Enzymes and their Role in Biotechnology

A.R. Teal BSc MSc

Department of Biological Sciences,
North East Surrey College of Technology

Dr P.E.O. Wymer BSc PhD PGCE

Welcome Trust
179 Great Portland Street, London W1N 5TB

The *Biochemistry Across the School Curriculum Group* (BASC) was set up by the Biochemical Society in 1985. Its membership includes education professionals as well as Society members with an interest in school science education. Its first task has been to produce this series of booklets designed to help teachers of 16+ level biology and related courses to teach the sections of the 16+ level syllabus which have a high biochemical content.

Other topics covered by this series include: *Essential Chemistry for Biochemistry, The Structure and Function of Nucleic Acids, Metabolism, Immunology, Photosynthesis* and *Recombinant DNA Technology.*

More information on the work of BASC and these booklets is
available from The Education Officer at the Biochemical Society,
59 Portland Place, London W1N 3AJ.

Comments on the contents of this booklet will be welcomed by the
series editor, Dr. Colin Wynn, School of Biological Sciences,
University of Manchester, Oxford Road, Manchester M13 9PY.

Contents

1. The Nature and Classification of Enzymes

i) Enzymes are catalysts

Enzymes are proteins with specific catalytic functions that are produced by all living cells. In common with other catalysts they are effective in small amounts, remain unchanged after the reaction and do not affect the position of equilibrium of a reversible reaction. They simply increase the speed at which equilibrium is achieved.

Compared with inorganic catalysts, enzymes are very efficient. For example, while iron filings can catalyse the decomposition of hydrogen peroxide, the enzyme **catalase**, specifically catalyses this reaction. The enzyme contains a single iron atom strategically sited within the protein structure. One milligram of iron in catalase can produce a rate of peroxide decomposition that could only be achieved by several tonnes of iron filings. Thus the protein part of the catalase and its associated structures greatly enhance the inherent catalytic power of the iron. Enzymes do not require the extremes of temperature and pressure often associated with inorganic catalysts. In the Haber process for ammonia synthesis, elevated temperatures (500°C) and pressures of several hundred atmospheres are used to ensure a sufficiently rapid rate, and an iron based catalyst is required. In contrast, nitrogen fixing bacteria can convert the inert dinitrogen molecule into ammonia at ordinary temperatures and pressures. This is due to the activity of the enzyme complex **nitrogenase**, which contains iron and molybdenum essential for activity. Reducing power and energy in the form of ATP are also required.

Most enzymes are much larger molecules than the substrates they act on, and only a small portion of the enzyme called the **active site** comes into direct contact with the substrate. The rest of the enzyme molecule functions to maintain the correct shape of the active site.

ii) Enzyme specificity and the active site

Unlike chemical catalysts that display only limited selectivity most enzymes are specific. The active site of an enzyme typically consists of 3-12 amino acid residues organised into a precise three-dimensional arrangement in a pocket or crevice in the protein. This site has a strong affinity for the substrate because the chemical nature of these amino acid residues and their three-dimensional arrangement form a region that complements certain groupings on the substrate molecule. Examples of the reactive groups of protein side chains that participate in the active site include the thiol group of cysteine, the imidazole group of histidine and the hydroxyl group of serine (see Fig. 1).

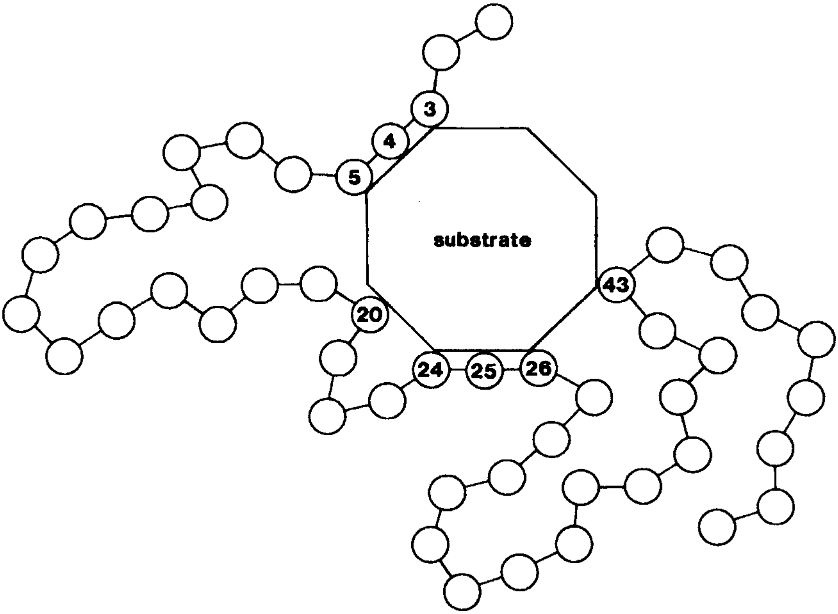


Fig. 1 Diagrammatic representation of substrate complexing with an enzyme active site

The active site of **lysozyme**, an enzyme that attacks the carbohydrate polymer murein present in the cell walls of certain bacteria, is a cleft into which the substrate fits. Carboxylic acid groups of glutamate and aspartate residues participate in the breaking of the susceptible bond of the substrate. **Chymotrypsin**, which hydrolyses certain peptide bonds in proteins, depends for its activity on histidine and serine residues which are near to each other in the active site.

The degree of specificity shown by enzymes for their substrates varies considerably, but usually there is discrimination between stereoisomers. For example, the fungal enzyme **glucose oxidase** will catalyse the oxidation of D-glucose but not L-glucose to gluconic acid. It even distinguishes between α and β forms: the rate of β-D-glucose oxidation is about 157 times faster than that for α-D-glucose. Most intracellular enzymes only work on one particular substrate although certain enzymes work on a range of related compounds. An example is an isomerase from *Streptomyces* or *Bacillus* species. It is a xylose isomerase, but can also convert glucose into fructose. Glucose and xylose have similar structures, but the former is a hexose and the latter a pentose. No comparable glucose isomerase has so far been isolated and so this enzyme is exploited by the food industry to produce high fructose syrups, although its catalytic rate with glucose is low.

iii) Enzyme Classification

Many enzymes have been named by simply adding the suffix **–ase** to the name of the substrate. Thus amylase hydrolyses amylose, L-asparaginase hydrolyses L-asparagine and β-galactosidase hydrolyses the terminal β-D-galactose residues in β-galactosides such as lactose. However some enzymes have been given uninformative names, e.g. catalase, and the names of some enzymes do not conform with this system, e.g. trypsin, rennin. A more rational system of classification divides them into six groups based on the type of reaction that they catalyse. Within each group, various sub-groups are possible helping to specify the type of reaction more clearly. The six major classes are:

1. **Oxidoreductases:**	catalyse oxidation-reductions
2. **Transferases:**	catalyse the transfer of a group from one compound to another
3. **Hydrolases:**	catalyse hydrolysis reactions
4. **Lyases:**	catalyse the addition of a group across a double bond
5. **Isomerases:**	catalyse intra-molecular rearrangements
6. **Ligases:**	catalyse bond formation between two compounds using the energy derived from the cleavage of a pyrophosphate bond such as is found in ATP

2. Mechanism of Enzyme Action

i) Enzymes form complexes with their substrates

Enzymes catalyse reactions by forming a complex with their substrates at the active site, and the mode of action of an enzyme can therefore be represented as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_{cat}} P$$

where E = enzyme, S = substrate, ES = enzyme-substrate complex,
P = product

The first step involves the enzyme binding the substrate rapidly and reversibly to give an ES complex. The rates of the individual forward and backward reactions are k_1 and k_2 respectively. Subsequent chemical changes occur at the active site involving the breaking and forming of chemical bonds to form the products. The rate is characterised by a rate constant k_{cat}. Evidence for such behaviour can be obtained by carrying out a set of determinations of overall velocity with a fixed amount of enzyme and different amounts of substrate. Obviously the temperature and pH should remain constant. The result is shown in Figure 2.

The velocity increases up to a certain point, after which no further increase occurs: this is the maximum velocity, V_{max}. The enzyme is now saturated with substrate, and every molecule of enzyme is performing catalysis at its maximum rate under the conditions of the experiment. Mathematical analysis of such a curve (a rectangular hyperbola) produces the following general equation:

$$y = \frac{a \cdot x}{b + x}$$

where a and b are constants.

If this is applied to the velocity (v) against substrate concentration [S] curve above, this becomes

$$v = \frac{a \cdot [S]}{b + [S]}$$

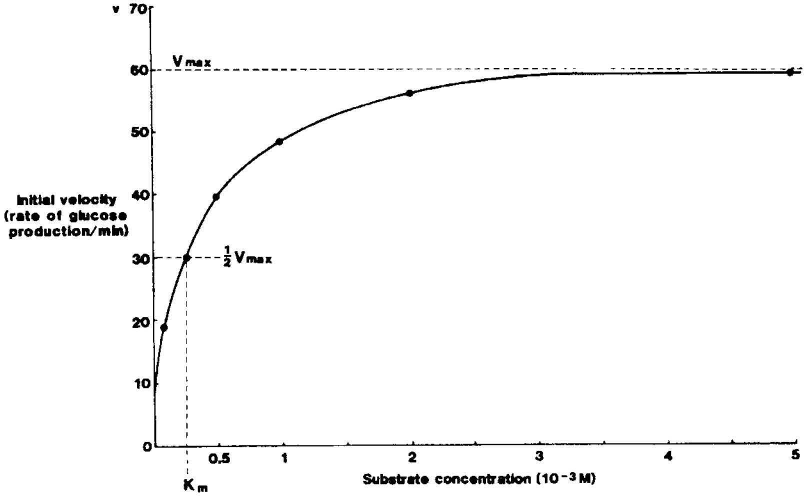


Fig. 2 Variation of initial velocity of an enzyme reaction with substrate concentration

A simple kinetic analysis by Michaelis and Menten demonstrated that for enzyme catalysed reactions, a is equal to V_{max} while b is equal to a constant K_m, now called the Michaelis constant (see below), i.e.

$$v = \frac{Vmax \cdot [S]}{K_m + [S]}$$

ii) Enzyme kinetic constants are of considerable use

Depending on the precise mechanism of the enzyme's action, K_m is a complex function involving the rate constants k_1, k_2 and k_{cat}. In experimental terms it is numerically equal to the concentration of the substrate at which the velocity of the reaction is half of the maximum velocity V_{max}.

$$\text{i.e. when} \qquad v = \frac{V_{max}}{2}, \quad \text{then } [S] = K_m$$

K_m is a useful and fundamental characteristic for an enzyme and a particular substrate. It can be viewed as an index of how easily the enzyme can be saturated by the substrate (i.e. the affinity) under defined conditions of temperature and pH. The smaller the value of K_m the more readily the enzyme may be saturated with substrate. If the active site of the enzyme can bind and react with several molecules of a similar structure, i.e. there are a number of potential substrates for the enzyme, then each substrate is likely to have a different K_m value. The K_m values for a number of natural and synthetic substrates of β-galactosidase are given below.

Substrate	K_m
lactose (a natural substrate)	1×10^{-3}M
p-nitrophenyl β-galactoside	2×10^{-4}M
p-aminophenyl β-galactoside	4×10^{-3}M
4-methyl umbelliferyl β-galactoside	2×10^{-4}M

The synthetic substrate *p*-aminophenyl β-galactoside has the lowest affinity for the enzyme while 4-methyl umbelliferyl β-galactoside has a much higher affinity.

V_{max} is not however a fundamental characteristic for an enzyme and its value will depend on the amount of enzyme present. If this is standardised at one mole of enzyme, the theoretical V_{max} obtained then is called the **turnover number** or **molar activity**. This is a useful measure of the catalytic power of an enzyme. Its value quantifies the number of substrate molecules transformed by one molecule of enzyme per minute. Carbonic anhydrase has the highest molar activity of any known enzyme and the molar activities of various enzymes under optimum conditions are given below:

Enzyme	*Turnover Number*
carbonic anhydrase	36×10^6
catalase	5.6×10^6
β-galactosidase	12×10^3
chymotrypsin	6×10^3
lysozyme	60

iii) Many enzymes need non-protein components

Some enzymes consist solely of protein but others require additional components, referred to as **cofactors** for activity. The catalytically active enzyme-cofactor complex is called a **holoenzyme**, while if the cofactor is removed, the remaining inactive protein is referred to as an **apoenzyme**. Most cofactors are either inorganic ions, coenzymes, or prosthetic groups.

Inorganic metal ions may be an integral part of the enzyme protein structure or may associate with its substrate, aiding binding and enhancing catalytic activity. Thus the Fe^{2+} found associated with the haem group in peroxidase and catalase is tightly bound within the enzyme protein, while Mg^{2+} complexes with ATP^{4-} and is an essential component of reactions involving this molecule such as those catalysed by phosphotransferases.

Coenzymes are organic substances of relatively low molecular weight compared with the enzyme protein. Many coenzymes contain a vitamin molecule as part of their structure. They are only loosely bound to the enzyme and function effectively as one of the substrates of that enzyme. They have specific functions, such as the transfer of hydrogen (nicotinamide adenine dinucleotide, NAD^+, in dehydrogenase reactions) or the transfer of acyl groups (coenzyme A in fatty acid metabolism). Many different enzymes catalysing different reactions have the same coenzyme; for example over a hundred dehydrogenases are known that have NAD^+ as coenzyme.

When the coenzyme is tightly bound to the enzyme molecule and does not leave the enzyme molecule after a cycle of catalysis, it is referred to as a **prosthetic group**. Flavin adenine dinucleotide (FAD) is another carrier of hydrogen atoms associated with oxidase enzymes such as glucose oxidase. The haem group of catalase and peroxidase with its porphyrin ring is also regarded as a prosthetic group. The cofactor requirements of a number of enzymes is given below.

Enzyme	*Type*	*Cofactors*	*Name*
Subtilisin (a protease)	Hydrolase	none	
Lactic acid dehydrogenase	Oxidoreductase	NAD^+	coenzyme
Glucose isomerase	Isomerase	Co^{2+}, Mg^{2+}	activating ion
β-Galactosidase	Hydrolase	Na^+, K^+	activating ion
Peroxidase	Oxidoreductase	Haem containing Fe^{2+}	prosthetic group
Glucose oxidase	Oxidoreductase	FAD Fe	prosthetic group inorganic ion

iv) Enzymes are influenced by pH and temperature

Enzymes being proteins, are sensitive to changes in the environment in which they work. A change in either the hydrogen ion concentration (pH) or temperature can profoundly affect the activity of an enzyme. This is very important in the industrial use of enzymes. The pH must be controlled and industrial processes usually try to get the maximum rates of activity by choosing the highest temperature at which the enzyme is active but is not denatured.

Most enzymes have a characteristic pH at which the velocity of the catalysed reaction is maximal and above and below this optimum pH the velocity declines (see Fig. 3).

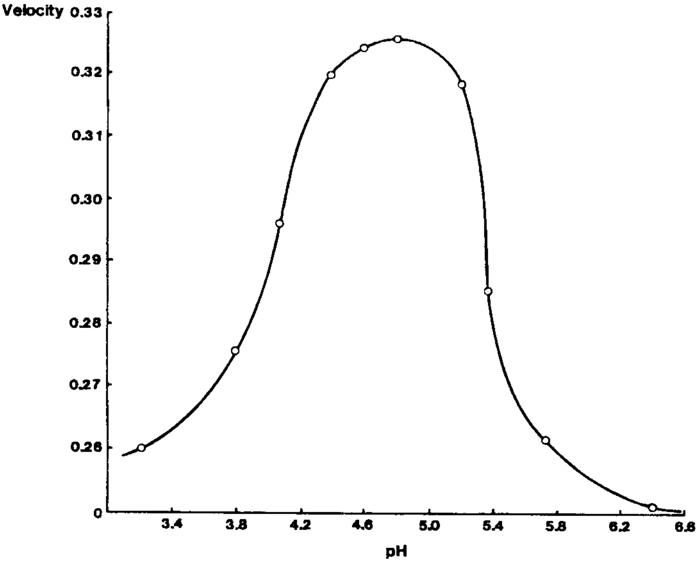


Fig. 3 The pH optimum for β-glucosidase

This response depends on a number of factors. As the pH changes, the ionisation of groups both at the active site and on the substrate can alter, influencing the rate of formation and decomposition of the E-S complex. The optimum pH of an enzyme need not be identical with that of its normal intracellular surroundings, indicating that the local pH can exert a controlling influence on enzyme activity. Further the optimum pH for activity need not coincide with the pH at which the enzyme is most stable, and one effect of a pH change can be to cause the irreversible denaturation of the enzyme protein.

Thus while glucose oxidase has a pH optimum of 5.5 coinciding with its pH stability range of 4.0-6.0, lysozyme shows optimal activity over a pH range of 6.0-7.0 yet it is maximally stable between pH 4.0-5.0.

The effects of temperature on the activity of an enzyme are complex and can be considered as two forces acting simultaneously but in opposite directions. As the temperature is raised, the rate increases, but at the same time there is a progressive inactivation (denaturation) of the enzyme protein. This becomes more pronounced as the temperature increases, so that an apparent temperature 'optimum' is observed (see Fig. 4).

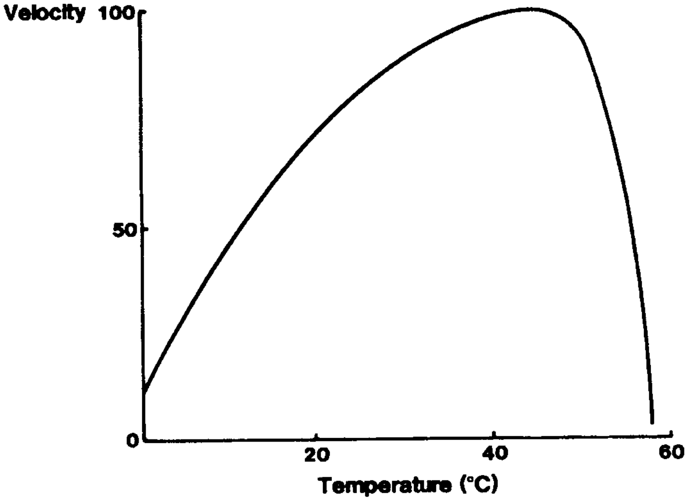


Fig. 4 The effect of temperature on enzyme activity

Thermal denaturation is time-dependent, and for an enzyme the term 'optimum temperature' has little meaning unless the time of its exposure to temperature is recorded. The thermal stability of an enzyme can be determined by first exposing the protein to a range of temperatures for a fixed time, and subsequently measuring its activity at one favourable temperature, e.g. 25°C (see Fig. 5).

The temperature at which denaturation becomes an important factor varies from enzyme to enzyme. Normally it is negligible below 30°C and starts to become appreciable above 40°C. Occasionally immobilisation of an enzyme such as the

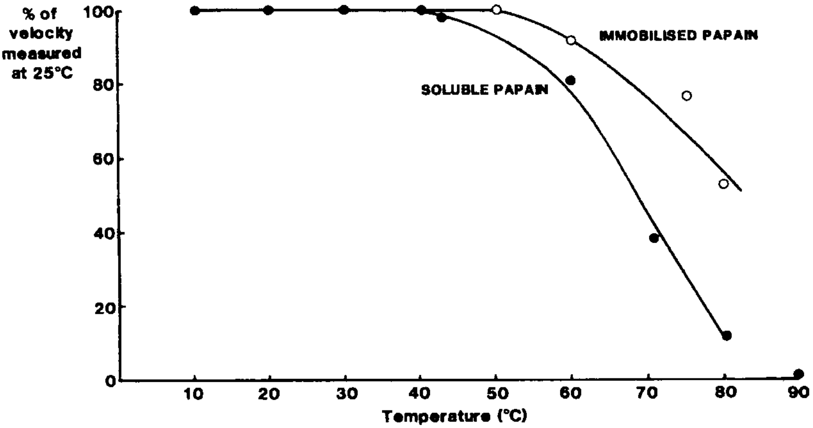


Fig. 5 The thermal stability for papain

protease papain onto an inert support can increase its thermal stability, but this is not a general rule.

A few enzymes can retain considerable activity at higher temperatures, e.g. those produced by thermophilic bacteria, and such properties are very important in biotechnology.

v) Enzymes are sensitive to inhibitors

Substances that reduce the activity of an enzyme catalysed reaction are known as inhibitors. They act by either directly or indirectly influencing the catalytic properties of the active site. Inhibitors can be foreign to the cell or natural components of it. In the latter instance, they can represent an important element of the regulation of cell metabolism.

Inhibitors are classified as **reversible** inhibitors when they react reversibly with the enzyme to establish an equilibrium between free and bound forms. An inhibitor with a structural similarity to the normal substrate so that it can bind reversibly at the active site is known as a **competitive inhibitor**. A characteristic of all such molecules is that they can be totally displaced from the active site again if high enough substrate concentrations are used, thus restoring full activity. Glucose oxidase, whose normal substrate is D-glucose can be inhibited by D-arabinose, a pentose with a very similar structure. With certain enzymes, high concentrations of either the substrate or product can be inhibitory. For example invertase activity is considerably reduced at high sucrose concentrations, while the β-galactosidase of *Aspergillus niger* is quite strongly inhibited by its product, galactose. Products of an enzyme reaction are some of the most commonly encountered competitive inhibitors.

Such considerations are important in developing commercial processes. Fungal cellulases have considerible potential in glucose production from cellulose containing materials. The reaction products, cellobiose (a disaccharide) and glucose exert inhibitory effects and to achieve maximum efficiency are removed either by ultra-filtration, or by the activity of another enzyme.

Other types of reversible inhibitor exist. **Non-competitive inhibitors** react with the enzyme at a site distinct from the active site. The binding of inhibitor does not interfere with substrate binding but does prevent its subsequent turnover. Most non-competitive inhibitors are not related chemically to the substrate, and the inhibition caused by them cannot be relieved by increasing substrate concentrations.

If an inhibitor binds permanently to the enzyme it is known as an **irreversible inhibitor**. Certain organophosphorus compounds such as diisopropylfluorophosphate (DFP) completely inhibit enzymes such as chymotrypsin and acetylcholinesterase by interacting covalently with an important serine residue at the active site of these enzymes. The physiological consequence of irreversible acetylcholinesterase inactivation is to interfere with neurotransmitter destruction at the synapses of nerves causing constant propagation of nerve impulses and eventual death. DFP was originally designed as a war gas: modified versions of this compound now find great use as pesticides.

3. The Origin and Purification of Enzymes

i) Enzymes are ubiquitous

Enzymes are essential components of animals, plants and micro-organisms, catalysing and co-ordinating the complex reactions of cellular metabolism. Over 2500 different enzymes have been isolated and described to date, but this probably only reflects one tenth of the full enzymic potential of nature.

The properties shown by an individual enzyme depend upon its source. For example, the enzyme β-galactosidase (lactase) which hydrolyses lactose, is found in animal organs, plants, fungi, yeasts and bacteria. The various forms of this enzyme do not all have the same molecular size and their pH and temperature optima also vary depending on their origin. Examples of the properties of β-galactosidases from several sources are given below.

Source of β-galactosidase	*pH optimum*	*Temperature optimum*	*Relative molecular mass (M_r)*
Bacterial: *Escherichia coli*	7.2	40°C	540×10^3
Fungal: *Aspergillus niger*	3.0–4.0	55–60°C	124×10^3
Yeast: *Saccharomyces fragilis*	6.6	37°C	201×10^3

In the dairy industry, whey is produced in abundance as a by-product of cheese manufacture and contains 5% lactose. β-Galactosidase can be used to hydrolyse the lactose in whey and milk to give a sweet syrup containing glucose and galactose that can be used by the food industry. Fungal lactases, with an acid pH optima can be used for acid whey hydrolysis, while the yeast and bacterial lactases are suited to milk lactose hydrolysis at pH 6.6.

Lactose also presents problems because it is a relatively insoluble sugar, crystallising from 16% (w/v) solutions at 25°C, making its transport as a concentrated solution difficult. Furthermore, 70% of the world's adult population cannot digest it due to a congenital deficiency of β-galactosidase in the gut. This gives rise to an intolerance of dairy products for a large population for whom lactose consumption can give rise to intestinal pain and diarrhoea. Thus there is considerable commercial interest in the enzymic removal of lactose from milk and other dairy products using β-galactosidase.

ii) Enzymes may be intracellular or extracellular

While many enzymes are retained within cells and integrated into specific subcellular compartments, others are released into the surrounding environment. Thus β-galactosidase from animals, plants, yeast and bacteria are intracellular enzymes, while fungi produce an extracellular form. Some fungi are also able to excrete large amounts of cellulases into culture media but these generally stay bound to the cell wall. In animals the cells that line the kidney tubules secrete the proteolytic enzyme urokinase into the urine, from which it can be extracted. Certain body fluids contain lysozyme which acts as a natural antibiotic destroying the cell walls of many Gram positive bacteria. This enzyme is abundant in hen egg white, from which it is produced.

The majority of enzymes in industrial use are extracellular proteins from fungal (*Aspergillus* sp) or bacterial (*Bacillus* sp) sources and it is easier and therefore cheaper to purify such an enzyme for commercial use. Examples include α-amylase, cellulases, dextranase, proteases and amyloglucosidase. Many other enzymes for non-industrial use are intracellular and are produced in much smaller amounts by the cell. Examples include asparaginase, catalase, cholesterol oxidase, glucose and glucose 6-phosphate dehydrogenase.

iii) Enzyme purification is important

Enzymes generally occur in the cell in association with other proteins, nucleic acids, polysaccharides and lipids. The activity of the enzyme in relation to the total protein present (specific activity) can be determined. A variety of methods are used to remove contaminating material so the enzyme is purified and its specific activity increases. Enzymes used as diagnostic reagents and in clinical therapeutics are normally prepared to a high degree of purity, because great emphasis is placed on the specificity of the reaction being catalysed. This contrasts with many industrially used enzymes where the degree of purification is less important. The higher the purity, the greater the cost of bulk enzyme production. This can be of considerable importance when industrial applications are considered. Even the cheapest of bulk enzymes, e.g. proteases for washing powder formulation, can contribute to between 5-10% of the final product value. Thus in the large scale extraction of bacterial enzymes, the use of crude hen egg white to cause cell lysis is often equally as effective and less expensive than using pure lysozyme.

The major difference between isolating an intracellular and an extracellular enzyme is that cell lysis is required. Once the cells have been broken to release the intracellular protein the same types of purification techniques are applicable. A variety of methods for cell disruption are available. The procedure adopted to give a cell-free preparation (homogenate) is carefully chosen to try and ensure maximum cell disruption with minimum enzyme damage.

iv) Enzyme purification involves the separation of proteins

A number of stages are involved in enzyme purification and these are summarised in Figure 6.

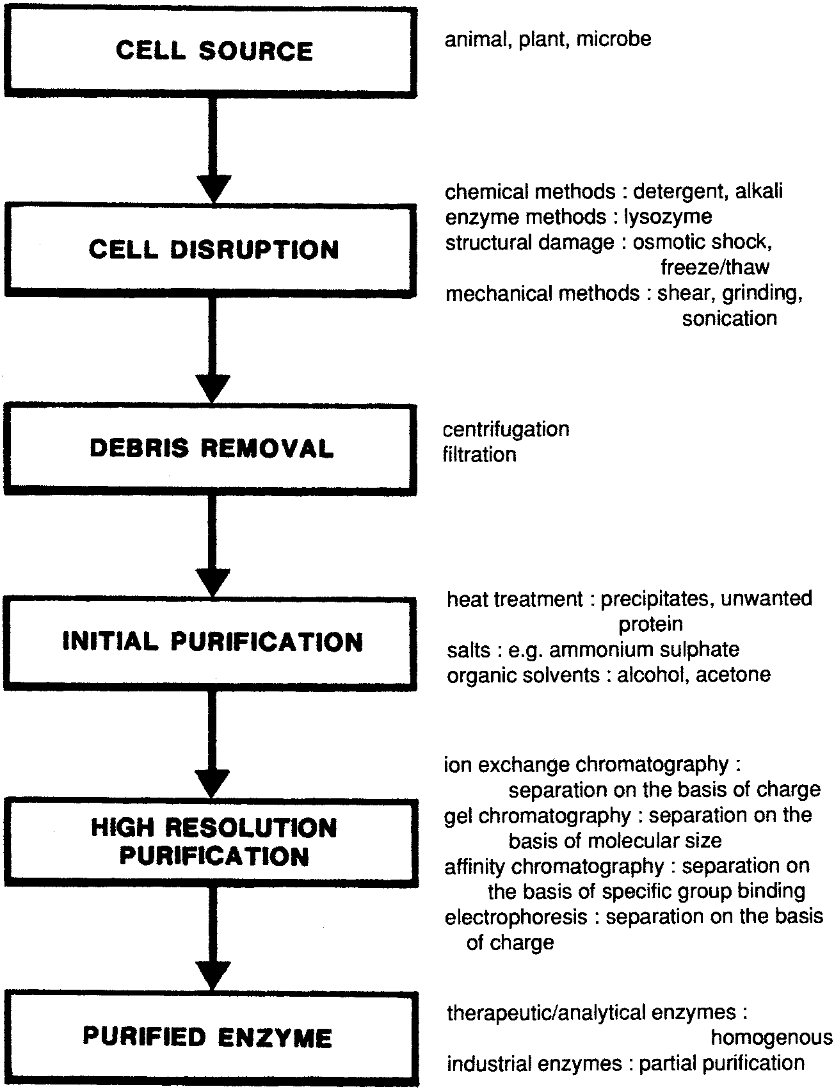
Purification methods rely on the fact that most enzymes are globular, charged macromolecules. However, as the enzyme is purified it is usually more readily denatured. Not only can the polypeptide chain partially unfold, but also when an enzyme is exposed to reagents such as organic solvents, aggregation or precipitation can occur with consequent loss of activity. The presence of contaminating proteases in an initial homogenate may hydrolyse the enzyme or it may be poisoned by the presence of metal ions or oxygen.

Many of the methods initially used to extract the enzyme from the cell were developed empirically. When more is known about the intracellular location of the enzyme, its chemical nature and requirements for maintaining catalytic activity, a more rational approach to method selection becomes possible. During the extraction the temperature is normally held at around 0°C and careful control of pH is essential.

Once the cell debris has been removed, the enzyme in solution is frequently partially purified and concentrated by the use of ammonium sulphate. This salt is ideal because of its high solubility, low toxicity to enzymes and low cost. As the percentage saturation of the salt increases, different enzyme proteins precipitate from solution and can be recovered.

Subsequently a large number of techniques may be used for further purification, and steps involving chromatography are standard practice.

Ion exchange chromatography is often effective at early stages in the fractionation. The protein solution is added to an insoluble polymer e.g. cellulose, containing ionic groups whose charge will determine the type of mobile ion (cation or anion) it attracts. Proteins whose net charge is opposite to that of the ion exchange material will bind and be displaced from solution. A subsequent change in pH or the introduction of salt can alter the electrostatic forces, allowing the protein to be released again into solution.



animal, plant, microbe

chemical methods : detergent, alkali
enzyme methods : lysozyme
structural damage : osmotic shock, freeze/thaw
mechanical methods : shear, grinding, sonication

centrifugation
filtration

heat treatment : precipitates, unwanted protein
salts : e.g. ammonium sulphate
organic solvents : alcohol, acetone

ion exchange chromatography : separation on the basis of charge
gel chromatography : separation on the basis of molecular size
affinity chromatography : separation on the basis of specific group binding
electrophoresis : separation on the basis of charge

therapeutic/analytical enzymes : homogenous
industrial enzymes : partial purification

Fig. 6 Methods of enzyme purification

Gel filtration can be used at later stages, to resolve molecules on the basis of molecular size. Highly cross linked gels (Sephadex) are used whose particles exclude large protein molecules while allowing smaller ones to diffuse within them. Complete separation can occur because large molecules follow a shorter path (i.e. can occupy a smaller fraction of the column volume) than smaller ones and are recovered first.

Affinity chromatography represents the most important recent advance in enzyme purification. The biological affinity of an enzyme for a substrate analogue bound to a solid support is exploited. Only proteins that recognise the immobilised substrate analogue are retained: other molecules pass through unretarded. Subsequently a solution containing a competitive inhibitor can be introduced to release and recover the bound molecule. This technique is capable of rapidly and highly successfully isolating an enzyme and provides a high degree of purification in a single step.

4. Uses and Applications of Enzymes

i) Enzymes have a wide commercial application

Because of their enormous catalytic power in aqueous solution at normal temperatures and pressures, enzymes are of great commercial and industrial importance. Enzymes such as proteases have been used for centuries in cheese making and in the leather industry. Even the use of enzymes to wash clothes is not new: pancreatic enzymes were patented in 1913 for this purpose, but the unstable nature of the preparation limited its use to pre-soak operations. In 1953-64 however, Novo Industries of Denmark developed an alkaline protease, subtilisin, which is secreted in large amounts from *Bacillus subtilis*, for use in bioactive detergents. It is stable at high pH in the presence of phosphate and other detergent ingredients and its proteolytic activity is retained up to a temperature of 60°C.

A major concern of modern biotechnology is the production of enzymes and their industrial application. In 1981 it was estimated that the world market for commercially produced enzymes was around 65,000 tons and valued at £200 million. Enzymes are currently used in the following areas:

(a) Food and Agricultural Industry
(b) Pharmaceutical Industry
(c) Chemical Industry
(d) Analytical Methods
(e) Medical Research.

Table I summarises the current use of various enzymes.

ii) Enzymes from microrganisms

About 200 enzymes have so far found a practical application, and the majority of these originate from a limited number of fungi, yeast and bacteria. When a new enzyme is required these organisms are often first considered as a source. Stringent regulations exist for food grade enzymes, and if a microbial source is to be used, production is limited to food compatible microorganisms. Microbes are the preferred source for enzyme production because:

(a) Animal and plant sources require careful and planned collection and storage of raw material. Microbes represent a convenient source independent of an external supply of raw materials. They can be grown up in fermenters whose capacity can easily be altered to match fluctuations in enzyme demand.

Table I Application of Enzymes

Enzyme	*Reaction*	*Source*	*Application*
Industrial			
α-Amylase	Starch hydrolysis	Bacillus sp	Conversion of starch to glucose or dextrans in food industry
Amyloglucosidase	Dextrin hydrolysis	*Aspergillus* sp	Monomeric glucose production
Glucose isomerase	Glucose to fructose conversion	*Streptomyces* sp	Production of high *fructose syrups*
β-Galactosidase	Lactose hydrolysis	*Aspergillus* sp	Hydrolysis of lactose in milk or whey
Proteases	Protein digestion	*Bacillus* sp	Laundry aid
Penicillin acylase	Benzoyl side cleavage	*E. coli*	6-APA production for semi-synthetic use
Aminoacylase	Hydrolysis of acylated L-amino acids	*Aspergillus* sp	Resolution of racemic *mixtures*
Medical			
L-Asparaginase	Removal of L-asparagine essential for tumour growth	*E. coli*	Cancer chemotherapy particularly leukaemia
Urokinase	Plasminogen activation	human urine	Removal of fibrin clots
Analytical			
Glucose oxidase	Glucose oxidation	*Aspergillus niger*	Detection of glucose in blood
Luciferase	Bioluminescence	marine bacteria/ firefly	Bioluminescent assays for ATP etc.
Peroxidase	Dye oxidation using H_2O_2	Horseradish	Quantification of hormones or antibodies
Urease	Urea hydrolysis to CO_2 and NH_3	Jack beans	Measurement of urea in body fluids
Manipulative			
Lysozyme	Hydrolysis of 1-4 glycosidic bonds	Hen egg white	Disrupts mucopeptide of bacterial cell walls
Nucleases	Hydrolysis of phosphodiester bonds of nucleic acid	Bacteria	Genetic manipulation

(b) Microbes grow on a wide range of substrates and their enzyme producing potential is therefore enormous. Inducer molecules can be added to fermenters to enhance enzyme production. Repression of enzyme synthesis by natural feedback mechanisms can be avoided by limiting end product accumulation and replacing rapidly utilised sources of carbon and nitrogen with nutrients that can be consumed more slowly, e.g. starch or soya bean meal.

(c) Microbes can be treated with mutagens (chemical or physical) to produce minor changes in enzyme structure that can result in altered enzyme characteristics, for example development of insensitivity to a natural inhibitor. While such changes may be detrimental to the organism, they can be exploited industrially, and it can be advantageous to bypass regulatory mechanisms that would normally prevent the overproduction of an enzyme. Thus enhanced amounts of enzyme can be produced in such mutants.

iii) Enzymes from animal cells are important

A wide spectrum of biological molecules are produced from animal sources including hormones, antibodies and polypeptide growth factors. However only a few of the thousands of enzymes produced in animal cells have a current potential market. Then they are often used as therapeutic agents or analytical reagents.

Such an example are the **plasminogen activators**. These are a group of proteolytic enzymes that act on an inactive blood precursor molecule called plasminogen and by removing part of it, produce the active enzyme plasmin. Plasmin destroys the fibrin network of a blood clot. Urokinase is the best known human plasminogen activator, but it is expensive to produce and a course of treatment could cost £5000, requiring urokinase to be extracted from 5000 litres of urine. In contrast, plasminogen activators produced by tissues are more specific in their action, and can be produced by human cells grown in cell culture. While this offers the potential for a cheap and efficient fibrinolytic drug, the large scale culture of animal cells does present greater problems than those encountered with microorganisms. **Streptokinase**, a bacterial enzyme, can also be used to activate plasminogen, but it is relatively non-specific in its action and may produce a severe immunological reaction from the patient.

An alternative approach to anticoagulant therapy is to use snake venom enzymes. **Arvin**, a protease obtained from the Malayan pit viper enzymically disperses the fibrin precursor fibrinogen, thereby reducing its role in clot formation. Even the medicinal leech is making a comeback, and several products are being developed from these animals to control blood clotting and promote research on connective tissues and arthritis.

iv) Enzyme technology is rapidly developing

Recent developments in microbial genetics have created a new potential for enzyme production. The enzyme industry is expected to expand as genetic engineering (recombinant DNA technology) is applied to the microbial production of enzymes.

The techniques of genetic engineering can be used to manipulate DNA such that multiple copies of a particular gene encoding an enzyme of commercial value can be made. This is achieved by attaching the gene of interest to a small bacterial DNA molecule that can replicate independently of the chromosomes. The extra-chromosomal DNA (plasmids) found in many bacteria can be conveniently isolated for this purpose. The gene is spliced into the plasmid forming a recombinant DNA molecule that can be inserted into a suitable host (e.g *E. coli* or *B.subtilis*). The plasmid molecules are then allowed to replicate at a higher rate than the chromosomal DNA producing several hundred copies per cell. Large amounts of the protein encoded by the cloned gene can be made, provided it can be expressed i.e. transcribed into RNA and then translated into protein by the bacterial cell. Cloned prokaryotic genes can be expressed in a bacterial host if suitable regulatory sequences are present before the start of the coding sequences.

Eukaryotic genes can also be cloned in bacterial hosts but production of an active protein does not always occur because many eukaryotic enzymes are only active if they are modified after translation. This can involve adding sugar residues or peptide cleavage, and prokaryotes cannot carry out these modifications. However yeast and fungi which can carry out this post-translational modification are now being developed as hosts for gene cloning. A number of biologically active mammalian proteins have been produced using yeast, including the enzymes lysozyme, chymosin (rennin) and tissue plasminogen activator. Because of the low cost and ease of bulk yeast fermentations, yeast is a particularly attractive host for proteins required in large quantities.

Gene cloning also provides the opportunity to redesign enzymes in a rational way. Termed enzyme engineering, this involves altering a precise amino acid residue in the native enzyme to improve its characteristics. Methods exist to modify a specific base in a DNA strand (site directed mutagenesis) and then using recombinant-DNA technology, to produce a redesigned enzyme — although at present the result of such amino acid engineered changes cannot be predicted.

These, developments are already being applied to industrial situations. **Chymosin** (rennin) is one of the most important proteases in the food industry. It coagulates milk for cheese manufacture by limited proteolysis. Traditionally chymosin was obtained from the fourth stomach of the unweaned calf, but its relative scarcity had led to the use of substitutes, often of microbial origin. Recently microorganisms have been genetically engineered to produce rennin.

α-Amylase from *B. subtilis* is denatured by heat. The α–amylase gene from a thermophilic organism can be inserted into *B. subtilis* so that a thermostable enzyme can be obtained. The enzyme can therefore be used at higher temperatures to speed up the conversion of starch to glucose.

Subtilisin, used in bioactive detergents has had a key amino acid residue substituted with a suitable alternative using enzyme engineering. This makes the enzyme more resistant to oxidation.

5. Enzyme Stability and Immobilisation

i) Enzyme stability is important

Enzyme instability is one of the key factors that prevents their wider use. Enzyme structures have evolved in response to the metabolic requirements of cells and are well suited to this role. If these same enzymes are now considered as industrial catalysts, they may be exposed to unnatural non-physiological environments. Chemicals such as organic solvents, elevated temperatures and pH values outside their normal *in vivo* values can unfold (denature) the enzyme with consequent loss of activity. Enzymes that are stable to high temperatures may often be more resistant to organic solvents or extremes of pH and there is considerable interest in thermostable enzymes. By allowing processes to occur at higher temperature than normal, reaction rates are elevated and the risk of microbial contamination is reduced. **Thermophilic bacteria**, that can grow in the temperature range 65-75°C and survive at high temperatures produce thermostable enzymes. Their potential is difficult to exploit directly as methods have not been found to cause them to overproduce a particular enzyme. This can be overcome using recombinant-DNA techniques, and transferring the gene for a thermostable enzyme from a thermophile into an appropriate organism, such as *B. subtilis.*

The expense associated with enzyme use increases if the enzyme is considered to be a soluble reagent which is lost at the end of the process and not recovered. Enzymes for industrial processes are far more valuable in an insolubilised form, since the enzyme should be more stable and capable of being separated easily from the reactants and possibly re-used.

ii) Enzyme immobilisation has advantages

Immobilisation of an enzyme on to an inert insoluble polymer offers the potential for enhancing the stability of an enzyme. Other advantages include:

(a) Enzymes can be retained and re-used, reducing overall costs.
(b) Enzymes can easily be added to or removed giving greater control over the reaction. Problems of separating the catalyst from the products are practically eliminated.
(c) Continuous processes using columns of immobilised enzyme become more practical and automation is possible.
(d) Enzymes may be stabilised against heat or solvent effects.

Enzyme immobilisation may enhance enzyme stability because once attached, the intensity of conformational changes that the protein can undergo may be reduced and hence denaturation made less likely. Unfortunately this is not always true and depending on the method of immobilisation, the enzyme may not be stabilised at all. It is also possible to immobilise whole cells rather than individual enzymes. This is very useful if the enzyme of interest is membrane bound and unstable in a solublised state. In such a situation, permeability barriers must be destroyed by proper treatment of the cells and any undesirable reactions eliminated. Thus most commercial preparations of **glucose isomerase** comprise the enzyme immobilised within the cells that produced them. The immobilisation process usually involves heat treatment which inactivates unwanted enzymes, but barely alters the activity of the glucose isomerase. Intact cells also offer the opportunity to immobilise living multienzyme reactions. The continuous production of ethanol by immobilised growing yeast and the production of vinegar using immobilised acetic acid bacteria are two such examples.

iii) Enzymes can be immobilised to a wide range of materials

There exist a large range of insoluble materials that can bind enzymes and a variety of techniques for achieving this. A few examples of the way in which β-galactosidase has been immobilised are given below:

Material	*Method of attachment*
Agar gel	entrapment
Cellulose	adsorption/covalent
DEAE-cellulose	adsorption/covalent
Polyacrylamide	entrapment
Glutaraldehyde	polymerisation
Porous alumina	adsorption/covalent
Porous glass	adsorption/covalent

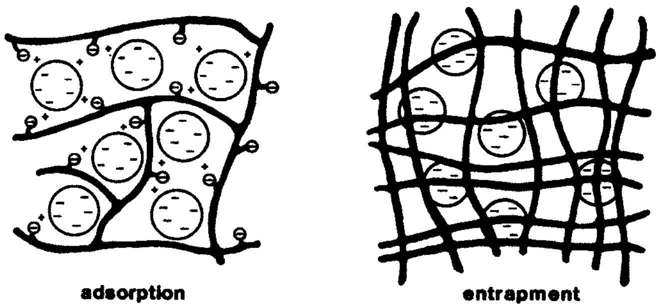
Both inorganic and organic materials can be used. Inorganic materials have the advantage of not being susceptible to microbial attack, as well as having a greater structural and operational stability. Common organic supports for covalent coupling include cellulose derivatives which have free hydroxyl or amino groups that can participate in linking with groups on the enzyme. It has been found that in order to maintain an enzyme in an active state after immobilisation, the amount of bound water is an important factor. This places hydrophilic gels (e.g.

agar, polyacrylamide) that effectively entrap enzymes in a very favourable position among immobilised enzyme matrices.

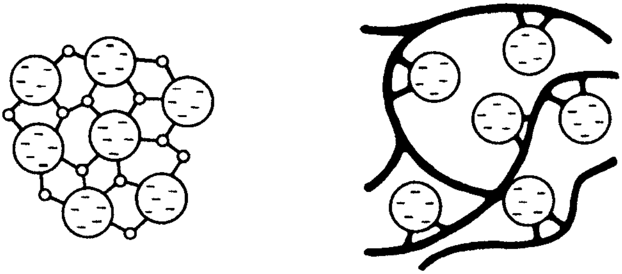
Frequently immobilisation causes a change in the properties of the enzyme. As well as influencing the stability of the preparation, its optimum pH and affinity for substrate can alter. This relates directly to the new **microenvironment** provided by the polymer matrix. The substrate not only has to diffuse through the matrix to the active site, but if it carries a net charge, it can be profoundly influenced by the presence of ionic groups in the polymer.

iv) Enzymes can be immobilised in a number of ways

Four principal methods exist to immobilise enzymes (see Fig. 7). Each has its own advantages and disadvantages and no one method is ideal for all situations. Sometimes more than one approach is adopted in an attempt to maximise enzyme activity and stability.



adsorption **entrapment**



cross-linking **covalent bonding**

Fig. 7 Four principal methods to immobilise enzymes

(a) **Adsorption.** The oldest technique. The types of binding forces are weak, e.g. ionic bonding, hydrogen bonding and hydrophobic interactions. It is simple and inexpensive and no chemical reagents are needed. While the enzyme is less likely to be denatured as a consequence, desorption can occur quite easily. Being non specific, other materials may also be adsorbed as well.

(b) **Entrapment**. A popular technique. The enzyme is trapped within the internal structure of a polymer. As the enzyme is retained rather than bound, there is very little change in its catalytic properties. In many instances no reactive chemical reagents are used. **Alginate**, a polysaccharide from brown seaweeds, forms a viscous solution in which whole cells and enzymes can be suspended. The addition of calcium ions causes gel formation and entrapment under mild conditions. In contrast **polyacrylamide** gels involve the use of toxic monomers and these may inactivate the enzymes. All these methods are limited to enzymes whose substrates are of small molecular size so that they can diffuse through the pores of the matrix.

(c) **Cross-linking**. Covalent linkages between macromolecules can be formed using bifunctional cross-linking reagents such as glutaraldehyde. Some enzymes may lose biological ativity when linked in this way, however.

(d) **Covalent bonding**. A commonly used but expensive method. Chemical reagents are used to activate groups associated with the polymer matrix, so that these can react with amino acid residues on the protein to form a stable covalent linkage. The active site of the enzyme has to be protected to ensure only non-essential enzyme residues are involved. The enzyme will not leak from the preparation.

6. Examples of Enzymes at Work

i) Enzymic production of high fructose syrups

Starch is the major carbohydrate reserve of most higher plants, occurring as water insoluble granules. It comprises two glucose polymers: amylopectin, a highly branched polysaccharide that makes up 75-85% of most starches; and amylose, a linear polysaccharide. Corn starch is a very cheap feedstock, especially in the USA. Its enzymic hydrolysis to glucose followed by partial isomerisation to fructose, is the basis of a highly successful industrial process involving the large scale use of microbial enzymes (see Fig. 8). The final product, **high fructose syrup**, is used to replace sucrose as a sweetener in soft drinks and confectionery. Glucose has only 70% of the sweetness of sucrose, while fructose is twice as sweet as glucose. Thus if glucose can be converted into fructose, its commercial value is greatly increased.

Three enzymes are involved, bacterial α-amylase, fungal amyloglucosidase and bacterial glucose isomerase. The process is summarised in Figure 8. A starch slurry is briefly heated to 105°C, the granules swell and gelatinise giving a paste. α-Amylase from *Bacillus licheniformis* is added at this stage; it is a very thermostable enzyme and will initiate starch hydrolysis and reduce the viscosity of the solution. The temperature is then lowered to 90°C and hydrolysis continued for 1-2 hours. α-Amylase is an endoenzyme that hydrolyses bonds within the polysaccharide molecule so that long chains are broken into smaller branched or linear units (maltodextrins).

The next step involving the saccharification of dextrins to glucose is carried out by a soluble amyloglucosidase from *Aspergillus niger*. The substrate is cooled to 55-60°C and acidified to pH 4.5 before the enzyme is added. This process is carried out in large reactor tanks and 48-72 hours are needed to achieve maximum glucose levels. Amyloglucosidase is an exoenzyme removing glucose in a stepwise manner from the non-reducing chain ends of dextrins. When a branch point is reached, however, glucose is released at a much slower rate. Immobilised amyloglucosidase has been developed to reduce the space required for this step, but it is not extensively used as the maximum glucose content achieved is less than that obtained with the soluble enzyme.

The resulting glucose syrup, after concentration and refining, can be used as such or converted into fructose. This process involves glucose isomerase,

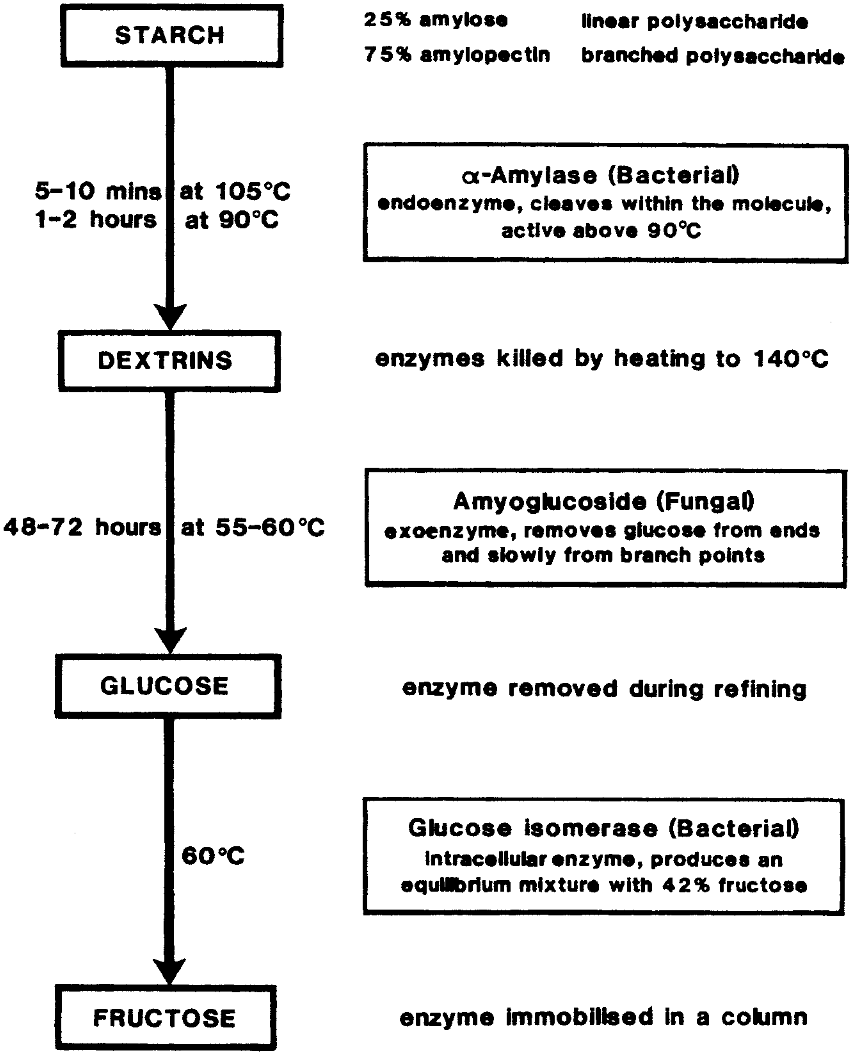


Fig. 8 Enzymic hydrolysis of starch

produced by *Bacillus* or *Streptomyces* sp. Glucose isomerase is an intracellular enzyme bound to cell membranes and it is expensive to both extract it and use it as a soluble reagent. It is therefore normally immobilised by either ionic binding to DEAE-cellulose or cross-linking the cells with glutaraldehyde. The enzyme is immoblised in rigid granules, packed into a column, and is fully stable at 60°C in a 50% w/v solution of glucose substrate for between 1000-2000 hours. This is a very successful application of an immobilised enzyme in the food industry.

The process yields a high fructose syrup containing 42% fructose and 55% glucose, with the same sweetening power as sucrose. While the total annual production of high fructose syrup is estimated at 4 million tons, its use in Europe is limited, because of an overproduction of sucrose from sugar beet.

Fructose has the same calorific value as glucose but as a nutrient for humans it can give rise to metabolic side effects. Whereas glucose utilisation requires insulin, fructose utilisation is less controlled and is not dependent on this hormone. The rapid metabolism of fructose by the liver promotes lactic acid production which can be harmful in certain situations. Currently there is a demand for low-calorie sweeteners and the development of **Aspartame**, a dipeptide composed of aspartic acid and phenylalanine methyl ester has met with great success. Being 180 times sweeter than sucrose it can be used in small non-nutritive amounts. The commercial production of aspartic acid necessary for this compound involves the enzyme **aspartase**, which is retained within immobilised *E. coli* cells and utilises fumaric acid and ammonia as substrates. The future use of Aspartame may profoundly affect the demand for high fructose syrups.

ii) Enzymes in the production of semi-synthetic penicillins

The search for new, more effective and non-toxic antibiotics is important to help combat both naturally resistant microorganisms and those that have acquired resistance through mutation. The natural antibiotic penicillin G (Pen G) is produced on a multi-tonne scale and manufactured by fermentation using the organism *Penicillium chrysogenum.* Given water, nutrients and phenylacetic acid, the organism will generate Pen G over a period of ten days as a secondary metabolite in the fermentation media, when the growth of the organism has slowed. A basic outline of its production is given in Figure 9.

Pen G is a strong antibacterial agent whose effectiveness depends on the presence of the β-lactam ring in the molecule. Increasing numbers of bacteria are becoming resistant to it because they produce a β-lactamase enzyme, which opens

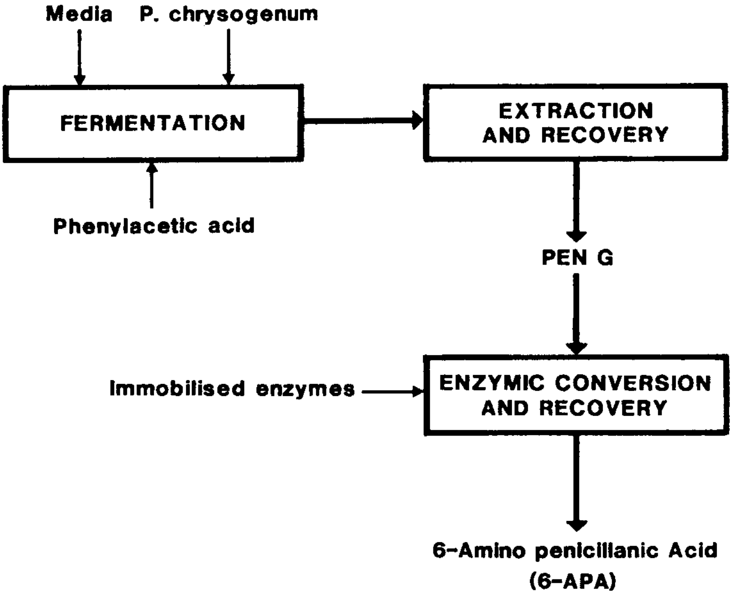


Fig. 9

up the ring and produces a biologically inactive product, penicilloic acid. This is summarised in Figure 10.

To combat this and to optimise the biological activity of the penicillin nucleus, various pharmaceutical companies are now producing a range of semi-synthetic penicillins. These are prepared by first converting Pen G into 6-amino penicillanic acid (6-APA) using the microbial enzyme penicillin acylase. 6-APA is the primary building block on which various chemical side groups can be added, to produce a large number of compounds known collectively as the semi-synthetic penicillins. Thus 6-APA can be reacylated with phenylglycine to give ampicillin which has a broader anti-bacterial spectrum than Pen G.

Penicillin acylase from *E. coli* can be immobilised by adsorption or covalent binding to organic or inorganic carriers and used in the enzymic hydrolysis of Pen G to 6-APA. Incidentally, one of the reagents used to introduce a new side chain, D-phenylglycine, may also be produced by means of the enzyme amino-peptidase. This makes it possible to separate the racemic mixture D,L-phenylglycine into its optical isomers.

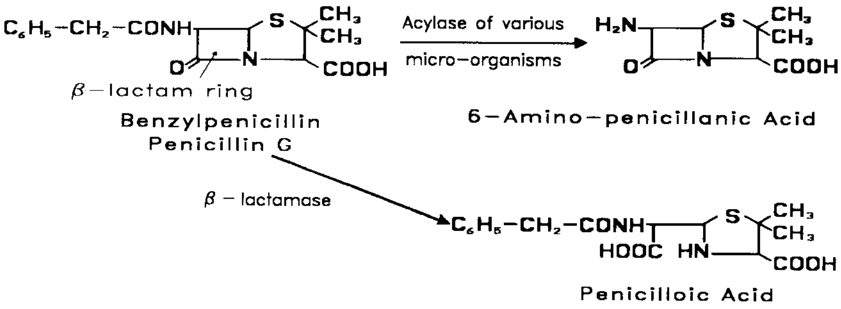


Fig. 10

iii) Enzymes in analysis — Glucose oxidase

Because of their sensitivity and specificity, enzymes are widely used as reagents in analysis. Glucose oxidase amply illustrates the diversity of analytical systems that can be developed from a single enzyme. The reaction it catalyses is as follows:

$$\beta\text{-D-glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

The quick and accurate measurement of glucose is of great importance both medically and industrially. A simple quantitative procedure can be devised by coupling the production of hydrogen peroxide to the activity of the enzyme peroxidase.

$$\underset{\substack{\text{chromagen}\\ \text{a hydrogen donor}\\ \text{(colourless)}}}{DH_2} + H_2O_2 \xrightarrow{\text{peroxidase}} 2H_2O + \underset{\substack{\text{coloured}\\ \text{compound}\\ \text{(colour)}}}{D}$$

Peroxidase can oxidise an organic chromogen (DH_2) to a coloured compound (D), utilising the hydrogen peroxide. The amount of D produced is a direct measure of the amount of glucose reacted and therefore it can be measured quantitatively using a colorimeter. This method of glucose analysis is highly specific and is used routinely in many laboratories. The specificity of the enzyme allows glucose to be assayed in the presence of other sugars, e.g. in a biological fluid, without the need for an initial separation.

Both glucose oxidase, peroxidase and DH_2 can be immobilised on a cellulose fibre pad. This forms the basis of the glucose dipsticks (e.g. 'Clinistix') which were developed to enable diabetics to monitor their own blood or urine glucose levels. The amount of glucose can be determined semiquantitatively using a photometer.

Glucose oxidase was one of the first enzymes to be incorporated into the design of an **enzyme electrode**. The enzyme can be immobilised using either polyacrylamide or glutaraldehyde onto the surface of an oxygen electrode. This measures the amount of oxygen dissolved in a solution. When the glucose oxidase–oxygen electrode probe is placed into a glucose solution the glucose diffuses into the immobilised enzyme layer and immediately oxygen is consumed. The principle of the enzyme electrode is summarised in Figure 11.

The electrode responds to oxygen uptake and monitors this amperometrically. Providing that the glucose concentration in solution is below the K_m for the immobilised enzyme, the rate of oxygen uptake is proportional to the glucose concentration.

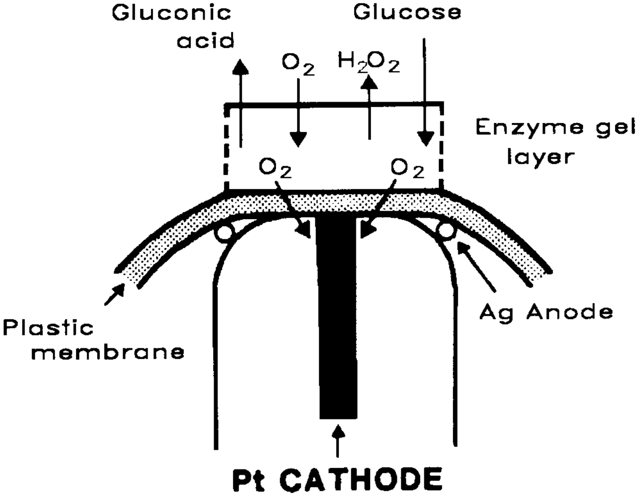


Fig. 11

The term **biosensor** has been introduced to describe the association of a biomolecule, such as an enzyme, with a transducer which produces an electrical signal in response to substrate transformation. The scope and range of these systems can be expanded if several enzymes are immobilised together and linked so that the product of one is the substrate for another. Thus the concentration of high molecular weight polysaccharides such as starch can be determined by co-immobilising amyloglucosidase with glucose oxidase in a membrane, and adding α-amylase to the starch solution. Dextrins and maltose are produced from the starch, and the low molecular weight products diffuse into the enzyme membrane. Amyloglucosidase converts them to β-D-glucose which is oxidised by the indicator enzyme glucose oxidase.

Other enzyme sensors have been described, for example sucrose determination can be achieved by co-immobilising invertase and glucose oxidase. Biosensors are now being commercially developed to provide a rapid, sensitive and specific measurement of molecules that are of either clinical or industrial importance, or that can act as pollutants of the environment.

7. Not all Enzymes are Proteins

While most biochemical reactions are catalysed by proteins, it has been observed that RNA, traditionally associated with the transfer of genetic information from DNA to protein, may act as an enzyme. **Ribozyme** is the term given for enzyme-like RNA, and its implication is that proteins can no longer be considered exclusively responsible for the complete catalytic activity of a cell.

RNA catalysis was first observed in the protozoan *Tetrahymena* during RNA processing. Eukaryotic genes are 'split' and contain intervening sequences (introns) between the coding sequences (exons). Once a gene is transcribed into a large RNA molecule, the introns are removed and exons spliced together to form a functional RNA molecule. During the processing of ribosomal RNA, an intron has been shown to catalyse both its own removal and the splicing together of adjoining exons, with no assistance from protein enzymes. A strategy analogous to that used by enzymes is involved. Binding sites are generated by the three-dimensional folding of the RNA intron for a molecule of GTP and the end of one exon, so they are brought together in close proximity. This facilitates the cleavage of a phosphodiester bond between the intron and exon and the subsequent splicing together of RNA exons.

While this example of RNA self-splicing has many enzyme-like properties, a ribozyme has been discovered that operates on other molecules rather than itself. **Ribonuclease P** is a tRNA-processing enzyme found both in bacteria and in higher cells. It is unusual in that it is not a pure protein, but is an association of a small RNA molecule with a small protein. The RNA molecule is the catalytic component and will cut the tRNA precursor at a specific point while the protein lacks this ability.

In all examples to date, the substrate for ribozyme has been RNA; it is unlikely that it can form a good active site with other biological molecules. Yet the consequences of RNA having the dual capacity for both carrying genetic information and demonstrating catalytic activity are only beginning to be realised. It may well be, for example, that the RNA component of the ribosome has a direct catalytic role in the synthesis of proteins. From an evolutionary point of view, perhaps the first enzymes consisted of catalytic RNA molecules, indeed the possibility exists that the early replication of RNA without the assistance of functional proteins was crucial to the development of life.

8. Practical Work

Below is a selection of published practicals that could form the basis of work for A-level biology students. Experiments have been chosen to supplement the ideas and concepts developed in the preceding sections.

Boiret M, Marty A, Deumie M. (1985) *Distribution of activity of tyrosinase in the mushroom.* Biochemical Education, Volume 13, No 2, pp 82-84

Conlon HD, Walt DR. (1986) *Immobilisation of enzymes in polymer supports.* Journal of Chemical Education, Volume 63, No 4, pp 368-370

Myers A. (1987) *Suitable class experiments in biochemistry for high school chemistry and biology courses.* Biochemical Education, Volume 15, No 4, pp 179-183

Ragatz BH, Werth DK, Bonner JF. (1984) *Factors influencing the rate of an enzyme catalysed reaction.* Biochemical Education, Volume 12, No 2, pp 60-64

Russo SF, Moothart L. (1986) *Kinetic study of the enzyme lactase.* Journal of Chemical Education, Volume 63, No 3, pp 242-243.

Woolridge E, Turchi S, Edwards JR. (1986) *The peroxidase-glucose oxidase enzyme system in the undergraduate laboratory.* Biochemical Education, Volume 14, No 2, pp 82-83.

9. Books

Bickerstaff GF. (1987) *Enzymes in industry and medicine.* New Studies in Biology. Edward Arnold.

Gacesa P. (1987) *Enzyme technology.* Open University Press.

Higgins IJ. (1985) *Biotechnology: principles and applications.* Blackwell Scientific.

Ingle MR. (1986) *Enzymes, energy and metabolism.* Studies in Advanced Biology 3. Blackwell Scientific.

Priest FG. (1984) *Extracellular enzymes.* Aspects of Microbiology. Van Nostrand Reinhold/American Society Microbiology.

Primrose SB. (1987) *Modern biotechnology.* Blackwell Scientific.

Smith E. (1981) *Biotechnology.* IOB Studies in Biology 136. Edward Arnold.

Smith JE. (1985) *Biotechnology principles.* Aspects of Microbiology. Van Nostrand Reinhold/American Society Microbiology.

Woodward J. (1985) *Immobilised cells and enzymes.* Practical Approach Series. IRL Press.

Wynn CH. (1979) *Structure and function of enzymes.* IOB Studies in Biology 42. Edward Arnold.

Yanchinkski S. (1985) *Setting genes to work.* Penguin.

10. Teaching Resources

Recommended resources for use in teaching programmes concerning enzymes are listed below. For convenience they are divided into the following sections:

(i) **Practical kits**
(ii) **Audio-visual aids**
(iii) **Computer software**
(iv) **Industrial publications**

The prices quoted are correct at the time of going to press.

i) Practical kits

GB Biotechnology Ltd

Enzymes for school biotechnology — 5 Enzyme experiments. Teacher/Technician Booklet. Packets of enzymes.	£19.55

Griffin & George

Enzyme immobilization set — Immobilization of yeast cells as pellets. Investigating properties.	£26.46
Enzyme production set — Production of enzymes by three different organisms.	£ 9.03
Enzyme teaching kit — Urease; Amylase. Effects of pH, temperature, substrate concentration.	£20.12
Enzymes in industry — Investigations using pectinase, rennet, protease.	£27.80
Enzymes in the home — Investigations using rennet, proteases, biological washing powders.	£28.28
Fermentation kit — Beer and diastase production.	£19.50

Philip Harris Ltd

Basic enzymology kit — Invertase, Effects of temperature, substrate, enzyme concentration.	£14.95
Cellulose breakdown kit — Demonstration of cellulase production and activity.	£16.90
Cheesemaking kit — Cheese Production using rennet and microbes.	£3.25
Effect of pH on enzymes kit — Effects of pH on invertase and amylase.	£15.25

Effect of temperature on enzymes kit — Effects of temperature on amylase, invertase.	£11.85
Enzyme biotechnology kit — Investigation using rennet, protease, pectinase, wash powder enzymes.	£14.95
Enzyme specificity and inhibition kit — Invertase, amylase, inhibition by aniline.	£17.75
Fat breakdown kit — Investigations using lipase producing organism and enzyme preparation.	£15.75
Immobilised enzyme kit — Yeast entrapment, immobilised glucose isomerase and invertase.	£18.75
Introduction to enzymes — pH and temperature effects on activity of 3 enzymes.	£13.85
Microbial activity kit — Illustrates starch, protein, fat, glucose, nitrite catabolism.	£18.25
Nitrogen cycle kit — Outlines the transformations occurring in the soil.	£27.80
Pectin breakdown kit — Pectin breakdown and comparison of soils.	£14.85
Protein breakdown kit — Catabolism of casein and gelatin.	£15.75
Starch breakdown kit — Amylase assay using soil and other organisms.	£12.75

ii) Audio-visual Aids

Griffin & George

Enzymes — Effects of temperature, substrate, specificity, lock & key hypothesis (Slide set).	£ 6.54

Guild Sound and Vision Ltd

Enzymes S202 06F — Molecular biology, enzyme structure and function. Open University, Biology Form and Function Course (Video — off air licence available)	purchase cost £100.

Philip Harris Ltd

Enzymes, Novo Industry — Overhead transparency diagrams of enzymes in a variety of industrial uses.	£12.50
Food from microbes — Familiar food, Part 1, Microbial conversion of resources to food.	Film strip £20.50

Slide Centre

Enzymes — Structure, active site, effects of environmental variables, use in industrial production.	(Slide set) £7.00

iii) Computer software

D A Bender

Enzyme assay — Simulation of laboratory exercises.	£12.50

Elsevier-Biosoft

J Garratt & P Groves — *Enzlab* — Simulation exercises in designing and carrying out enzyme kinetic experiments.	£40.00
P Williams — *Enzpack* — A teaching program about enzyme kinetics and calculations.	£40.00

Philip Harris Ltd

Enzymes in industry, home and environment labdisc.	£8.95
Cycles in nature 1, labdisc	£8.95

IRL Press Ltd

Protein structure and enzyme activity — Structure and function, review questions.	£42.00

iv) Industrial publications

ABM Brewing and Food Group

Enzymes for industry — Properties and uses of enzymes

Enzymes for the brewing industry — Allied brewing and distillation, enzyme properties and usage in brewing.

Biocon UK Ltd

Biocon product list — Enzymes in dairying, alcohol production, baking.

Cytozyme (UK) Ltd

Burning issue — Enzymatic stubble treatments.

Seed plus — Enzyme hydrolysed protein complexes for seeds.

Stubble digester extra — Cellulose decomposition.

Genzyme Biochemicals Ltd

Genzyme — Assay methods, series of leaflets about products or services offered.

Gist — Brocades NV

Taking a look at the invisible — Enzymes for food, fabrics, detergents, etc.

Novo Enzyme Products Ltd

Enzymes at work
Enzymes for fruit juice processing
Enzymes for wine and grape juice
Novo enzymes for fuel alcohol
Novo enzymes for silk degumming
Novo enzymes for the alcohol industry
Novo enzymes for the brewing industry
Novo enzymes for the dairy industry
Novo enzymes for the detergent industry
Novo enzymes for the production of baked goods
Novo enzymes for the starch industry
Novo enzymes for the sugar industry
Novo enzymes for the textile industry

11. Addresses of Suppliers

Addresses of suppliers are listed below. Industrial publications are usually available free of charge, on written application.

ABM Brewing and Food Group
Poleacre Lane, Woodley, Stockport, Cheshire SK6 1PQ
D A Bender
Dept. Biochemistry, University College, Gower Street, London WC1E 6BT
Biocon UK Ltd
Erdiston, Tenbury Wells, Worcs.
Cytozyme (UK) Ltd
Bourn, Cambridge, CB3 7TX
Elsevier-Biosoft
68 Hills Road, Cambridge CB2 1LA
GB Biotechnology Ltd
4 Beaconsfield Court, Sketty, Swansea, West Glam. SA2 9JU
Genzyme Biochemicals Ltd
Springfield Mill, Maidstone, Kent ME14 2LE
Gist – Brocades NV
PO Box 1, 2600 MA Delft, Holland
Griffin & George
Bishop Meadow Road, Loughborough, Leics. LE11 0RG
Guild Sound and Vision Ltd
6 Royce Road, Peterborough, Cambs. PE1 5YB
M T Heydemann
Edward Arnold (Publisher's) Ltd., 41 Bedford Square, London WC1B 3DQ
IRL Press Ltd
PO Box 1, Eynsham, Oxford, Oxon. OX8 1JJ
Novo Enzyme Products Ltd
9 Lion and Lamb Yard, West Street, Farnham, Surrey GU9 7LL
Philip Harris Ltd
Oldmixon, Weston Super Mare, Avon.
Rickitt Educational Media
Ilton, Illminster, Somerset TA19 9HS
Slide Centre
Ilton, Illminster, Somerset TA19 9HS

12. Acknowledgements

We would like to thank the following for giving us permission to reproduce diagrams in the text of this booklet.

Page 2 Fig. 1 Diagrammatic representation of substrate complexing with an enzyme active site. From: *Biological science* by Green, Stout and Taylor, Volume I (1984) Cambridge University Press.

Page 5 Fig. 2 From: *The theory of enzyme tests* by H Mattenheimer. Boehringer Mannheim GmbH.

Page 9 Fig .4 From: *The measurement of enzyme activity in the digestive gland of slug* Arion ater. by W A L Evans. In: *Experiments in physiology and biochemistry*, Kerkut G A (ed.) Volume I (1968). Academic Press.

Page 10 Fig. 5 From: *Immobilised enzymes and their application in the food and beverage industry.* by H H Weetal, Process Biochemistry, Volume 10, No 6 (1975). Turret-Wheatland Ltd.

Page 31 Fig.11 Diagram of enzyme electrode. Reprinted by permission from Nature, Volume 214, pp 986-988. Copyright © 1967. Macmillan Magazines Ltd.