduced rate of nonshivering thermogenesis. Such individuals therefore tend to be cold sensitive. Indeed, whereas normal individuals increase their rate of thyroid hormone activation on exposure to cold, genetically obese animals and obese humans fail to do so.

j. The Overexpression of PFK Does Not Increase the Rate of Glycolysis

PFK has long been thought to be the controlling enzyme of glycolysis. It was therefore expected that increasing the level of expression of PFK in yeast cells via genetic engineering techniques would increase the rate of glycolysis independent of the demand for glycolytic products. It has been amply demonstrated, however, that this is not the case. Although PFK is a major regulatory enzyme of glycolysis, its catalytic activity *in vivo* is controlled by the concentrations of the effectors that reflect the needs of the demand blocks that utilize its products.

Metabolic control analysis, in addition to helping us recognize that control can be shared by several enzymes in a pathway, has also alerted us to the difference between control and regulation. *Although PFK has a major role in regulating the flux through glycolysis, it is controlled, in vivo, by factors outside the pathway.* An increase in the *in vivo* concentration of PFK will therefore not increase the flux through the pathway because these controlling factors adjust the catalytic activity of PFK only to meet the needs of the cell.

5 METABOLISM OF HEXOSES OTHER THAN GLUCOSE

While glucose is the primary end product of the digestion of starch and glycogen (Section 11-2D), three other hexoses are also prominent digestion products: **Fructose,** obtained from fruits and from the hydrolysis of sucrose (table sugar); **galactose,** obtained from the hydrolysis of lactose (milk sugar); and **mannose,** obtained from the digestion of polysaccharides and glycoproteins. After digestion, these monosaccharides enter the bloodstream, which carries them to various tissues. *The metabolism of fructose, galactose, and mannose proceeds by their conversion to glycolytic intermediates, from which point they are broken down in a manner identical to glucose.*

A. *Fructose*

Fructose is a major fuel source in diets that contain large amounts of sucrose (a disaccharide of fructose and glucose; Fig. 11-13). There are two pathways for the metabolism of fructose; one occurs in muscle and the other occurs in liver. This dichotomy results from the different enzymes present in these various tissues.

Fructose metabolism in muscle differs little from that of glucose. Hexokinase (Section 17-2A), which converts glucose to G6P on entry into muscle cells, also phosphorylates fructose, yielding F6P (Fig. 17-35, *left*). The entry of fructose into glycolysis therefore involves only one reaction step.

Liver contains little hexokinase; rather, it contains glucokinase, which phosphorylates only glucose (Section 17-2A). Fructose metabolism in liver must therefore differ from that in muscle. In fact, liver converts fructose to glycolytic intermediates through a pathway that involves seven enzymes (Fig. 17-35, *right*):

1. Fructokinase catalyzes the phosphorylation of fructose by ATP at C1 to form **fructose-1-phosphate.** *Neither hexokinase nor phosphofructokinase can phosphorylate fructose-1-phosphate at C6 to form the glycolytic intermediate fructose-1,6-bisphosphate.*

2. Class I aldolase (Section 17-2Da) has several isoenzyme forms. Muscle contains Type A aldolase, which is specific for fructose-1,6-bisphosphate. Liver, however, contains Type B aldolase, which also utilizes fructose-1-phosphate as a substrate (Type B aldolase is also called **fructose-1-phosphate aldolase**). In liver, fructose-1-phosphate therefore undergoes an aldol cleavage (Section 17-2D):

Fructose-1-phosphate $\rightleftharpoons$
dihydroxyacetone phosphate + glyceraldehyde

3. Direct phosphorylation of glyceraldehyde by ATP through the action of **glyceraldehyde kinase** forms the glycolytic intermediate glyceraldehyde-3-phosphate.

4–7. Alternatively, glyceraldehyde is converted to the glycolytic intermediate dihydroxyacetone phosphate by reduction to glycerol by NADH as catalyzed by alcohol dehydrogenase (Reaction 4), phosphorylation to glycerol-3-phosphate by ATP through the action of **glycerol kinase** (Reaction 5), and reoxidation by NAD^+ to dihydroxyacetone phosphate as mediated by glycerol phosphate dehydrogenase (Reaction 6). The DHAP is then converted to GAP by triose phosphate isomerase (Reaction 7).

As this complex series of reactions suggests, the liver has an enormous repertory of enzymes. This is because the liver is involved in the breakdown of a great variety of metabolites. Efficiency in metabolic processing dictates that many of these substances be converted to glycolytic intermediates. The liver, in fact, contains many of the enzymes necessary to do so.

a. Excessive Fructose Depletes Liver P_i

At one time, fructose was thought to have advantages over glucose for intravenous feeding. The liver, however, encounters metabolic problems when the blood concentration of this sugar is too high (higher than can be attained by simply eating fructose-containing foods). When the fructose concentration is high, fructose-1-phosphate may be produced faster than Type B aldolase can cleave it. Intravenous feeding of large amounts of fructose may therefore result in high enough fructose-1-phosphate accumulation to severely deplete the liver's store of P_i. Under these conditions, [ATP] drops, thereby activating glycolysis and lactate production. The lactate concentration in the blood and the consequent low pH under such conditions can reach life-threatening levels.

Fructose intolerance, a genetic disease in which ingestion of fructose causes the same fructose-1-phosphate accumulation as with its intravenous feeding, results from a deficiency of Type B aldolase. This condition appears to be