Kinetic structure of a biochemical pathway

Many enzymes in the cell are organised into sequences, so that the reactions they catalyse are integrated into pathways or processes. In these pathways, a precursor or substrate is converted to a product, e.g. glucose is converted to lactic acid; amino acids are polymerised to form protein; glutamine is converted to aspartate. These pathways have both a thermodynamic and a kinetic structure. The thermodynamic structure is presented in Chapter 2. The kinetic structure is described here. There are three basic facts that must be appreciated before the kinetic structure is explained.

- Reactions in a pathway can be divided into two classes: those that are very close to equilibrium (near-equilibrium) and those that are far removed from equilibrium (non-equilibrium). This is discussed in Chapter 2 but is summarised here using kinetic principles to explain how enzyme catalysis can give rise to two separate types of reaction in one pathway.
- One of the enzymes that catalyses a non-equilibrium reaction approaches saturation with substrate, so that it is the flux-generating step, (i.e. the beginning of the pathway).
- The kinetic and thermodynamic structure of a pathway or process in a cell or a tissue can only be maintained because living systems are open: that is, they exchange matter and energy with the environment (Chapter 2).

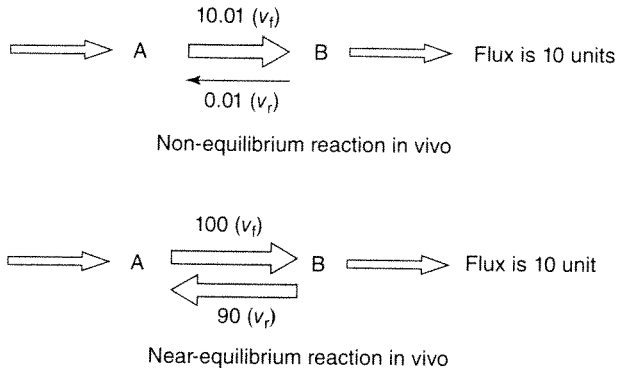


Figure 3.27 *Representation of the rates of the forward and reverse reactions for non- and near-equilibrium reactions in one reaction in a hypothetical pathway.* The values represent actual rates, not rate constants. The net flux through the pathway is given by (V_f-V_r). In the non-equilibrium reaction, the rate of the forward reaction dominates, so that the net flux is almost identical to this rate. In the near-equilibrium reaction, both forward and reverse rates are almost identical but considerably in excess of the flux.

Equilibrium and non-equilibrium reactions: a kinetic explanation

A reaction in a metabolic pathway is likely to be non-equilibrium if the maximum catalytic activity of the enzyme that catalyses the reaction is low in comparison with those of other enzymes in the pathway. In consequence, the concentration of substrate of this reaction is likely to be high whereas that of the product is likely to be low, since the next enzyme in the sequence readily catalyses its removal. Because the concentration of this product is low, the rate of the reverse component of the reaction is very much less than the rate of the forward component. This situation characterises a non-equilibrium process. Conversely, a reaction is near-equilibrium if the maximum catalytic activity of the enzyme is high in relation to those of other enzymes in the pathway; in this case, the rates of the forward and the reverse components of the reaction are similar and both are much greater than the overall flux through the pathway. A quantitative explanation should help to clarify and is presented in Figure 3.27. From this explanation, it should be clear why the terms *reversible* and *irreversible* are sometimes used in place of *equilibrium* and *non-equilibrium*. The latter terminology is used in this text. The concept of the flux-generating reaction is now discussed.

Flux-generating reactions

Consider an enzyme at the beginning of a pathway whose pathway-substrate concentration is much less than that required to saturate the enzyme (see Figure 3.7), e.g. similar to or lower than that of the K_m. As the catalysis proceeds, the concentration of substrate falls so that the activity of the enzyme decreases more and more. Consequently, the activity of such an enzyme cannot maintain a constant flux through a pathway, so that a steady state cannot be achieved.

In contrast, if the enzyme is saturated with its pathway-substrate, (i.e. zero order) a decrease in the concentration will not decrease its activity, so that it could generate a constant flux through the reaction and hence through the pathway. It is, therefore, an enzyme that can generate a constant flux: a non-equilibrium reaction that is saturated with pathway-substrate is termed a *flux-generating reaction*. Examples of some flux-generating reactions are given in Figure 3.19. The physiological significance of

such enzymes is discussed for specific pathways in later chapters. The kinetic significance in establishing a pathway and defining how the flux is transmitted through a pathway is now discussed.

Transmission of flux: the kinetic structure of the pathway

If a biochemical pathway possesses a flux-generating reaction and, by definition, it should, it follows that the flux through all reactions in the pathway must conform to that of the flux-generating step. To see how this works, consider an increase in the activity of enzyme E_1, the flux-generating reaction of the hypothetical pathway depicted in Figure 3.28. The immediate consequence will be a rise in the concentration of A, which will increase the rate of E_2, since it is not saturated with substrate. This will raise the concentration of B, and hence the activity of E_3, and so on along the pathway, so that the rates of all the reactions will increase in parallel with the rate of the reaction catalysed by E_1 and, in time, a new steady-state will be established. This mechanism of regulation of the activities of E_2 and E_3 etc. by changes in the concentrations of their substrates can be described as *internal regulation*, i.e. regulation is internal to the pathway. The activity of E_1 results, therefore, in a steady-state flux through the whole pathway which is described as a *transmission sequence*.

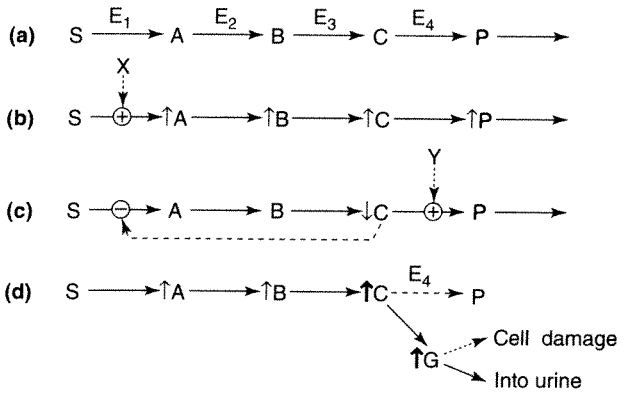


Figure 3.28 *A hypothetical pathway and modes of regulation*
(a) The hypothetical pathway in which E_1 is the flux-generating step.
(b) Factor X activates E_1, which results in increased concentrations of all the intermediates in the pathway (i.e. the transmission sequence).
(c) Factor Y activates E_4, concentration of C decreases, which stimulates E_1 (because it is an inhibitor).
(d) Enzyme E_4 is absent, so that C accumulates and it is then converted to G by a side reaction and G is excreted in urine, and can cause damage to the all or other tissues.

Regulation of flux through a pathway

If a compound (e.g. X, an allosteric activator) increases the activity of E_1, the flux through the transmission sequence will increase. This type of regulation is termed *external regulation* (i.e. it is achieved by a factor external to the pathway). However, a change in the activity of any other enzyme would not change the flux. For example, a change in flux through the pathway would not occur if only the activity of E_4 increased: it would result only in a decrease in the concentration of C, until the activity of E_4 decreased to its previous value. However, if E_4 communicated with E_1, such a change could modify the flux. Appropriate communication could come about if compound C is an allosteric inhibitor of E_1. Thus an increase in activity of E_4, via an effect of an allosteric regulator, would lower the concentration of C, which would then increase the activity of E_1, so that the flux through the transmission sequence would increase (Figure 3.28(c)). Such inhibition, from a final product or a precursor of the product of the pathway is common in the control of biochemical pathways, and it is known as *feedback inhibition* (see Figure 3.13).

From this discussion it should be clear that if the activity of any enzyme in the sequence was decreased, for any reason, to such an extent that its maximum activity fell below that of E_1, the concentration of its substrate would rise sufficiently to saturate the enzyme, when the activity could increase no longer. If there were no feedback regulation, i.e. no control-structure to the pathway, this increase would continue until the substrate was removed by a side reaction or escaped from the cell to be modified and excreted in the urine. Such a loss of enzyme activity is exceedingly rare. When it occurs, it is usually due to a genetic deficiency; that is, the enzyme is inactive due to an 'inborn error' or an *enzyme deficiency disease*. Since the enzyme is not part of a coordinated regulatory mechanism, nothing prevents the substrate concentration from increasing excessively, even to pathological levels (Figure 3.28(d)).

Well-known examples of enzyme deficiency diseases include:

- Phenylalanine hydroxylase deficiency, giving rise to phenylketonuria (PKU) (Box 3.9).
- Pyruvate dehydrogenase deficiency giving rise to lactic acidosis (Chapter 9).
- Glycogen phosphorylase deficiency in muscle gives rise to muscle weakness, frequent cramp and ease of fatigue (McArdle's syndrome). It also gives rise to hypoglycaemia if the liver enzyme is deficient (Chapter 6).

Box 3.9 Phenylketonuria

Phenylketonuria (PKU) is a group of inherited disorders caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH) that catalyses the conversion of phenylalanine to tyrosine, the first step in the pathway for catabolism of this amino acid. As a result, the concentration of phenylalanine in the liver and the blood increases. This high concentration in the liver increases the rate of a side reaction in which phenylalanine is converted to phenylpyruvic acid and phenylethylamine, which accumulate in the blood and are excreted in the urine.

The disease develops at 3 to 6 months of age and it is characterised by developmental delay, eczema, hyperactivity and mental retardation. Newborn babies are routinely screened for PKU in many countries. Treatment is a phenylalanine-restricted diet and supplementation with tyrosine.

Diet → phenylalanine → tyrosine →
↓
phenylpyruvate

Regulation of enzyme activity

Investigating the regulation of enzyme activity requires identification of the external regulators and how regulation of the enzyme activity affects the flux through a pathway. There are four important questions that must be answered before mechanisms of regulation can be usefully discussed. These are:

(i) Which enzyme(s) in a pathway is subject to external regulation?

(ii) What is the biochemical mechanism by which the enzyme activity is regulated by an external regulator?

(iii) How does regulation of the activity of an enzyme regulate the flux through a pathway?

(iv) What is meant by sensitivity in regulation?

These questions are considered below.

(i) The enzyme that catalyses the flux-generating step must be regulated to change the flux through the pathway. Enzymes that catalyse non-equilibrium reactions are more likely to be regulated by external factors than those that catalyse near-equilibrium reactions, so it is these enzymes that are studied in (ii).

(ii) The biochemical mechanisms by which enzyme activity is regulated are suggested by studying the properties of the enzyme in vitro. The proposed mechanism must then be investigated in vivo. (This approach is used to establish a mechanism for different pathways or processes, in many chapters in this book.)

(iii) Once the mechanism(s) has been identified, the means by which it can change the flux requires information on how each enzyme is involved in the transmission sequence.

(iv) In many biochemical or physiological processes, a weak stimulus produces a large response. For example, the increase in flux through glycolysis in the leg muscle of a sprinter leaving the blocks, to achieve a maximum power output, is approximately 1000-fold. Yet the factors that regulate glycolysis change nothing like 1000-fold. Similarly, the increase in the Krebs cycle from rest to maximum aerobic physical activity in muscle is approximately 50-fold. To understand how such marked changes in activity can be produced by small changes in the concentration of a regulator, the concept of sensitivity in regulation must be addressed.

Sensitivity in regulation is defined as the quantitative relationship between the relative change in enzyme activity and the relative change in concentration of the regulator. For example, if an enzyme activity needs to increase 100-fold to produce the necessary change in flux through the pathway, how large an increase in concentration of regulator is required? The greater the change in response of enzyme activity to a given change in regulator concentration, the greater is the sensitivity. This is defined mathematically as follows.

The concentration of a regulator (x) changes by Δx, so that the relative change in concentration is $\Delta x/x$. This results in a change in flux, J, by ΔJ, so that the relative change in flux is $\Delta J/J$. The sensitivity of the flux to the change in concentration of x is given by the ratio $\Delta J/J$ to $\Delta x/x$, i.e. $S = \dfrac{\Delta J/J}{\Delta x/x}$ where S is the sensitivity.

The next quantitative problem is to understand the basic mechanism of interaction between the regulator (in this case x) and its binding to the target enzyme (i.e. the enzyme that regulates the flux through the pathway).

Equilibrium-binding of a regulator to an enzyme

To modify the activity of an enzyme, or any protein, the regulator must bind to the protein and, in almost all cases, the binding is reversible. Such binding is described as equilibrium-binding.

$$E + X \rightleftarrows E*X$$

where E is the enzyme, X is the regulator and E* is the altered form of the enzyme. The asterisk indicates that the

binding of X has changed the conformation of the enzyme so that the structure of the catalytic site has changed to increase or decrease the catalytic activity.

The normal response of enzyme activity to the binding of the regulator (or the binding of the substrate) is hyperbolic, as described above. Unfortunately, this response is relatively inefficient for sensitivity in regulation of the activity of the enzyme. The maximum sensitivity, as defined quantitatively above, is unity. This is the part of the response that is first order (see Figure 3.7). For example, a twofold change in regulator concentration will change the enzyme activity by no more than twofold (i.e. the value of S, in the above equation, is unity). This interpretation may be difficult to accept from simply viewing the initial part of a hyperbolic curve. However, it must be appreciated that sensitivity is *not* the slope of the plot of activity versus concentration of substrate or regulator; it is the relationship between the *relative* change in activity and the *relative* change in concentration of the regulator.

Since the hyperbolic response is the simplest relationship between protein and regulator, it can be considered as the basic response with which any mechanism for improving sensitivity can be compared. Four such mechanisms are now examined.

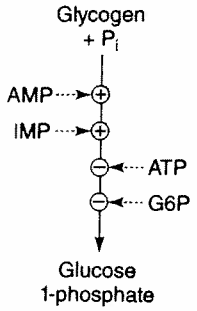


Figure 3.29 *Control of an enzyme activity by multiple allosteric regulators.* The enzyme glycogen phosphorylase *b* in muscle is regulated by changes in the concentrations of AMP and inosine monophosphate (IMP) (which are activators) and ATP and glucose 6-phosphate (G6P), which are inhibitors.

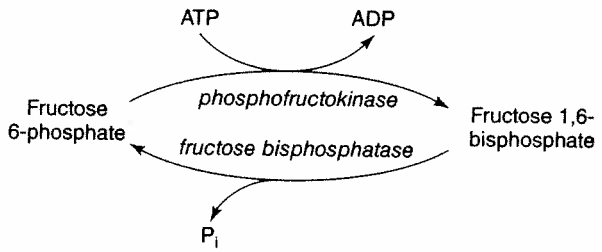


Figure 3.30 *The fructose 6-phosphate/fructose 1,6-bisphosphate cycle.* The forward reaction is catalysed by the enzyme phosphofructokinase, the reverse reaction by fructose bisphosphatase.

Mechanisms for improving sensitivity

Multiplicity of regulators

It is possible for an enzyme to be regulated by several different external regulators that all bind at different allosteric sites on the enzyme. In this case, if the concentrations of all the regulators change in directions to change the activity of the enzyme in the same direction, the effect of all external regulators could be cumulative (Figure 3.29).

Cooperativity

For many enzymes that play a role in regulation, the response of their activity to the substrate or regulator concentration is sigmoid, not hyperbolic. This phenomenon is known as *cooperativity* (see above). For part of the concentration range of the substrate or regulator, the effect on the enzyme activity is greater than that provided by the hyberbolic response, i.e. the sensitivity is greater than unity (see Figure 3.15(b)).

Substrate cycles

A totally different mechanism for improving sensitivity is known as the substrate cycle. It is possible for a reaction that is non-equilibrium in the forward direction of a pathway (i.e. A→B, see below) to be opposed by a reaction that is non-equilibrium in the reverse direction of the pathway (i.e. B→A). For example,

$$S \xrightarrow{E_1} A \underset{E_5}{\overset{E_2}{\rightleftharpoons}} B \xrightarrow{E_3} C \xrightarrow{E_4} P$$

The substrate cycle between A and B, is catalysed by enzymes E_2 and E_5 in the pathway S → P. The reactions must be chemically distinct and non-equilibrium and catalysed by different enzymes (i.e. E_2 and E_5, above). It is possible that these two opposing reactions are components of two separate pathways that function under different conditions (e.g. glycolysis and gluconeogenesis in the liver – see Chapter 6) but the reverse reaction (E_5 in the above example) may not be part of any other pathway but only present in the cell to provide a cycle for regulation of flux, through that reaction; that is, for improving sensitivity in regulation. An example is the fructose 6-phosphate/fructose bisphosphate cycle in muscle (Figure 3.30).

If the two enzymes are simultaneously active, A will be converted to B and the latter will be converted back to A, thus constituting the cycle. There are, thus, two fluxes: a

linear flux through the cycle, A to B, as part of the pathway by which S is converted to P, and a cyclical flux between A and B. Both fluxes are to a large extent independent and calculations show that the improvement in sensitivity is greatest when the cyclical flux is high but the linear flux is low, i.e. the ratio, cycling rate/flux, is high (Table 3.4, Figure 3.31).

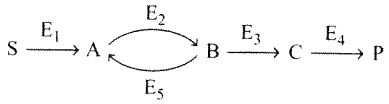
In some conditions, to achieve satisfactory regulation of flux, an enzyme activity may have to be reduced to values approaching zero. Even with a sigmoid response, this would require that the concentration of an activator be reduced to almost zero or that of an inhibitor be increased to an almost infinite level. Such enormous changes in concentration never occur in living organisms, because they would cause osmotic and ionic problems and unwanted side reactions; that is, they are physiologically unacceptable. However, the net flux through a reaction can be reduced to very low values (approaching zero) via a substrate cycle (Figure 3.32).

It is possible that, via a cycle, the direction of a flux can be completely reversed. An example is glucose metabolism in the liver: at a low blood glucose level the liver releases glucose, whereas at a high concentration of blood glucose the liver takes up glucose. This is the result of a substrate cycle between glucose and glucose 6-phosphate in the liver (Figure 3.32) (discussed in detail in Chapter 6).

Since the net result of a cycle, in addition to an increase in sensitivity, is the hydrolysis of ATP, it is unlikely that high rates of cycling will be maintained for any prolonged periods of time. One means of providing high sensitivity, but low rates of cycling transiently, is to increase the rate of cycling only when increased sensitivity. Chronically, is required. For example, a stressful condition increases the release of the stress hormones, adrenaline and noradrenaline. These hormones could increase the activity of both enzymes, i.e. those that catalyse the forward and reverse reactions in the cycle (e.g. by a change in a specific messenger, e.g. cyclic AMF). The role of these hormones is to prepare the body for 'fight or flight', i.e. increased physical activity. An increase in the rate of cycling and hence an increase in sensitivity in preparation for increased ATP generation, would be an advantage if fight or flight had to take place (Figure 3.31).

In some circumstances, substrate cycles may operate not only to regulate flux through biochemical pathways but to achieve the controlled conversion of chemical energy (i.e. ATP) into heat. This occurs in two conditions.

Table 3.4 Effect of an increase in the concentration of a regulator on net flux through a reaction that is regulated by a direct effect of the regulator on the activity of an enzyme. The hypothetical pathway is



The quantitive effect is examined when there is no substrate cycle and when there is substrate cycle.

Concentration of regulator (x)	Enzyme activities[a] (units/min)		Net flux A to B (J)	Relative fold increase in flux	Sensitivity (S)
	E_2	E_5			
No cycling (i.e. enzyme E_5 is inactive)					
Basal	10	zero	10		
Fourfold above basal	40	zero	40	4	1.0
Cycling (i.e. enzymes E_2 and E_5 are active)					
Basal	10	9.8	0.2		
Fourfold above basal	40	1.0	39	195	approx. 50.0

[a]The units are arbitrary.

E represents the enzymes catalysing the reactions in the pathway. Simultaneous activities of E_2 and E_5 produce a substrate cycle between A and B.

In the *no cycling* condition, enzyme E_5 is absent (or inactive).

In the *cycling* condition, the regulator not only increases the activity of E_2 but decreases that of E_5. However, the improvement in the relative increase is not much greater if E_5 activity does not change. The relative change in the concentration of regulation (i.e $\Delta x/x$) is 4.0 in both conditions; in the *no cycling* condition $\Delta J/J$ (the relative change in flux) is 4.0 but it is approx. 200 in the *cycling* condition. Consequently the values for $\Delta J/J/\Delta x/x$ (i.e. sensitivity, S) are unity and about 50, in the no cycling and cycling contitions, respectively.

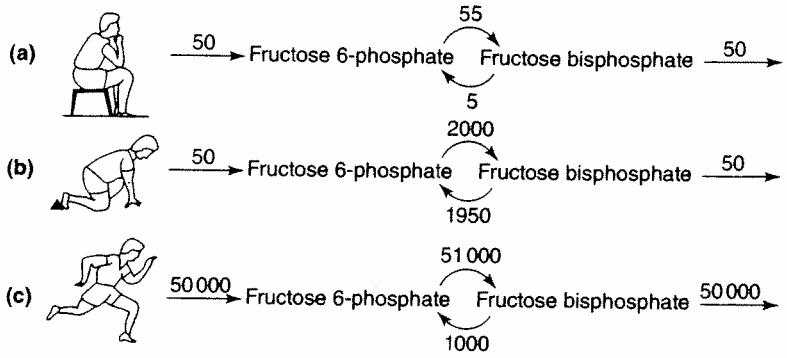


Figure 3.31 *Representation of the role of a substrate cycle improving the sensitivity of the regulation of the flux through the reaction in which fructose 6-phosphate is converted to fructose bisphosphate in muscle during sprinting.* The upper arrow represents phosphofructokinase activity and the lower arrow represents fructose bisphosphatase activity. **(a)** Resting before the sprint, when cycling rate is low, and flux is low; **(b)** on the starting block, when stress hormones increase the cycling rate markedly (i.e. 'preparation for flight or fight'); **(c)** about six seconds after the start of the sprint, when allosteric regulators have increased the activity of phosphofructokinase and decreased that of fructose bisphosphatase. The enzyme activities represent (a) the relaxed sprinter, (b) the stressed sprinter immediately before the race and (c) the sprinter at maximum speed. The activities are hypothetical. From this it can be seen that a 25-fold increase in activity of phosphofructokinase and a 50% decrease in that of fructose bisphosphatase, both caused by changes in allosteric regulator concentrations, at the beginning of the sprint, increase the glycolytic flux 1000-fold: a well-established biochemical fact. Indeed, this increase in sensitivity must be required in most sporting activities (Chapter 13). It must be noted that the activity of glycogen phosphorylase must also increase by a thousand fold. This is achieved by an interconvention cycle.

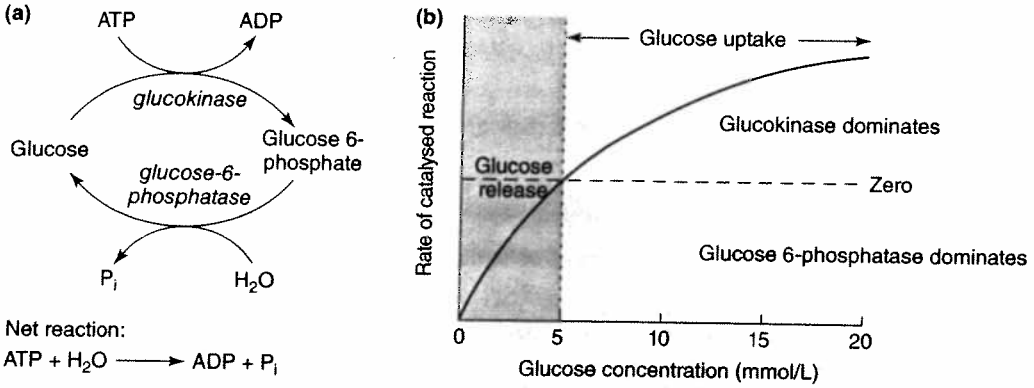


Figure 3.32 *Generation of a threshold response of a reaction in a pathway to a change in regulator concentration. The example is that is provided by the glucose/glucose-6-phosphate cycle.* **(a)** The glucose/glucose-6-phosphate cycle. **(b)** The net reaction is achieved by subtracting the activity of the enzyme that catalyses the reverse reaction from that of the enzyme catalysing the forward reaction. A threshold (vertical dotted line) is achieved since there is zero net flux, when both activities are identical. The example given is the cycle between glucokinase and glucose 6-phosphatase in liver. When glucose 6-phosphatase activity exceeds glucokinase activity, glucose is released from the liver; when glucokinase activity exceeds glucose 6-phosphatase activity, glucose is taken up from the blood. This remarkable effect is achieved solely by changing the activity of glucokinase, but not that of glucose 6-phosphatase. It depends solely on changes in the concentration of glucose, the substrate for glucokinase (see above and Chapter 6).

- To produce heat so as to maintain body temperature (known as non-shivering thermogenesis) (Chapter 9).
- To reduce body mass by 'burning off' stored fuel. This is put forward as one mechanism by which the amount of triacylglycerol stored in adipose tissue can be reduced (Chapters 7, 12 and 15).

Interconversion cycles

The topic of interconversion cycles in providing inhibition or activation of a target enzyme, the activity of which regulates the flux through a pathway, is discussed above. In brief, an enzyme exists in two forms, conventionally designated *a* and *b*, one being a covalent modification of the other. This is brought about, for example, by phosphorylation with ATP, so that one form is a phosphorylated modification of the other. Since only one of the two forms, *a*, has significant catalytic activity, the flux can be regulated by altering the amount of the target enzyme in this form (Box 3.7). The basis for improving sensitivity by this mechanism is discussed above and indicated in Figure 3.12. It is also discussed in Chapter 20 where enzyme interconversion cycles are compared with control of the concentration of a regulatory protein by protein synthesis and protein degradation.

It is important to point out that these four mechanisms are not mutually exclusive. Indeed, it is probable that, for some reactions, all four mechanisms play a role in regulation of flux and this combination could provide an enormous increase in sensitivity. An example is the regulation of the enzyme phosphorylase in muscle and liver, and hence the process of glycogenolysis (Chapters 6 and 12).