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Control of enzyme

Enzymes are powerful and specific catalysts for the many thousands of chemical reactions taking place in living organisms. However, it is vital that their activity can be regulated to meet a variety of circumstances.

This carbonated drink contains the synthetic sweetener aspartame, registered under the trade name Nutrasweet. The enzyme thermolysin catalyses the joining of aspartic acid and phenylalanine in the correct way.

KEY WORDS

Enzyme catalysis
Metabolic control
Optimum temperature
Optimum pH
Competitive inhibition
Non-competitive inhibition

Enzymes show some remarkable properties as catalysts. *First*, they are exceedingly powerful. They usually speed up the rate of reactions by huge factors, up to several million- or even billion-fold. This allows metabolism to proceed at a rate compatible with the needs of living organisms. *Second*, enzymes are specific. They catalyse the reaction of one or only a few substrates to give the required products with no side reactions. These properties arise from the precise tertiary structure of the enzyme and the way that its active site interacts with the substrate (Biological Sciences Review, Vol. 22, No. 2, pp. 22–25).

It is small wonder that enzymes are being increasingly exploited in the food and pharmaceutical industries. An example is the use of the enzyme thermolysin in the manufacture of the artificial sweetener aspartame, widely used in diet drinks. The enzyme can bring about the joining of two amino acids (aspartic acid and phenylalanine) in the correct fashion, whereas the use of man-made catalysts would lead to undesirable by-products.

There is a *third* remarkable property of enzymes, namely that their catalytic activity can be tightly regulated. This article explains why such control is important and outlines some of the ways in which it can be brought about.

Why does enzyme activity need to be controlled?

The most important reason is to allow metabolic control. If all the thousands of different enzymes in a cell were working at their maximum rate, there would be chaos. Some unwanted products would pile up and other, perhaps essential, ones would be in short supply. Think of a large factory where every production line was working flat out, irrespective of the demands for each product. All organisms need precise control over metabolism to be able to respond to different circumstances. For example, when you want to run out of the way of a large animal, or an approaching bus, you require the rapid and controlled use of your skeletal muscles. The breakdown of carbohydrate reserves such as glycogen provides the energy for your muscles to work. Once the emergency is past, you need to stop your muscles contracting and be able to replenish the reserves for the next emergency. All this is brought about by control of the activity of specific enzymes.

activity

Understanding how enzymes are controlled has important applications. A number of disease states can be controlled by drugs that lower the activity of selected enzymes. In addition, some essential enzymes in pathogens and humans are different, so we can make drugs that target the enzyme in the pathogen without affecting the human host. Finally, if we are using enzymes as catalysts in industry, we need to be able to control the activity of the enzyme at key steps in the process.

Factors that control enzyme activity

There are many different factors that control enzyme activity. We will look at four of them. The first two factors are temperature and pH, which change the environment in which the enzyme operates. The other two factors affect the amount of enzyme–substrate complex formed, either by changing the concentration of the substrate or by adding inhibitor molecules.

Temperature

In all chemical reactions the reacting molecules must have enough energy to overcome an activation energy barrier. Think of a high jumper needing to gain sufficient energy to jump over the bar. An increase in temperature leads to a greater proportion of molecules with the necessary energy, so the reaction rate increases. This is also true for enzyme-catalysed reactions, and for many enzymes the reaction rate approximately doubles for a rise in temperature of 10°C. However, above a certain temperature, the rate declines markedly, so there is an optimum temperature (see Figure 1).

The decline in rate at high temperatures is due to loss of the precise tertiary structure of the enzyme required for activity. For most mammalian enzymes, the optimum temperature is around 37°C. The high temperatures associated with a severe fever can prove fatal because of the loss of activity of key enzymes in metabolism. Conversely, hibernating mammals such as hamsters can allow their body temperature to drop by as much as 30°C. The consequent slowing down of metabolism allows energy reserves to last through the winter when food is scarce. In the laboratory, the rapid boiling of a solution containing an enzyme is an efficient way of stopping its activity.

The body temperature of hibernating mammals drops. This slows down their metabolism and allows their energy reserves to last through the winter.

pH

The term pH is used to denote the degree of acidity of a solution. The pH of a solution can affect the activity of enzymes considerably, for a number of reasons. Extremes of pH lead to a loss of the enzyme's tertiary structure and hence loss of activity. For most enzymes there is an optimum pH. At pH values around this optimum, the charge carried by important amino acid side chains at the active site affects the activity. A typical graph of rate against pH for two different enzymes is shown in Figure 2. In the human body, there is

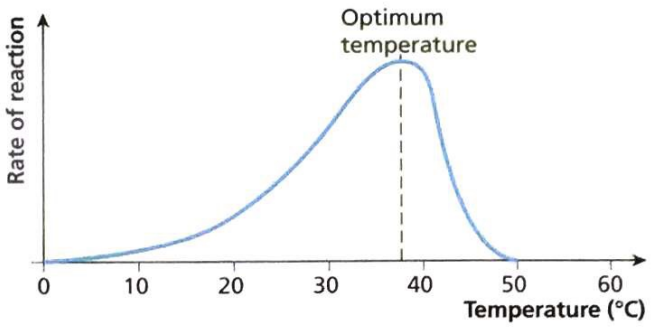


Figure 1 Graph of rate against temperature for a typical mammalian enzyme-catalysed reaction. The optimum temperature is indicated.

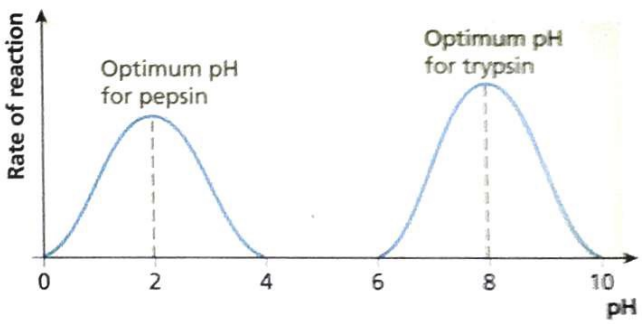


Figure 2 Graph of rate against pH for the digestive enzymes pepsin and trypsin. The optimum pH for each enzyme is indicated.

generally tight control of the pH, at or near neutral. For example, blood plasma pH is typically around 7.4. The stomach is one obvious exception to this rule: stomach pH is typically in the range 1–2. The digestive enzyme pepsin in the stomach has an unusual composition, with a high proportion of its amino acids being acidic, such as aspartic acid. This allows it to withstand these conditions and remain active (see Figure 2) in the first step in the digestion of dietary proteins. By contrast, the optimum pH for the digestive enzyme trypsin, which functions in the duodenum, is close to neutral pH.

Concentration of substrate

The rate or velocity of an enzyme-catalysed reaction depends on the concentration of substrate. Suppose you measured the rate of the enzyme-catalysed reaction at different substrate concentrations. If you then plotted velocity against substrate concentration, you would end up with a graph like the one shown in Figure 3.

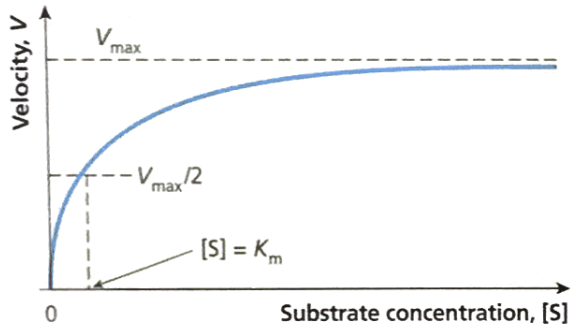
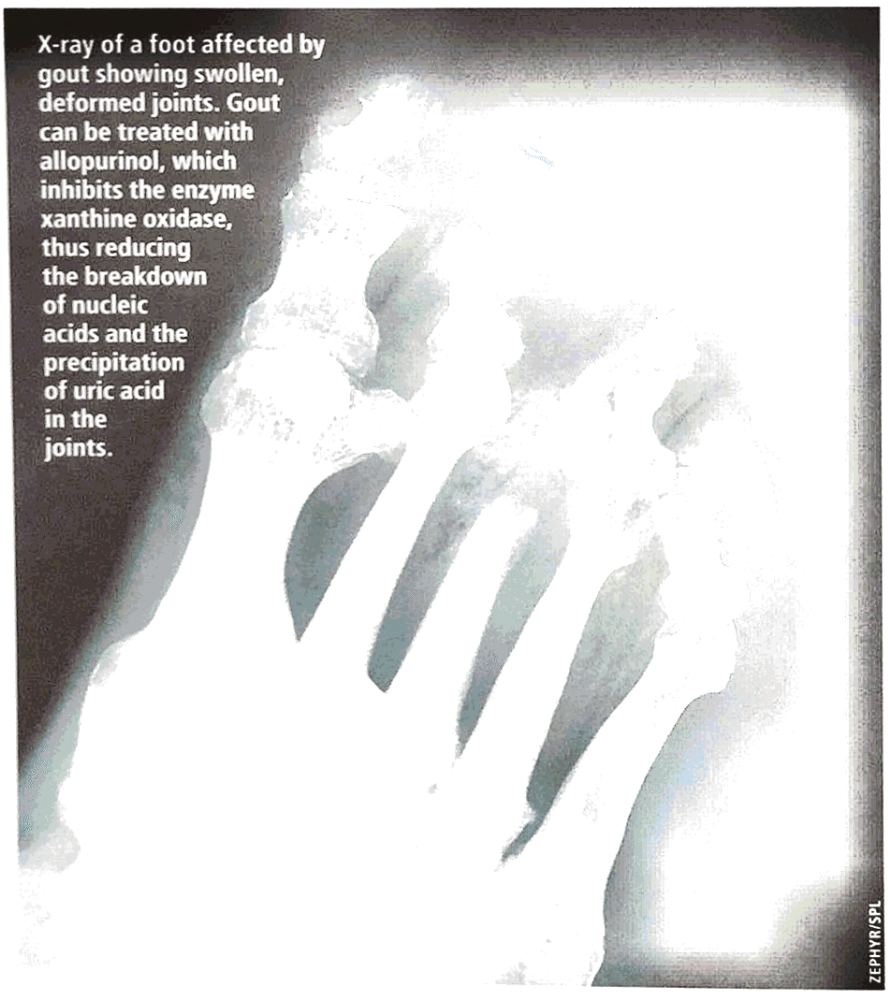


Figure 3 Graph of velocity or rate of reaction against concentration of substrate.


X-ray of a foot affected by gout showing swollen, deformed joints. Gout can be treated with allopurinol, which inhibits the enzyme xanthine oxidase, thus reducing the breakdown of nucleic acids and the precipitation of uric acid in the joints.

ZEPHYR/SPL

We can see that at low values of substrate, the rate increases almost linearly with the increase in substrate. Here the substrate concentration is a limiting factor (see pp. 30–32). However, at higher concentrations of substrate, the rate starts to level off towards a maximum, which corresponds to the maximum velocity or V_{max}.

The concentration of substrate which gives rise to a rate that is equal to half V_{max} is known as the K_m. In general terms, K_m is a measure of the affinity between the enzyme and the substrate. A low K_m corresponds to tight binding between enzyme and substrate, and a high K_m to weak binding. The reason that the rate starts to level off at high substrate concentrations is that all the active sites of the enzyme present in the solution are becoming fully occupied with substrate. Here the enzyme concentration becomes limiting. Addition of more substrate does not allow the reaction to go more quickly, since the active sites are already working flat out.

A good way to understand this is to think about the rate at which spectators gain access to a sports stadium by going through the single turnstile in operation. The rate is limited by the events at the turnstile, irrespective of whether there are 10 or 10000 spectators waiting outside. The only way of getting spectators in more quickly would be to have more turnstiles in operation.

Addition of inhibitors

An inhibitor molecule decreases the rate of an enzyme-catalysed reaction. There are two main classes of inhibitors, known as competitive and non-competitive.

Competitive inhibitors

Competitive inhibitors compete with the substrate for binding to the active site of the enzyme (see Figure 4A).

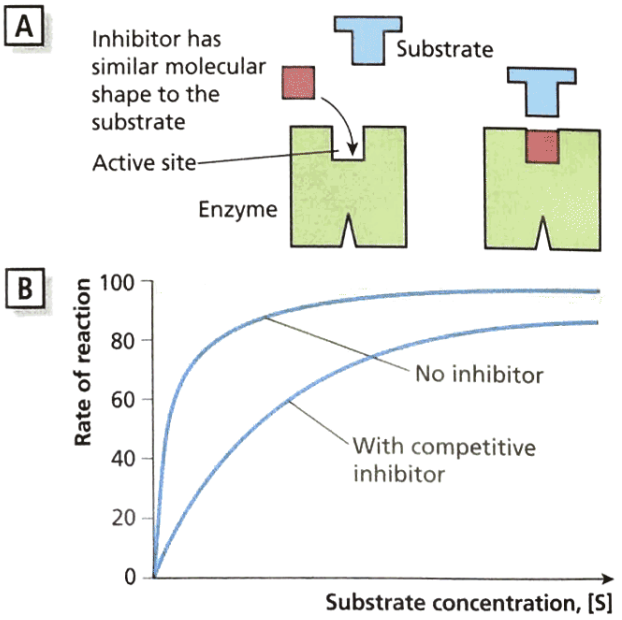


Figure 4 (A) The action of a competitive inhibitor, which binds to the active site of an enzyme. (B) Graph of rate against concentration of substrate for an enzyme in the absence and presence of a competitive inhibitor.

FURTHER READING

Scrutton, N. and Heyes, D. (2009) 'Enzymes: nature's catalytic machines', Biological Sciences Review, Vol. 22, No. 2, pp. 22–25.

A short article summarising the effects of changing conditions on enzyme catalysis, with some helpful diagrams:
http://tinyurl.com/43pbyfx

A chance to test your knowledge about enzyme inhibitors, containing a number of helpful animations:
http://tinyurl.com/h69t9

Generally this is because the inhibitor bears some structural resemblance to the substrate. However, the inhibitor lacks the chemical groupings needed to undergo the actual reaction. A way of envisaging this is to think of a piece of a model that lacks one of the connections to other parts. This wrong part could be built into the model during its assembly but would prevent the addition of further pieces in the correct way. There is thus a competition between substrate and inhibitor for binding to the active site. This is why the rate of the reaction can be increased or decreased simply by adjusting the relative concentrations of substrate and inhibitor. At high substrate concentrations the effect of the inhibitor can be overcome (see Figure 4B).

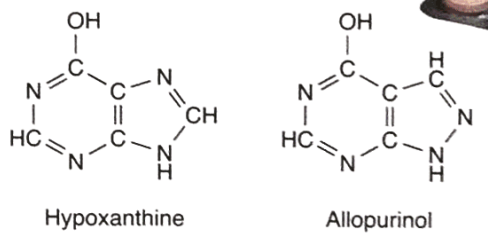
Competitive inhibitors are increasingly being developed as drug molecules to inhibit specific enzymes involved in particular disease states. For example, the painful condition of gout is caused by the precipitation of uric acid in the joints. Uric acid is formed by the breakdown of nucleic acids, and a key enzyme in this process is xanthine oxidase. The structures of the substrate (hypoxanthine) for the enzyme and the inhibitor (allopurinol) are similar (see Figure 5). Allopurinol is proving to be a useful treatment for gout, as it slows down the rate at which uric acid is produced.

Other enzymes for which competitive inhibitors have been developed include HMG-coenzyme A reductase and angiotensin-converting enzyme, which are involved in the synthesis of cholesterol and the regulation of blood pressure respectively. The statins (see Biological Sciences Review, Vol. 22, No. 3, pp. 7–9) and ACE inhibitors, which target these two enzymes respectively, are two of the most widely prescribed drugs in current medical use.

Non-competitive inhibitors

Non-competitive inhibitors do not compete with substrate. They are usually structurally unrelated to the substrate and bind to a site on the enzyme distinct from the active site. The binding of inhibitor leads to a structural change in the enzyme at the active site, making the substrate unable to bind to the active site (see Figure 6A). In this case, a proportion of the enzyme is removed from the system. Hence K_m remains the same, but the V_{max} decreases (see Figure 6B). The inhibition cannot be overcome by adding more substrate.

Statins target HMG-coenzyme A reductase while ACE inhibitors target angiotensin-converting enzyme.



Hypoxanthine

Allopurinol

Figure 5 Structures of the substrate (hypoxanthine) and inhibitor (allopurinol) of xanthine oxidase.

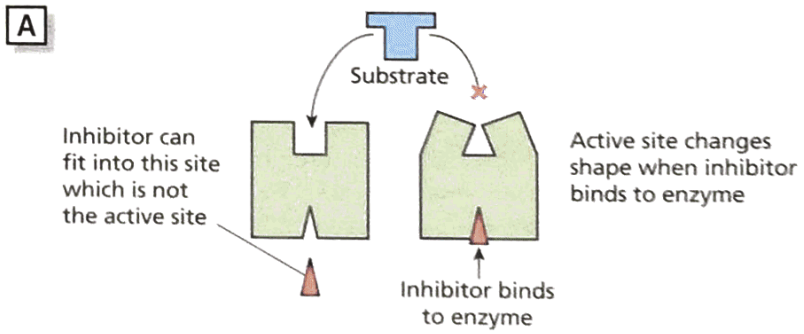
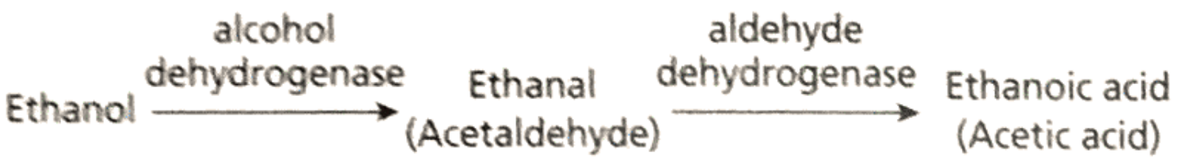


Figure 6
(A) The action of a non-competitive inhibitor, which binds to an enzyme at a site distinct from the active site. (B) Graph of rate against concentration of substrate for an enzyme in the absence and presence of a non-competitive inhibitor.

Non-competitive inhibitors are less common than competitive ones. An example is the drug disulfiram. This behaves as a non-competitive inhibitor towards aldehyde dehydrogenase (AlDH), which is involved in the metabolism of ethanol:




A non-competitive enzyme inhibitor can be used in the treatment of alcohol abuse.

FOTOLIA

Disulfiram has been widely used in the treatment of alcohol abuse, since inhibition of AlDH leads to the build-up of ethanal with its associated unpleasant symptoms, such as nausea and facial flushing, if ethanol is ingested.

This article has outlined some of the many ways in which the catalytic power of an enzyme can be regulated. Understanding these helps us not only to explain how the network of metabolic reactions in cells can be integrated, but also to devise ways in which the activities of some enzymes can be controlled to treat various diseases.

Nicholas Price retired recently from being professor of protein science at the University of Glasgow. He spent nearly 40 years working on the structure, mechanism and regulation of enzymes at the Universities of Oxford, Stirling and Glasgow.

KEY POINTS

- Enzymes are powerful catalysts, but their activity needs to be controlled.
- Enzyme activity can be affected by factors such as temperature, pH and concentration of substrate.
- Inhibitors are molecules that lower enzyme activity.
- There are two main types of inhibition: competitive and non-competitive.