

## Enzyme catalyzed reactions

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Enzymes are a specific group of proteins that are synthesized by living cells to function as catalysts for the many thousands of biochemical reactions that constitute the metabolism of a cell.

More than 2000 different enzymes are known, and it is likely that many more await discovery. Enzymes are required in metabolism because at physiological temperature and pH, un-catalyzed reactions would proceed at too slow a rate for the vital processes necessary to sustain life.

For example, the disintegration of foodstuff by the digestive system involves various enzymes, which catalyze the hydrolysis of proteins to amino acids, carbohydrates to sugars, etc., and this is normally accomplished within 3 to 6 hours depending on the amount and type of food. In the absence of enzyme catalysis, such hydrolysis would take 30 years or more to achieve.

Enzymes are catalysts and are governed by the same rules that apply to all catalysts including chemical catalysts that speed up reactions. However, enzymes have a number of unique properties that are not available to any great extent in chemical catalysts. These are: range, control, catalytic power, and specificity.

The range of reactions catalyzed by enzymes is very large due to immense versatility of enzymes, and the large number of different enzymes that are found in living systems: from hydrolytic reactions, polymerization, red-ox reactions, dehydrations, condensations, and transfer reactions to name a few.

### Catalytic power

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In common with all catalysts, enzymes are subject to the normal laws concerning the catalysis of reactions. Thus, the catalyst cannot speed up a reaction that would not occur in its absence, e.g. because the reaction is not thermodynamically possible.

The catalyst is not consumed during the reaction, and so relatively few catalyst molecules are capable of catalyzing the reaction a great many times. Lastly, the catalyst cannot alter the equilibrium position of a given reaction.

The vast majority of reactions proceed, eventually, to a state of equilibrium in which the rate of the forward reaction is equal to the rate of the reverse reaction. At equilibrium the substrate and product have specific equilibrium concentrations that are a special characteristic of the reaction. The position of the equilibrium may lie strongly to the

product side, for example 1% substrate : 99% product, or more toward the substrate side 80% : 20% or near the middle 50% : 50%.

For example the isomerization of glucose to produce the isomer fructose is catalysed by enzyme glucose isomerase. Starting from 100% glucose the reaction proceeds to equilibrium, which in this reaction is 45% fructose and 55% glucose. The catalyst cannot change the equilibrium position of the reaction, but it can reduce the time that the reaction normally takes to reach equilibrium. In these respects enzymes are no different from other catalysts.

During any reaction the reactants briefly enter a state in which the susceptible substrate is not completely broken and the new bonds in the product are not completely formed. This transient condition is called the transition state, and it is energy dependent because it requires energy to make and break chemical bonds. This represents an energy barrier to successful reaction, and is the reason why the vast majority of reactions proceed extremely slowly in the absence of external help.

Reactants can be helped towards the transition state by supplying heat energy, high pressure or extreme pH to weaken bonds or by the addition of catalysts. Enzyme catalysts are more effective than chemical catalysts at reducing the energy barrier to enable transition state formation, and thereby increase the rate of a reaction.

The efficiency of enzyme catalysis varies, but most enzymes can enhance the rate of an uncatalyzed reaction by a factor of many millions. One of the most efficient enzymes is carbonic anhydrase, which catalyses the hydration of up to 600,000 molecules of carbon dioxide per second under optimal conditions.

Carbonic anhydrase is found mostly in red blood cells where it plays a vital role in maintaining the acid-base balance in the body. The enzyme enables rapid transport of molecular CO<sub>2</sub>, formed by cellular respiration, from the site of formation (tissues) to the lungs for expulsion.

An indication of catalytic power is provided by the turnover number of an enzyme. When an enzyme is fully saturated with its substrate, then the turnover number is the number of substrate molecules converted to product per second.

The catalytic power of enzymes is due to the precise molecular interactions that occur at the active site, which lower the energy barrier and enable formation of the transition state.

## Control of enzyme activity

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A variety of mechanisms exist for controlling enzyme activity giving a full range from very fine to coarse control. Enzymes can be synthesized in inactive precursor state, which is then activated at the appropriate time and place (coarse control) e.g. trypsinogen synthesized in the pancreas, and is activated by peptide bond cleavage in the small intestine to form the active enzyme trypsin, when required to digest food.

Another more common mechanism of control is covalent modification by insertion of a small chemical group usually a phosphate group. For example glycogen phosphorylase is the first enzyme in degradation of glycogen to glucose. It exists in two forms called a and b (active and not active), which are phosphorylated and not phosphorylated. So the enzyme is regulated by the insertion or removal of a phosphate group on the enzyme. Fine control can be exerted by inhibiting and activating enzymes to reduce or increase the rate of enzyme activity.

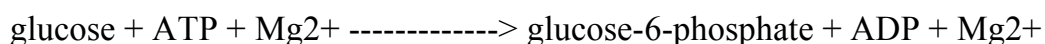
Many mechanisms for inhibiting enzymes are used in cells, and some enzymes called allosteric enzymes have complicated structures, which facilitate control by a range of small molecule effectors to activate or inhibit enzyme activity.

## Specificity

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Perhaps the most distinctive feature of enzyme-based catalysis is its specificity. Chemical catalysts display only limited selectivity, whereas enzymes show three levels of specificity: for the reactants, for the susceptible bond involved in the reaction, and the type of product produced.

The degree of specificity for substrate varies from absolute to fairly broad. For example, urease is absolutely specific for its substrate urea (NH<sub>2</sub>CONH<sub>2</sub>), and structural analogues (e.g. NH<sub>2</sub>SONH<sub>2</sub>) are not hydrolyzed by the enzyme. Hexokinase is less specific and shows group specificity for a small set of related sugar molecules.



As well as glucose, this enzyme will catalyse the phosphorylation of several other sugars such as mannose and fructose, but not galactose, xylose, maltose or sucrose.

In addition to substrate specificity, enzymes display remarkable product specificity, which ensures that the final product is not contaminated with by-products. Thus, in the above phosphorylation of glucose, the product is exclusively glucose 6-phosphate, and no other phospho-glucose (e.g. glucose-1-phosphate, or glucose-3-phosphate) is produced

during the reaction. The formation of by-products by side reactions is a significant problem associated with most of the less specific chemical catalysts.

Lastly, some groups of enzymes show a fine specificity for the susceptible bond involved in a reaction. This aspect is neatly illustrated by considering proteases, which hydrolyse peptide bonds between amino acids residues in polypeptide chains. Various proteases show specificity for peptide bonds that are located between particular amino acid residues. For example, the blood protease thrombin is very specific, and will only hydrolyse the particular peptide bond between arginine and glycine residues i.e. **-arg--gly-**.

The pancreatic protease trypsin will only hydrolyse a peptide bond if it is adjacent to an amino acid residue bearing a positive charge (i.e. lysine or arginine), **-lys--X-** or **-arg--X-**. Other proteases such as pepsin and subtilisin are much less specific, and will catalyse the hydrolysis of a large number of peptide bonds in a polypeptide chain.

Specificity is an inherent feature of enzyme catalysis because the reaction takes in a particular region of the enzyme that is designed to accommodate the specific participants involved in the reaction. This region is the active site, and it is normally a small pocket, cleft or crevice on the surface of the enzyme. It is designed to bring a few amino acid residues into contact with the substrate molecule.

The site has strong affinity for the substrate because the site amino acid residues are primed for interaction with groups or regions on the substrate molecule. Thus upon substrate binding, an enzyme-substrate (ES) complex is formed, involving non-covalent interactions between the substrate and the site amino acid residues. Consequently the substrate molecule must have the correct shape and/or functional groups to fit into the active site and participate in the interactions.

Enzymes with absolute specificity have very precise shape/interaction requirements that are only found in a particular substrate molecule. Enzymes with broad specificity have more flexible active site requirements, and therefore accept a wider range of substrate molecules. The site amino acid residues participate directly in the catalytic reaction, and are largely responsible for the high catalytic power associated with enzyme reactions.

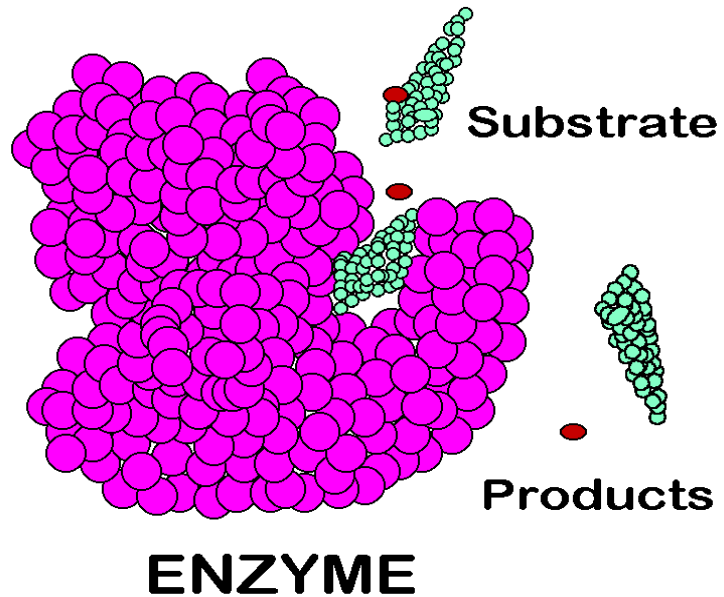
## Active site and ES complex

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The active site of an enzyme is the region that binds the substrate and contributes the amino acid residues that directly participate in the making and breaking of chemical bonds. The amino acid residues are called the catalytic groups. Enzymes differ widely in

structure, function & mode of catalysis so active sites vary, but possible to make some generalizations.

(1) Enzymes are usually very large in comparison with substrate, so only small portion of amino acid residues are near or in direct contact with substrate in Enzyme-Substrate (E-S) complex. Most of the enzyme involved in control & maintaining correct structural configuration of enzyme i.e. structural backbone.



(2) Active site is a 3-dimensional entity. Not a point or a plane usually an intricate pocket or cleft structurally designed to accept the structure of the substrate in 3-D terms.

(3) Substrate is bound by relatively weak forces.

(4) Most are clefts or crevices designed to exclude water from the active site and are surrounded with non-polar amino acid residues, which give the active site a non-polar environment. This appears essential for both binding and catalysis. Essential to exclude water (unless water involved in the reaction) because water disrupts bond breaking & making processes.

(5) Specificity. Active site provides specificity for its particular substrate, which is a characteristic feature of enzymes. **Enzyme-substrate (ES) complex**

Enzymes convert substrates to products by breaking and making chemical bonds and so seems logical that at some point in proceedings enzyme and substrate are chemically bound together briefly in an ES complex at the active site. Evidence for existence of an ES complex is:

(a) Suggested by observation of maximum velocity in enzymes. At constant  $[E]$  increasing the  $[S]$  will cause increase in reaction rate until a maximum velocity reached then further increases in  $[S]$  produce no further increase in activity. Maximum velocity is due to ES complex. At sufficiently high  $[S]$  all active sites are filled & working flat out so no more activity possible.

(b) Possible to isolate ES enzyme-substrate complex.

## Inhibition of enzymes

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Inhibition of enzymes is important for several reasons. Firstly, it serves as a major control mechanism in biological systems providing a means of regulating metabolic pathways. Also many drugs are used, which act by inhibiting specific enzymes in brain or body tissues, so it is essential to understand the mechanism of enzyme inhibition. Inhibitors are also used as tools to study the mechanism of enzyme action.

Basically there are two types of inhibitor:-

1 reversible, binds to the enzyme, but not permanently so inhibition can be transient.

2 irreversible, binds permanently so inhibition is complete.

Reversible inhibitors can normally be removed by simple dialysis or change in buffer or pH. Irreversible inhibitors normally bind covalently with the enzyme, and cannot be removed easily. There are three principal forms of reversible inhibition:

(a) competitive; (b) non-competitive; (c) uncompetitive.

## Competitive inhibition

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A competitive inhibitor normally strongly resembles the substrate for the enzyme in shape and size and therefore competes with the substrate for the substrate binding site on the enzyme.

So it reduces the rate of reaction by lowering the proportion of enzyme molecules that have bound substrate.

No products  $\leftarrow$  EI  $\leftarrow$  I + E + S  $\rightarrow$  ES  $\rightarrow$  E + Products

I = Inhibitor, E = Enzyme, S = Substrate

So the more I present, the more EI formed, and the less product produced.

It is common to have the product of a reaction being a competitive inhibitor of the substrate because of the structural resemblance of the two and in many cases the product of a reaction regulates the activity of an enzyme by a feedback mechanism.

Actual level of inhibition caused by a competitive inhibitor is strictly dependent on the relative  $[I]$  and  $[S]$  because they compete with each other e.g. at low  $[I]$  the inhibition can be overcome by adding large  $[S]$  to swamp out inhibition by  $I$ . This increases the amount of  $ES$  over  $EI$ . Because it is necessary to add more and more substrate to overcome the inhibition this affects  $K_m$  for the substrate. i.e.  $K_m$  will be higher.

We can use this knowledge to find out the kind of inhibition being observed. By measuring the reaction rates at different  $[S]$  and  $[I]$  we can obtain a double reciprocal plot characteristic for competitive inhibition. So the plot for competitive inhibition is characterized by straight lines of different slope all intersecting at a single point on the  $1/v$  axis i.e. y-axis. In competitive inhibition  $V_{max}$  is not altered, but  $K_m$  is altered, and this is characteristic of competitive inhibition.

## Non-competitive inhibition

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In this type the  $S$  and  $I$  bind together on the enzyme, but at different binding sites,  $S$  at active site and  $I$  at another site. The binding of the  $I$  exerts an effect on the active site via a conformational change to reduce the enzyme activity possibly by affecting the structure of the active site so that it does not function efficiently. Consequently  $V_{max}$  is altered, but not  $K_m$  (binding of  $S$ ).

The typical Lineweaver-Burk plot is characterised by a series of lines meeting on the x-axis showing  $K_m$  constant, but varying  $V_{max}$ . So for non-competitive inhibition  $V_{max}$  is altered, but  $K_m$  constant.

## Un-competitive inhibition

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In this type the  $I$  does bind at the active site, but only AFTER the  $S$  has bound to the active site so it does not compete with the  $S$ . Thus even when the  $[S]$  is saturating and all  $E$  is in  $ES$ , the inhibitor can still bind to the active site and produce an inactive  $ESI$  complex.

The  $I$  only binds to  $ES$  so  $I$  stimulates formation of  $ES$  and increases binding of substrate to enzyme so  $K_m$  is reduced. However the  $ESI$  complex is non-productive so  $V_{max}$  is lowered.

The double reciprocal plot is a series of parallel lines indicating lower  $K_m$  and decreasing  $V_{max}$ , and is characteristic of un-competitive inhibition.

<http://www-biol.paisley.ac.uk/courses/stfunmac/glossary/enzymes.html>