Enzymatic Activity: Control

Introductory article

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Enzymes are the metabolic catalysts that effect a multitude of physiological processes and responses. Tight control of enzyme activity is therefore essential in maintaining the steady state of all organisms.

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Introduction

Tight control of enzyme activity is essential in maintaining the steady state of all organisms. Depending upon the metabolic state or needs of a cell, enzymatic activities can be either upregulated or downregulated. At any given time, not all of the body's metabolic pathways are operating at full capacity. Instead, these systems must be coordinated to prevent either uncontrolled growth or catabolism of the body. If left uncontrolled, opposing metabolic pathways (e.g. glycogen synthesis vs glycogen breakdown; glycolysis vs gluconeogenesis) would work in conflict. Instead, the body works to regulate efficiently these metabolic processes. This is accomplished by controlling the activity of the enzymes, or metabolic catalysts, within the cell. Regulation of gene transcription, protein phosphorylation, proteolytic destruction and allosteric effectors are examples of ways enzyme activity can be modulated. The disruption of proper enzymatic control can adversely affect the body's natural homeostatic or steady state and eventually lead to severe pathological conditions.

Concentrations of Substrate and Product Affect Enzymatic Activity

Enzymes are highly specific catalysts. Thus, an enzyme will only react with selected substrates, and only if the substrate molecule is correctly oriented. An important consideration in enzymatic activity is substrate concentration. The more substrate available, the more likely it is that an enzyme will come into close proximity with a substrate molecule to produce a productive interaction. Enzyme activity is therefore dependent on the concentration of the substrate *relative* to the efficacy/affinity of the enzyme for the substrate (Michaelis constant; K_m).

Multiple enzymes are frequently required for end-product synthesis (see **Figure 1**). Typically, only one or two enzymatic steps will be closely regulated. These control points of a metabolic pathway generally occur early in a reaction scheme, and are often the rate-limiting steps. The rate-limiting enzyme or step in a reaction determines the amount of end-product to be formed. Regulation of the rate-limiting enzyme occurs in order to conserve energy and metabolites, some of which may be toxic in higher quantities. How then are these key enzymes regulated?

Feedback inhibition allows enzymes to be directly sensitive to the presence of reaction products. An excellent example is hexokinase. This enzyme phosphorylates glucose to form glucose 6-phosphate in the glycolysis pathway. If the glucose 6-phosphate is not further utilized, however, it will bind to hexokinase to inhibit its activity. The inhibition of enzymatic activity by the end product of the reaction is referred to as negative feedback control (see **Figure 1**). By inhibiting enzyme 'a', the unnecessary utilization of substrate 'A' as well as the accumulation of the final product 'E' is prevented.

Frequently, feedback inhibition occurs through a steric mechanism whereby the product occupies a portion of the active site of the enzyme, thus preventing substrate binding. In this way, the regulation is the product of simple mass action relationships. That is, if the product accumulates, the enzyme itself senses this fact and exhibits reduced activity.

Allosteric Effectors

Allosteric control is another mechanism of regulation. In this case, compounds called effectors modulate activity by binding noncovalently to the enzyme in a location distinct from the active site. Allosteric enzymes are typically multisubunit (oligomeric) proteins with more than one active site. The effectors act by altering the affinity of the

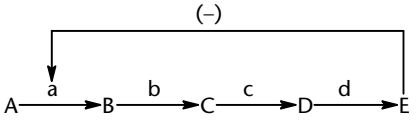

Figure 1 Feedback inhibition. As concentrations of product E builds up, they act to inhibit/regulate the catalytic activity of enzyme 'a'.

enzyme for its substrate and/or changing its catalytic activity. The altered catalytic activity is the result of a change in the tertiary and quaternary structure of the allosteric protein upon effector binding. Rate-limiting enzymes are frequently subject to allosteric regulation. One of the most well-understood examples is aspartate transcarbamoylase (ATCase, EC 2.1.3.2) from the bacterium *Escherichia coli*. ATCase presents an example of a heterotropic allosteric interaction because the effect of one ligand has a dramatic effect on the binding of a different ligand.

ATCase is necessary for the synthesis of the pyrimidines (cytosine, thymine and uracil). Cytosine and thymine hydrogen-bond with guanine and adenosine, respectively, during DNA replication. The biosynthesis of the pyrimidines begins with the formation of *N*-carbamoylaspartate from the amino acid aspartate and carbamoyl phosphate. ATCase catalyses this reaction and this step is rate limiting for the entire pathway. Therefore, as the needs for pyrimidine and DNA biosynthesis change, ATCase is allosterically regulated accordingly to meet the requirements.

An allosteric protein like ATCase has two distinct small-molecule-binding sites. The substrates (aspartate and carbamoyl phosphate) bind in the active site within the catalytic domain, while effector molecules (adenosine triphosphate or cytidine triphosphate) bind elsewhere within the regulatory domain. Adenosine triphosphate (ATP) serves to activate ATCase and is therefore referred to as a positive effector. In contrast, cytidine triphosphate (CTP) acts as a feedback inhibitor of ATCase. High levels of CTP compete directly with ATP for access to a site within the regulatory domain and cause a decrease in pyrimidine production. **Figure 2** depicts the sigmoid kinetics observed for ATCase when either ATP or CTP is bound. When ATP is bound, ATCase more efficiently utilizes aspartate. That is, the concentration of substrate [S] needed to produce the same reaction velocity (V_0) as ATCase in the absence of CTP is decreased. This is indicated by the shift to the left in the kinetic profile.

As stated, subtle changes in either substrate availability or end product formation can trigger conformational changes in the enzyme structure that are necessary for observation of the kinetic differences shown in **Figure 2**. ATCase fluctuates between two different conformational states. When the CTP is bound, ATCase is maintained in a weak binding state (T state) for its substrate. The T states of allosteric proteins have high K_m values for their respective substrates. A weak binding state makes it more difficult for the pyrimidines to be synthesized and CTP is therefore referred to as a negative effector. Likewise, ATP is a positive effector because its binding to ATCase increases the enzymatic utilization of the substrate aspartate.

As discussed earlier, ATCase is a typical heterotropic allosteric enzyme. Homotropic allosteric interactions occur when substrate binds to one subunit of the allosteric protein. The substrate then alters the binding of an identical substrate molecule to another subunit. Homotropic interactions are typically positive. The binding of one ligand increases the ability of another to bind.

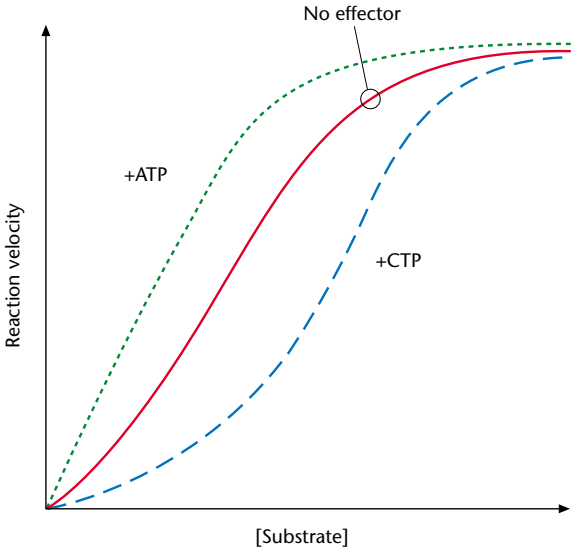

Figure 2 The kinetic profile for the allosteric protein ATCase. Shown is the sigmoid reaction velocity versus substrate concentration plots for ATCase when no effector is bound (solid line), when the positive effector ATP is bound at the allosteric site (dotted line) and when the negative effector CTP is bound to the allosteric site (dashed line). ATP serves as a positive effector to shift the curve to the left, meaning that lower substrate concentrations of aspartate and carbamoyl phosphate are required to achieve the same reaction velocity. CTP is a negative effector in this reaction, showing that higher substrate concentrations are required to achieve the same reaction velocities observed when ATP, or no effector, is bound.

The tetrameric haemoglobin protein necessary for oxygen transport in blood exhibits a classic homotropic cooperative interaction. Haemoglobin is composed of four identical subunits, each with a binding/active site for oxygen. The binding of oxygen to the active site of one subunit causes a conformational change of the subunit to which it bound. The change in this subunit is translated to other subunits in the protein. The changes make it more conducive for oxygen to bind to the other three subunits of haemoglobin and thus create a more efficient enzyme. The binding of both hydrogen ions (H^+) and carbon dioxide (CO_2) also regulates the activity of haemoglobin; these molecules exert a negative effect on the oxygen carrying capacity of haemoglobin.

Reversible Covalent Modification

Feedback inhibition and allosteric control are utilized by a cell or organism for short-term (seconds) metabolic regulation. Sometimes more prolonged (minutes) direct modulation of enzyme activity may be required. Finally,

under conditions of long-term control, regulation of the transcription of the source gene may occur over the time frame of hours or days. The short-term (seconds) level of regulation is frequently accomplished through the covalent modification of an enzyme. The most common type of covalent modification is phosphorylation. The sites for this modification are the side-chains of specific serine, threonine and tyrosine residues within a given protein. Unlike the proteolytic alteration of an enzyme to be discussed later, the process of phosphorylation is reversible. Other reversible covalent modifications occur: for example, adenylation/deadenylation, methylation/demethylation and acetylation/deacetylation. However, for the present discussion, we will focus our attention on the phosphorylation/dephosphorylation modifications as examples.

The addition and the subsequent removal of the phosphate group are distinct reactions. ATP usually serves as the donor of the phosphoryl ($-PO_3$) group. The terminal (gamma) phosphoryl group of ATP is transferred to the specific amino acid residues mentioned above. The transfer is mediated by a group of enzymes known as protein kinases. One class of protein kinase is responsible for the phosphorylation of particular serine and threonine residues, while another class of kinase (protein tyrosine kinases) phosphorylates specific tyrosine residues. Dephosphorylation of the amino acid residues that are covalently bound to a phosphoryl group is accomplished by a group of enzymes called phosphatases (see **Figure 3**). Proteins that exist extracellularly are not generally subjected to reversible phosphorylation because of the lack of ATP.

An example of reversible covalent modification is provided by the enzyme tyrosine hydroxylase (TH, EC 1.14.16.2). TH is the rate-limiting enzyme in the biosynthesis of catecholamine neurotransmitters (dopamine, noradrenaline and adrenaline). This enzyme is subject to nearly all versions of enzyme regulation. For example, it has been demonstrated that Ser40 is phosphorylated by protein kinase A (PKA). This leads to an increase in the enzymatic activity of the enzyme. It is important to mention that not all serine and threonine residues in TH are phosphorylated by PKA. PKA only phosphorylates serines and threonines that are contained within the consensus amino acid motif Arg-Arg-X-Ser (or Thr)-Z, where X is any small amino acid residue and Z is a hydrophobic residue.

The process of phosphorylation-dependent activation begins with the binding of an external signal molecule, such as a hormone or a neurotransmitter, to a membrane-bound receptor. This binding activates the enzyme adenylate cyclase, which is localized on the intracellular surface of the plasma membrane. Adenylate cyclase converts ATP to cyclic AMP (cAMP). Two cAMP molecules then bind to each of the two regulatory subunits of PKA, causing them to dissociate from the catalytic domains of PKA. This process releases the two catalytic domains of PKA from the inhibitory control of the regulatory subunits and results in a catalytically active kinase that can mediate the phosphorylation of TH at Ser40. As mentioned earlier, protein phosphatases reverse protein activation by hydrolysing the phosphoryl group at Ser40. Removal of the phosphoryl group restores the hydroxyl group and produces a reduction of TH enzymatic activity. In the case of TH, a specific enzyme termed protein phosphatase 2A dephosphorylates Ser40. The covalent modification of enzymes is different from many other mechanisms for enzyme regulation because it permits metabolic pathways to be controlled by compounds that are structurally unrelated to the metabolic byproducts.

Stimulation and Inhibition by Control Proteins

To this point, our characterization of enzyme regulation has focused primarily on the enzyme itself. That is, the

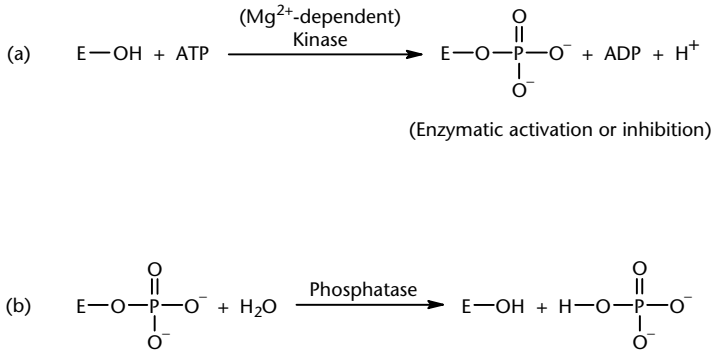

Figure 3 (a) The reversible phosphorylation of an enzyme, leading to enzymatic activation or inhibition. Specific serine, threonine and tyrosine residues in an enzyme (E) covalently accept a phosphoryl group from donor ATP. A group of enzymes known as kinases mediate phosphorylation. This mechanism of regulation is reversible. (b) Cleavage of the amino acid phosphoryl group is performed by a group of proteins known as phosphatases.

interaction of enzyme with substrates and feedback inhibitors and covalent posttranslational modifications. The availability and/or activities of control proteins can also directly modulate enzyme activity. One of the key examples of this is the Ca^{2+}-activated protein calmodulin. This small (12 000 Da) protein is a high-affinity chelator of Ca^{2+}. Upon binding Ca^{2+}, however, it undergoes conformational changes that allow it to bind to other proteins. In this way, calmodulin is a specific control protein that is sensitive to the intracellular levels of calcium.

Among the many targets of the Ca^{2+}/calmodulin control is the calcium/calmodulin-dependent protein kinase (CaMPK). This serine/threonine kinase is activated by calmodulin, which, in turn, is activated by calcium. Calcium is an intracellular second messenger released in response to extracellular signals. Interestingly, the tyrosine hydroxylase enzyme previously described to be phosphorylated and regulated by PKA (on Ser40) is also phosphorylated by CaMPK (on Ser19). The tandem Ca^{2+}/calmodulin control plays a regulatory role in a number of other physiological situations, including muscle contraction, glycogen breakdown and calcium pumping.

The protease inhibitors are another example of direct-control proteins. These proteins bind as false substrates to their cognate enzymes. While these protease inhibitors bind with high affinity, they are very poor substrates (low K_m, but also low k_{cat} values). These two types of control proteins (calmodulin and protease inhibitors) illustrate how seemingly unrelated proteins can have a dramatic effect on a given enzyme.

Proteolytic Activation

Proteolytic activation is an irreversible covalent modification used to regulate enzymatic activity. Unlike phosphorylation (reversible covalent modification), no energy, or ATP, is generally required for proteolytic activation. The relevance of this type of regulation is illustrated in cells that create proteolytic (digestive) enzymes. If the cells synthesized these enzymes in an active form, serious problems would arise, ultimately leading to the self-destruction of the cell. Proteolysis of the cell is not observed because the enzymes are produced in an inactive form known as a zymogen or proenzyme. These inactive precursors must be irreversibly modified to become active. Many molecules undergo this phenomenon including digestive enzymes, blood-clotting factors and hormones.

Zymogen activation occurs by means of proteolytic cleavage of a peptide bond. This may be a result of catalytic (e.g. enzymatic) or chemical conditions (e.g. pH). The resulting protein can then go on to autocatalyse its own activation, or contribute to the activation of other zymogens, resulting in a cascade of activation events. One of the best-studied systems that exhibit such behaviour is the formation of digestive enzymes by the pancreas.

The pancreas produces a variety of digestive enzymes as zymogens, which it then secretes into the duodenum of the small intestine via the pancreatic duct. These proenzymes include trypsinogen, chymotrypsinogen, proelastase and procarboxypeptidase. Proteolytic activation is achieved by the enzyme trypsin, whose actions result in the formation of the active forms of trypsin, chymotrypsin, elastase and carboxypeptidase from their respective zymogen precursors. How is this possible? First, active trypsin is formed by removal of the N-terminus by enteropeptidase, a protease made in the duodenum. Trypsin can then go on to autocatalyse its own activation by cleaving itself specifically or to activate the other zymogens. For instance, cleavage of chymotrypsinogen by trypsin, specifically at the peptide bond between Arg15 and Ile16 results in an active chymotrypsin molecule through the formation of a substrate-binding site. Cleavage of this peptide bond creates a positively charged amino acid at Ile16 that forms a salt bridge interaction with Asp194. This causes a conformational change in residues 193–195, thereby constructing the active site. Further autocatalytic cleavages occur that remove residues 14 and 15, and then 147 and 148. It should be noted that the initially cleaved amino terminal fragment is still associated with the active chymotrypsin by a disulfide bond between residues 1 and 122. Association of the cleaved fragment is not the case for all enzymes that undergo proteolytic activation. Other examples of zymogen activation are the blood-clotting cascade and the induction of programmed cell death (apoptosis) as mediated by the caspase proteases.

Proteolytic activation is an irreversible modification. For this reason, mechanisms must be available to turn off the activating enzyme. This is accomplished by irreversible binding of specific inhibitory control proteins known as protease inhibitors. These inhibitors bind tightly to enzymatic active sites forming stable, noncatalytic complexes, thereby disabling the enzymes (**Figure 4**). The production of these inhibitors is under distinct regulatory control (primarily at the level of gene expression).

It is noteworthy that, in most instances, proteolysis results in the destruction of an enzyme, not its activation. For this purpose, cells maintain a cohort of nonspecific proteases whose role is to degrade proteins. Examples include the digestive proteases trypsin and chymotrypsin. These enzymes exist for the sole purpose of degrading dietary protein. In a less global example, proteases exist for the purpose of regulating the turnover of enzymes (and their associated activities). One way this is accomplished is by first attaching a targeting protein called ubiquitin onto the enzyme/protein that is to be degraded. Ubiquitin then serves as a beacon for the 26S proteosome to breakdown the 'tagged' protein.

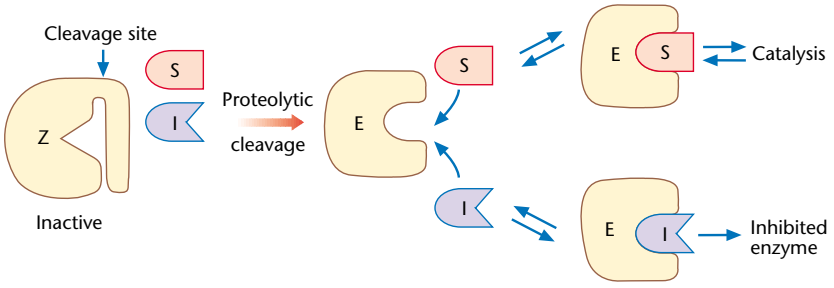

Figure 4 Upon proteolytic cleavage, the zymogen (Z) undergoes a conformational change resulting in an active enzyme (E) with its active site exposed. Substrate (S) can now access the catalytic core. Irreversible proteolytic inhibition may also occur owing to the binding of specific protease inhibitors (I).

Transcriptional Control of Enzyme Activity

In most cases, the modification of enzyme activity is accomplished via short-term regulation (seconds to minutes). Under certain circumstances, long-term regulation at the gene level is necessary. This is frequently accomplished by altering the rate of transcription of the gene encoding the enzyme in question. That is, the levels of the specific mRNA encoding the enzyme will increase if more activity is needed and mRNA levels will decrease if less enzyme is required.

Let us consider TH as an example once again. Under certain physiological conditions such as prolonged stress, the body responds by transcribing DNA to produce more TH mRNA. This occurs, in large measure, through the interaction of the steroid hormone, cortisol, with its receptor, followed by a direct induction of TH gene expression. The increase in TH 'message' is then directly reflected by increased TH protein and enzymatic activity.

Compartmentalization

Cells contain many subcellular compartments that are specialized to perform certain metabolic functions. For instance, mitochondria are used for energy production, while ribosomes specialize in protein production. Compartmentalization allows for different chemical and/or physical environments within a cell. Compartmentalization is necessary to regulate enzymes that require a certain environment, not to mention the availability of enzymatic substrates and products. From the previous discussion of proteolytic enzymes, one should recall that enzyme compartmentalization also acts to protect a cell from degradation. As such, compartmentalization provides another means for the control of enzymatic activity.

The importance of compartmentalization is clearly illustrated by the lysosomes of a cell. Lysosomes are membrane-bounded, cytoplasmic organelles that contain a wide variety of hydrolytic enzymes that function at an acidic pH. Lysosomes function in digesting essentially all types of biological macromolecules such as proteins, carbohydrates, lipids and nucleic acids. As such, confinement of the lysosomal hydrolases to the lysosome prevents possible destruction of the entire cell. The acidic conditions also provide a means of denaturing macromolecules, changing their structure and rendering them susceptible to degradation and recycling. In this case, then, compartmentalization protects the cell and provides the optimal (acidic) environment for enzyme activity.

Our discussions have focused on compartmentalization within cells, but the principle also occurs on a grander scale within organisms (i.e. in terms of tissues/organs). All cells may contain the genetic material to produce the milieu of enzymes required to sustain life, but in a complex multicellular organism, expression is tightly controlled to maximize efficiency while conserving energy and preventing buildup of potentially toxic metabolic byproducts. For example, tryptophan hydroxylase (TPH), the rate-limiting enzyme in the biosynthesis of the neurotransmitter serotonin, is primarily found expressed in cells of the nervous system, pineal gland and digestive tract. For it to catalyse the addition of a hydroxyl group to the amino acid tryptophan, a phosphorylated tetrameric TPH enzyme requires several cosubstrates including tetrahydrobiopterin, and molecular oxygen (O_2). Although the body needs to maintain an adequate level of serotonin, byproducts of its biosynthesis are toxic and therefore the body limits those cells that produce TPH.

Summary

At any given time, there are thousands of different metabolic processes occurring within a cell. This article has reviewed some of the mechanisms cells utilize to regulate the activity of the enzymes that are driving these reactions. Depending upon needs, a cell can stimulate either synthesis or degradation of its intracellular enzymes. Additionally, enzymatic activities can be controlled by the concentrations of both substrates and reaction products.

Other mechanisms, such as allosteric control, covalent modification, proteolytic activation or the compartmentalization of proteins, allow the cell to efficiently modulate the overall metabolic processes of an individual cell. These processes can operate independently of each other and/or in a synergistic manner, with the major objective of maintaining a well-balanced, homeostatic state.

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