# FACTFILE:
# GCE BIOLOGY

## ENZYMES

Rewarding Learning

## Enzyme Applications

### Learning outcomes

Students should be able to:

- demonstrate knowledge and understanding of diagnostic reagent strips using enzymes or inhibitors as biosensors (for example glucose monitoring strips or pregnancy tests);
- demonstrate knowledge and understanding of the importance of enzymes as biomarkers of disease including that some enzymes are only present or active during disease processes and that detecting the presence of these enzymes in clinical samples such as blood, urine and sputum can be used for diagnosis or monitoring of disease;
- demonstrate knowledge and understanding of applying inhibitors as therapeutic drugs, including the activity of enzymes that contribute to disease processes, which can be targeted with active site-directed inhibitors.

### 1. Enzymes as biosensors

Biosensors are defined differently by different sources, but here the term is used to mean a device which uses a biological material in the detection of a substance of interest.

Clinistix is a well-known example of a diagnostic reagent strip using an enzyme. They are used to test for the presence of glucose in urine and the mechanism of action involves the enzyme glucose oxidase. A paper pad soaked in the enzyme is attached onto a nitrocellulose strip and this is dipped in urine to test specifically for glucose. The glucose oxidase catalyses a reaction which results in the production of hydrogen peroxide from glucose. A second enzyme (peroxidase) is also included on the reagent strip, which uses this hydrogen peroxide to change the colour of a dye. The colour change can be compared with a reference chart in order to estimate the concentration of glucose in the urine.

*Girl with blood glucose meter*

In order to monitor the level of glucose in the blood, glucose oxidase can be incorporated into another type of biosensor called a blood glucose meter. The enzyme is on a disposable 'test strip' which is inserted into the electronic blood glucose meter and a drop of blood from, for example a finger prick, is applied to the strip. In this case the reaction catalysed by glucose oxidase results in an electrical current being produced and the device converts this to a digital reading for blood glucose.

Pregnancy tests detect the level of a glycoprotein called hCG (human chorionic gonadotrophin), which is a hormone released from the placenta shortly after implantation. It is actually an antibody which detects the presence of hCG and this antibody is linked to an enzyme. This enzyme, as it moves

through the test strip, causes a colour change in a region described as the 'test zone'. This is often seen as a blue line on the test. The pregnancy test described is an example of an ELISA (enzyme-linked immunosorbent assay).

Enzyme inhibition can also be used in a biosensor application. For example the inhibition of an enzyme by heavy metal ions such as mercury, cadmium and chromium, can be used to test a sample of soil water for their presence. Heavy metal ions are dangerous pollutants, precisely because of their effect on enzymes. Urease and glucose oxidase are commonly employed in these biosensors.

## 2. Enzymes as biomarkers of disease

Many diseases involve changes in the amount of particular biological molecules in affected tissues and the measurement of these molecules can prove very useful both in diagnosing and monitoring the disease. Many molecules can be used as 'biomarkers', and some of these molecules are enzymes.

One example is the enzyme elastase, which is released from white blood cells during respiratory infections. This enzyme hydrolyses a structural protein called elastin within the alveolar walls. The result of this is that the 'recoil' function of the lungs, which is an important part of exhalation, is reduced. Hence exhalation takes longer and less gas exchange takes place during each breath. Breakdown of elastin is seen in several respiratory conditions including; cystic fibrosis, emphysema and COPD (chronic obstructive pulmonary disorder). The level of elastase found in sputum can be used as an indicator of disease progression in conditions such as these.

The levels of certain cardiac enzymes change dramatically in the hours following a heart attack. These can act as biomarkers to confirm a heart attack if the attack has been a mild one, or to establish the stage of recovery. An example of a cardiac enzyme is myocardial muscle creatine kinase. These biomarkers are sometimes called 'cardiac markers'.

Another example is the appearance in the urine of enzymes which are normally found on the brush border of the epithelial cells of the proximal convoluted tubule of the kidney nephron. These 'tubular enzymes' can leak into the urine as a result of cell damage and testing for them can be useful in early detection of acute kidney injury (formerly referred to as acute kidney failure).

## 3. Treating disease through enzyme inhibition

In many diseases, enzymes which normally perform roles that maintain health can become damaging, perhaps through over-production or through the interaction with environmental triggers. In these instances, treating the disease often involves prescribing medication containing a known inhibitor of the enzyme in question. The table on the next page lists some examples of diseases and their treatment.

*Alpha-1 antitrypsin (A1AT) may be a substance familiar to biologists: A1AT deficiency is a human genetic disease and Dolly the sheep, the first successful mammal clone, was cloned from a sheep which had been genetically modified to produce A1AT in its milk. A1AT is produced naturally in healthy individuals and acts as an inhibitor of elastase in the lungs, preventing the breakdown of elastin. Some people are at risk of lung tissue damage because they have other conditions, such as CF, which result in frequent infections. White blood cell activity is amplified and the amount of elastase produced outweighs the amount of A1AT. In other cases, people may take in harmful substances which affect A1AT working and therefore elastase activity is not kept in check. This is the case in smokers; cigarette smoke negatively affects the synthesis of A1AT, so elastase is able to cause much more damage than it would in non-smokers. The use of A1AT as a treatment for respiratory diseases is currently in clinical development.

**Further information**

Animation showing how a pregnancy test works

Information on ACE inhibitors

Information on enzymes, A-level detail. Final section on Enzymes in Medicine has some useful information

| Disease or condition | Damaging enzyme | Action of enzyme | Inhibitor | Notes |
|---|---|---|---|---|
| Heart disease | ACE (angiotensin converting enzyme) | Converts angiotensin I to angiotensin II, which contributes to vasoconstriction of arteries, resulting in increased blood pressure | ACE inhibitor | ACE is part of the normal blood pressure regulating mechanism |
| High cholesterol | HMG-CoA reductase | Involved in the production of cholesterol | Statins (HMG-CoA reductase inhibitors) | Statins are the most commonly prescribed medication in the UK. |
| Headache, toothache, etc | cyclooxygenase | Involved in the production of prostaglandins, chemicals which bring about inflammation | Paracetamol and other non-opioid analgesics inhibit cyclooxygenase | |
| Type 2 diabetes | DPP-4 (dipeptidyl peptidase 4) | Destroys the gastrointestinal hormone incretin, which helps the body produce insulin only when needed | DPP-4 inhibitor | DPP-4 is a T-cell activator, and has several other metabolic roles |
| HIV | Protease | Following synthesis of large viral proteins, cuts them into functional molecules so that virus particles can be assembled | Protease inhibitor | |
| | Reverse transcriptase | Catalyses the formation of a DNA copy of the viral RNA genome | Nucleotide reverse transcriptase inhibitors | Inhibition involves faulty versions of DNA nucleotides, so that viral DNA is produced incorrectly and can't be incorporated into the host cell DNA. |
| | Integrase | Inserts viral DNA into host cell DNA | Integrase inhibitor | |
| Various mental health disorders | Monoamine oxidases | Break down various neurotransmitters, including serotonin, adrenaline, melatonin and dopamine | Monoamine oxidase inhibitors | First class of antidepressants to be developed; now used for depression, schizophrenia, and other conditions including Parkinson's Disease. |
| Respiratory disease | Elastase | Hydrolyses elastin in alveolar walls | Elastase inhibitors, including alpha-1 antitrypsin*(in clinical development) | Elastase is produced by white blood cells, and helps them to move through tissues to the site of infection |

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