Making Use of Biology

83p

Contents

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ISOLATION of ENZYMES

Intracellular and extracellular enzymes

Whilst there are many ways of classifying enzymes, one of the most straightforward is a classification based on whether the enzyme functions within or outside the cell which produces it.

Intracellular enzymes are produced inside cells and work inside cells. Examples include DNA polymerase which is involved in DNA replication in the nucleus, all of the enzymes used in cellular respiration, and all of the enzymes involved in photosynthesis.

Extracellular enzymes are produced inside cells but released from cells to work outside of the cell. The most familiar are the digestive enzymes which are produced in cells of the digestive tract or in cells within digestive organs and released into the lumen of the digestive tract. These include salivary amylase produced by cells in the salivary glands of the mouth, and pancreatic lipase and amylase produced by cells of the pancreas and released into the small intestine. In fungi and many bacteria, extracellular enzymes are secreted onto food materials to allow extracellular digestion. Such enzymes are often of great commercial interest.

Enzymes in industry: advantages

Enzymes are biological catalysts, and have a number of unique properties which distinguish them from inorganic catalysts. These properties, which are listed below, make them ideal for commercial use, especially on grounds of economy.

- Enzymes are highly specific to one substrate. Specificity ensures that only the desired reactions will take place, leading to the production of a pure product. This is particularly crucial in the pharmaceutical industry where enzymes are used to indicate the presence of one substance within a complex mixture.
- Enzymes work at low temperatures (usually below $40^{o}C$) compared to some inorganic catalysts which need temperatures of over $500^{o}C$. This reduces the cost of many processes and also makes them safer. In some industrial processes, however, enzymes that work at temperatures over $60^{o}C$ are used.
- Enzymes work at atmospheric pressure whilst some inorganic catalysts will only function at much higher pressures. Again this reduces cost.
- Enzymes are less toxic than many inorganic catalysts, making them more suitable for a variety of processes, particularly in the food and drink industry.

Enzymes in industry: disadvantages

Enzymes do, however, have some disadvantages compared to inorganic catalysts.

- Enzymes are proteins with a complex 3D shape upon which their activity depends, therefore their efficiency will be affected if the temperature or pH changes from the optimum. At extreme pHs and high temperatures they are irreversibly denatured.
- Enzyme action may be inhibited by other substances present in a mixture, including the products of their own reactions.

Application of enzymes in biotechnological processes

Examples of commercial applications of enzymes:

- Dairy products: forms of rennin and rennin substitutes, e.g. chymosin (rennilase) used to cause milk to coagulate in the production of cheese
- Fruit juices, wines: pectinases used in the removal of pectins and starches from juice and wines to prevent cloudiness.
- Brewing: amylases and proteases to digest polysaccharides and proteins in beer and to prevent cloudiness.
- Sugar: use of amylases and other carbohydrases to produce fructose and glucose from corn (maize) starch. In the processing of cane sugar (sucrose) carbohydrases are also used to remove polysaccharides.
- Meat: proteases (papain) used to tenderize meat by partially digesting protein fibres before sale.
- Baking: carbohydrases used to weaken gluten and produce soft textured cakes and breads.
- Additives: emulsifiers and flavourings for foods may be produced using enzymes.
- Alcohol production (for drinking and for fuel): amylases and other carbohydrases are used to convert unfermentable starches to fermentable sugars which yeast can utilise. In the production of alcohol for fuel the starting point is agricultural waste, particularly straw.
- Animal feeds: various enzymes are used to improve the nutritional value of foodstuffs.
- Paper industry: used in biobleaching, extraction of cellulose from wood (biopulping) and even in de-inking of recycled paper.
- Leather industry: various proteases used to remove hair from hides and to soften leather.
- Detergents: proteases, amylases and lipases used in 'biological' washing powders break down food stains on clothes. Also cellulases help to digest loose fibres in cotton, preserving the texture and appearance.
- Pharmaceutical, analytical and medical: a range of enzymes produced for use in testing kits such as the ELISA test, in biosensors (detecting glucose levels), and test sticks for glucose, proteins and uric acid.
- Waste treatment: various enzymes used to break down wastes including lignin, cellulose and other solid and toxic materials.
- Genetics: restriction enzymes (endonucleases) are used to cut DNA for use in genetic engineering, genetic fingerprinting. Polymerases are used to amplify DNA (section 11.3).

Enzymes as analytical reagents

Enzymes are ideal tools for use in biochemical analysis in both industry and medicine. They have indeed revolutionised many clinical tests which aim to detect the presence of certain compounds in biological fluids (blood, plasma, urine etc) and are widely used in hospitals and laboratories. They are also used in industrial processes, including the production of ethanol and other materials in fermenters. The two main advantages of enzymes over other analytical reagents are:

- Enzymes have a high specificity: They will only react with one specific substrate, even if that substrate is found in a complex mixture.
- Enzymes have a high sensitivity: they can catalyse reactions even when the substrate is present in very low concentrations.

Enzymes are used in three main types of system:

Diagnostic test strips: basic plastic strips containing a pad with an immobilised enzyme(s) (see below for discussion of immobilised enzymes), show a colour change if the substrate is present, and give a semi-quantitative result via a graded colour change. These are very cheap but can only be used once.

Enzyme electrodes or **'biosensors'**: contain an immobilised enzyme, a transducer and a tiny electric circuit. The enzyme catalysed reaction produces a product which generates an electric current in the transducer. The current is proportional to the amount of product (and hence substrate) present and is converted into a digital read out. These are small and reusable tools.

Enzyme analytical reactors: Large scale laboratory tools using soluble enzymes and colour changes to test hundreds of samples per day.

Examples of enzymes in enzyme electrodes used in medicine and industry:

Enzyme(s)	substrate/anylate
Alcohol dehydrogenase:	alcohol
Cholesterol esterase and cholesterol oxidase	cholesterol
Glucose oxidase and peroxidase	glucose
Urease	urea

Using enzymes to test for glucose

One of the most common uses of enzymes as analytical reagents in hospitals is in tests for blood glucose. The level of glucose in the blood is closely regulated in a homeostatic fashion by the pancreatic hormones insulin and glucagon. The normal level is around 90-150mg/100cm^3 of blood. Both high and low levels of glucose are harmful and are useful indicators of disease. In particular very high levels of glucose in the blood following meals are characteristic of the disease diabetes. This disease is also characterised by the presence of glucose in the urine. Glucose levels in blood and urine can be detected using the two enzymes: glucose oxidase and peroxidase.

For these reasons the development of immobilised enzymes has improved enzyme technology enormously. Immobilised enzymes are enzymes that have been attached to another (insoluble) material in some way and hence prevented from moving freely within the substrate mixture. They may be physically immobilised within a system by being attached to a fixed membrane or gel made of a range of substances including silica, starch, and cellulose. Processes used to attach the enzymes are adsorption, covalent bonding, and entrapment. In other cases the enzyme may move within the substrate but only once encapsulated within beads of another material. In the 'attached' systems enzymes never become mixed with the product, whereas encapsulated enzymes are mixed in with the final product but are easily extracted by filtering or other methods.

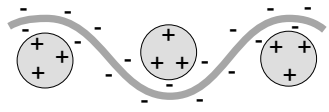
There are many advantages to immobilising enzymes, in addition to cost and ease of extracting a pure product.

- Immobilised enzymes may be more easily added to and removed from reactors to allow the rate of reaction to be more carefully controlled. This means that enzymes can be used to partially, but not completely, digest a substrate.
- Immobilised enzymes can lead to much greater production efficiency. Primarily they can be used in a continuous enzyme reactor where substrate is constantly added, and product constantly produced, without the need to add or extract enzyme. The best example of this is in the production of high fructose corn syrup described below.
- Immobilised enzymes tend to be more stable. In particular they are less likely to be inactivated or denatured by high temperatures (they are more thermostable), e.g. glucose isomerase discussed below is stable at 65^{o}C when immobilised but is denatured at 45^{o}C when free; by changes in pH, or the presence of other chemicals.
- Immobilised enzymes can also be used for longer before their activity decreases, and are less likely to lose activity during periods of storage.

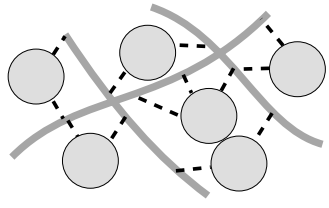
A disadvantage of immobilising enzymes is that the rate of reaction may be slower because the enzyme has reduced kinetic energy and is often partitioned from the substrate by a membrane through which the substrate must diffuse.

Immobilised enzymes-different systems

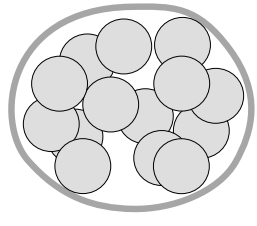
Absorbtion



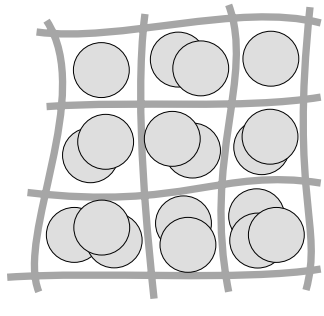
Covalent bonding



Encapsulation



Entrapment



The importance of thermostability

The continued profitability of the 'high fructose corn syrup' (HFCS) industry and other industries relying on enzyme based reactions has depended on the discovery of increasingly thermostable (heat tolerant) enzymes which can be used at temperatures up to and above 60 ^{o}C. Many of these enzymes come from bacteria which inhabit hot springs or live deep in the earth's core, where enzymes tolerant of temperatures up to 250^{o}C have been discovered. Enzyme thermostability is an essential feature of such industries for the following reasons:

- The higher the temperature, the lower the chance of contamination with micro-organisms which are generally inhibited at 60^{o}C.
- The higher the temperature the faster the rate of reaction. This makes the plant more efficient, as more substrate can be processed in a given time.

Forensic Examination of Blood

Contents

Principles of immunology

- Definition of antigen and antibody. The immunological response of B-lymphocytes
- ABO blood group antigens and agglutination

Genetic fingerprinting

- Genetic fingerprinting
  - restriction enzymes
  - electrophoresis
  - radioactive DNA probes

Polymerase chain reaction

- The polymerase chain reaction and its importance in obtaining increased amounts of DNA for analysis

PRINCIPLES OF IMMUNOLOGY

Definition of antigen and antibody

In order to remain healthy and able to function properly, the human body must protect itself from foreign material, particularly disease-causing organisms, known as **pathogens** Initially it must aim to prevent pathogens from entering the body in the first place, and then it must be able to defend itself against them if they do enter. The first lines of defence are therefore the barriers to infection.

If pathogens do manage to pass through these barriers and enter the body tissues including the blood, an immune system is necessary to attack and destroy these pathogens and so prevent serious disease or death. There are two branches of the immune system in operation within the body which co-operate with each other to some extent.

◆ SUMMARY OF BARRIERS TO INFECTION

- impermeable skin which covers the body, unless broken, when the blood clotting process is rapidly activated to plug the wound and prevent pathogen entry
- the mucous membranes of nose and respiratory tract trap pathogens in sticky mucus
- anti-bacterial enzymes in saliva, sweat and tears
- stomach acid (pH 2).

These are:

- **Non-specific immunity** which is not targeted at specific types of pathogen but at foreign material in general. This involves phagocytosis and inflammation and is immediate.
- **Specific immunity** which uses B and T lymphocytes to target specific pathogens. Such responses are more complex and are often delayed. B-lymphocytes are concerned with **humoral immunity**, involving antibodies, whilst T- lymphocytes are concerned **cell-mediated immunity** with the direct killing of infected cells.

Immunology is the study of the mechanisms by which the B and T lymphocytes recognise and and destroy foreign material which enters the body. In all cases, foreign or 'non-self' material (in the form of pathogens, in the form of human cells from other individuals (as in blood transfusions or organ transplants, or, in the case of allergic reactions, in the form of molecules on common products like peanuts) is distinguished from 'self' material by the presence of 'non-self' antigens.

Antigens

Antigens are defined as molecules which, when foreign to an individual, stimulate an immune response by lymphocytes in the body. They are often protein or glycoprotein molecules found on the cell surface membrane of 'non-self' cells, but a range of other molecules also act as antigens. Antigens on the cell surface are important in cell recognition. Under normal circumstances the immune system of an individual will recognise all of their own cells as being 'self' owing to the familiar types of antigens present. Any cells (or other materials) with different 'non-self' antigens are rapidly detected by white blood cells known as B-lymphocytes and T-lymphocytes and targeted for destruction.

The immunological response of B lymphocytes

B-lymphocytes are involved in the production of antibodies in response to foreign antigens which is known as humoral immunity.

On the cell surface membrane of B-lymphocytes are a number of specific antigen receptors or membrane bound antibodies. These are sites which may become attached to antigens on the surface of pathogens or other foreign material, leading to a sequence of events in which free antibodies are produced to destroy the foreign material.

There are many thousands of specific types of B-lymphocyte and each is capable of recognising only one specific antigen. This allows specific antibodies to be produced in response to a vast range of antigens. For example, there is one type of B-lymphocyte which will attach to the bacterium causing TB and cause the release of antibodies against TB, and another type which attach to a red blood cell of blood group B in the body of a person with blood group A, and cause the release of antibodies against the group B antigens. This specificity is due to slight differences in the shape of the antigen receptor protein on each type of B-lymphocyte.