Cell Biology

Sub-topic 1.4: Proteins

On completion of this topic I will be able to state that:

- All proteins are made of chains of amino acid molecules.
- The sequence of amino acids in a protein codes for and determines the protein’s shape.
- The shape of a protein determines its function.
- Proteins provide a vital structural role in all cell membranes.
- Other examples of proteins are: hormones, antibodies, enzymes and receptors on cells.
- Enzymes function as biological catalysts because they speed up the rate of all cellular biochemical reactions in living organisms.
- Enzymes are chemicals that are made by all living cells.
- Although enzymes take part in a reaction they remain unchanged at the end of it and can be used repeatedly.
- The active site of an enzyme is the location on the enzyme's surface which matches the shape of the substance that it works on (it's substrate).
- The shape of the enzyme’s active site is complementary to its specific substrate and results in specific product(s) working like a lock and a key.
- Enzymes are affected by temperature.
- The temperature at which the enzyme is most active is called the optimum temperature. The optimum temperature for most human body enzymes is 37°C.
- Temperatures of around 45°C or above will alter the shape of the enzyme. In this state, it is said that the enzyme has been denatured and it will no longer be able to carry out its function, affecting the rate of the reaction.
- Enzymes work within a range of pH values. Each enzyme is most active at its optimum pH.

I will also be able to:

Apply skills of scientific inquiry and draw on knowledge and understanding of the key areas of this Unit to carry out an experiment/practical investigation by:

1.1 Planning an experiment/practical investigation
1.2 Following procedures safely
1.3 Make and record observations/measurements correctly
1.4 Present results in an appropriate format
1.5 Draw valid conclusions
1.6 Evaluating experimental procedures
Protein Structure

Proteins are made up from subunits called **amino acids**. There are twenty different amino acids. These are represented below as different shapes, with each different shape representing a different amino acid. The individual amino acids are held together by **peptide bonds** to form long **polypeptide chains**. These chains form the protein molecule.

The structure of a protein depends on the **specific sequence** of the amino acids. You have already learned that this is determined by the order of bases on the DNA molecule. **The sequence of amino acids determines the shape and function of the protein.** If the order of bases is incorrect the protein shape will be altered and it may not be able to do its job. Proteins perform a wide variety of functions in all living organisms.

The diagram below shows how differently shaped proteins are formed.

![Diagram of protein structure](image-url)
Proteins are essential for proper development and functioning of cells and organisms. They can have many shapes and roles, some of which are shown in the table below.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
<th>Shape</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Structural protein</strong> (e.g. collagen, keratin)</td>
<td>Collagen makes up the body's cartilage. Keratin makes up hair and nails.</td>
<td>Composed of fibres.</td>
</tr>
<tr>
<td><strong>Enzymes</strong> (e.g. amylase, pepsin, lipase, phosphorylase)</td>
<td>Involved in metabolism. Break down or build up specific substances.</td>
<td>Globular proteins with a shape that fits snugly with the chemicals they work on.</td>
</tr>
<tr>
<td><strong>Hormones</strong> (e.g. insulin)</td>
<td>Act as chemical messengers in the body. They are produced by endocrine glands and circulate in blood. Insulin controls blood glucose levels.</td>
<td>Small, globular proteins.</td>
</tr>
<tr>
<td><strong>Antibodies</strong></td>
<td>Combine with foreign proteins (antigens) and mark them to be destroyed by the body's immune system.</td>
<td>Globular proteins, shaped to recognise and bind with the foreign protein.</td>
</tr>
<tr>
<td><strong>Receptors</strong></td>
<td>Found on the surface of particular cells which allow hormones to target these cells.</td>
<td>The shape of a receptor protein on a cell surface allows the cell to respond to a specific hormone.</td>
</tr>
</tbody>
</table>
Enzymes

Many different chemical reactions occur in living cells. The rate of these reactions is controlled by a special group of proteins called enzymes. Enzymes are known as 'biological catalysts' because they are chemicals that are made by living cells. They speed up the rate of cellular reactions and are unchanged in the process. A catalyst is a substance that lowers the energy needed for a chemical reaction to take place (activation energy), speeds up a chemical reaction and is not changed by the reaction itself.

Enzymes lower the activation energy needed for metabolic reactions to proceed in cells.

![Activation energy graph]

**Activity 1**
Watch as your teacher demonstrates the use of a catalyst.

1. Name the catalyst used, reactant and products of the reaction.

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Catalyst</th>
<th>Products</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2. Explain the function of the catalyst in this reaction.

__________________________________________________________________________________

__________________________________________________________________________________
Catalase in Living Cells

The enzyme catalase, is found in a variety of tissues. Its job is to break down the hydrogen peroxide, produced in living cells, into harmless oxygen and water.

**Activity 2: Experiment**

**Aim:** To demonstrate that living tissue contains the enzyme catalase.

**Collect:**
- 5 test tubes,
- hydrogen peroxide
- 1g samples of 3 different living tissues
- 1g sample of dead tissue
- 5ml syringe
- wooden splint
- Bunsen burner
- safety goggles

**Method:** Set up the experiment as shown below:

```
1. Place one of the tissues in test tube 1.
2. Test for oxygen production with a glowing splint and record the result in the table below.
3. Repeat using each of the other tissues.
```

**Results:**

<table>
<thead>
<tr>
<th>Test tube</th>
<th>Tissue</th>
<th>Is oxygen produced?</th>
<th>Is catalase present?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>carrot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>potato</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>boiled liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>control</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Conclusion:**

____________________________________________________________________

____________________________________________________________________
Evaluation:
How did you ensure your experiment was valid (fair)?
________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

There are two types of enzyme controlled reaction:

(a) **Degradation** reactions;
this happens when large complex molecules are broken down into smaller simpler ones. 
e.g. all the break down reactions that occur as food travels through the digestive system in the human body.

(b) **Synthesis** reactions;
this occurs when small, simple molecules are built up into large complex ones. e.g. 
glucose being built up into starch during the process of photosynthesis in plants.
Activity 3: A Degradation (breakdown) Reaction

Amylase is an enzyme that breaks down starch (a large molecule) into maltose sugar (a smaller molecule). Benedict’s reagent can be used to test for maltose. The blue Benedict’s reagent will turn to brick red once heated if maltose is present.

**Aim:** To demonstrate a degradation reaction using the enzyme amylase.

**Collect:**
- starch suspension
- amylase enzyme
- 2 test tubes
- Benedict’s reagent
- 2 syringes
- safety goggles

**Method:** Set the experiment up as shown in the diagram below.

Before starting the experiment, test the starch suspension with Benedict’s reagent to make sure there is no maltose sugar present. An orange colour will be produced if sugar is present.

1. Using a syringe add 5ml of starch solution to each test tube.
2. Add 2ml of amylase enzyme to test tube A.
3. Add 2ml of water to test tube B.
4. Test both samples after 10 mins with Benedict’s reagent.

**Results:**

<table>
<thead>
<tr>
<th>Test tube</th>
<th>Presence of maltose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At start</td>
</tr>
<tr>
<td>A</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion: (What you have found out?)
________________________________________________________
________________________________________________________
________________________________________________________

Evaluation: (Review of your practical)
Why did you use different syringes for the different liquids?
________________________________________________________
________________________________________________________
________________________________________________________

Write a word equation for this degradation reaction in the box below.

\[
\begin{array}{c c c}
\text{substrate} & \text{enzyme} & \text{product} \\
\end{array}
\]

Key Questions:

1. Give two ways in which enzymes differ from other catalysts.
   (a)________________________________________________________
   (b)________________________________________________________

2. Explain why enzymes are vital to all living organisms.
   __________________________________________________________
   __________________________________________________________

3. State the temperature that enzymes in the human body will work at their best?
   ______ °C
**Activity 4: A Synthesis (build up) Reaction**

The enzyme **phosphorylase** is found in potato cells and can be easily extracted. Phosphorylase builds up glucose -1- phosphate into starch.

**Aim:** To investigate a **synthesis** reaction using the enzyme **phosphorylase**.

**Collect:**
- safety goggles
- dimple tray
- 2 droppers
- beaker of water
- phosphorylase (potato extract)
- glucose-1- phosphate solution

**Method:**

1. Ensure there is **no starch** at the start of the experiment by putting 2 drops of potato extract into one of the dimples and adding 2 drops of iodine solution. If starch is present the iodine will change from brown to blue/black.

2. Wash the tray and dry thoroughly.

3. Set up the experiment as shown in the diagram and description below.

4. Add 3 drops of glucose-1-phosphate (G-1-P) and three drops of the enzyme phosphorylase (potato extract) into each of the dimples in the top row.

5. Using three drops each time, put the liquids into the other two rows.

6. Add 2 drops of iodine to the 0mins time column only; repeat every 10mins, adding iodine solution to the appropriate column, until 30mins.

**Result:**

Record your results on the dimple tray diagram using the key to indicate the level of starch present.

<table>
<thead>
<tr>
<th>Key</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>++++</td>
<td>a lot of starch</td>
</tr>
<tr>
<td>+++</td>
<td>starch</td>
</tr>
<tr>
<td>++</td>
<td>some starch</td>
</tr>
<tr>
<td>+</td>
<td>a little starch</td>
</tr>
<tr>
<td>-</td>
<td>no starch</td>
</tr>
</tbody>
</table>

Wear **safety goggles** and follow all teacher instructions carefully.
Conclusion:
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
____________________________________________________________________

Write out a word equation for this synthesis reaction.

\[
\begin{array}{ccc}
\text{substrate} & \rightarrow & \text{product} \\
\end{array}
\]

\[
\begin{array}{ccc}
\text{enzyme} \\
\end{array}
\]

Evaluation:

Rows 2 and 3 were set up to act as controls for this experiment.

(a) **Describe** what is meant by a control experiment.

____________________________________________________________________
____________________________________________________________________

(b) **Explain** what the controls prove in this experiment?

____________________________________________________________________
____________________________________________________________________
____________________________________________________________________
Digestive Enzymes

Digestion in the gut involves the chemical breakdown of food. Large insoluble molecules are degraded with the help of enzyme action into smaller soluble ones.

There are three main groups of digestive enzymes produced at various sites of the digestive system.

- **Carbohydrases** - break down carbohydrates into sugars such as maltose and glucose

  ![Starch to Sugars](image)

- **Proteases** - break down proteins into polypeptides then into amino acids

  ![Protein to Amino acids](image)

- **Lipases** - break down fats into fatty acids and glycerol

  ![Fat to Glycerol + Fatty acid](image)
Enzyme Action

During an enzyme controlled reaction the molecule on which the enzyme works, called the **substrate**, joins temporarily at the **active site** on the surface of the enzyme. This *facilitates* the chemical reaction which can now take place quickly and forms the **product** or **products**. This active site has a **definite shape** and the substrate molecule must have the **specific corresponding shape**. The **shape** of the active site of an enzyme molecule is **complementary** to its **specific substrate**. Substrate molecules fit into the active site like a key fitting into a lock. This enzyme-substrate complex that is temporarily formed is able to *facilitate* the chemical reaction taking place.

### A Degradation Reaction

![Image of enzyme action](image)

**Key Questions**

1. Explain why the diagram above illustrates a **degradation** reaction.

   ____________________________________________________________________________

   ____________________________________________________________________________

2. Draw a similar diagram in the box below to represent a **synthesis** reaction.

   ![Blank diagram for synthesis](image)

3. Explain why enzymes are referred to as specific.

   ____________________________________________________________________________

   ____________________________________________________________________________
Factors Affecting Enzyme Activity

Enzymes are made of proteins, the structure and shape of all proteins can be affected by changes in temperature and pH. Enzymes can be denatured resulting in a change to their shape. This will have a major effect on the rate of the reaction.

**pH and Enzyme Activity**

pH is a measure of the acidity or alkalinity of a substance. Although many enzymes are most active at around neutral pH (pH 7), different enzymes have a pH at which they are most active. This is called their optimum pH.

![pH Scale Diagram](image)

The diagram below shows the effect of pH on enzyme activity.

Describe what is happening at each pH A, B and C.

A  ______________________________________________________________

B  ______________________________________________________________

C  ______________________________________________________________
The graph below shows the optimum pH and the working range of three different enzymes.

![Graph showing optimum pH and working range for three enzymes: pepsin, plant amylase, and catalase.]

Complete the table below using the information from the graph above.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Optimum pH</th>
<th>Working pH Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.8 - 5</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>catalase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1. Draw a conclusion about the working range of the three enzymes.

____________________________________________________________________
____________________________________________________________________

2. State the pH range where both amylase and catalase will function:

Amylase and catalase will both function in the pH range from ____ to ____ .

4. Complete the following table by providing the correct substrate and product(s) for each of the enzymes listed:

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Enzymes</th>
<th>Product(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>catalase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>protease</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lipase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>phosphorylase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>amylase</td>
<td></td>
</tr>
</tbody>
</table>
Investigating The Effect of pH on the Activity of Pepsin

When egg albumen is heated, the proteins in the clear fluid egg white change becoming hard and insoluble. The heating process denatures these proteins turning them into an opaque solid.

Glass tubes were filled with egg albumen and then heated until cylinders of solid white albumen were produced. The albumen cylinders were then removed from the glass tubes and placed onto graph paper to determine their length:

Pepsin is an enzyme that is found in the human stomach which promotes the breakdown of insoluble protein molecules into soluble peptides. The action of the enzyme pepsin on the egg albumen cylinders at different PH’s was investigated.

During the investigation and under suitable conditions, the pepsin will digest the insoluble egg protein, the decrease in length of the cylinders will indicate the amount of digestion. The greater the decrease in length of the cylinders, the more active the enzyme pepsin.

The diagrams on the following page show the experimental set up and the results of this investigation. Read carefully and then answer the key questions on page 17.
Investigating the Effect of pH on the Activity of Pepsin

Length of egg albumen (mm)

egg albumen cylinders placed in a test tube containing pepsin solutions with different pH

pepsin solution + buffer solution

pH of each solution: 3 4 5 6 7

egg albumen cylinders removed after 24 hours at 37°C and placed back on the graph grid

Length of egg albumen (mm)

A decrease in length indicates that digestion has occurred
Key Questions

1. State the name of the:
   a) substrate - ___________________
   b) enzyme - ___________________
   c) product(s) - ___________________
   in the egg albumen investigation.

2. a) Complete the results table to show the change in length in mm and the % change in length of each cylinder at each pH:

<table>
<thead>
<tr>
<th>Cylinder</th>
<th>pH</th>
<th>Length at Start (mm)</th>
<th>Length after 24 hours (mm)</th>
<th>Change in length (+/- mm)</th>
<th>% change in length</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>30</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>30</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>30</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E</td>
<td>7</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

b) Collect graph paper and draw a line graph to show the % change in length of the egg albumen cylinders at different pH. The completed graph should have:
   1. suitable labels on the x and the y axis
   2. suitable scales on the x and y axis
   3. points plotted accurately using a small + or a x
   4. a straight line drawn connecting each of the plot points

3) Write a suitable conclusion for this investigation including an explanation of how the change in pH of the solutions relates to the activity of the enzyme used.
Temperature and Enzyme Activity

At high temperatures, enzymes can be \textit{denatured} resulting in a change to their shape. This will also change the shape of the active site. The specific substrate molecule can therefore no longer bind with the shape of the active site of the enzyme and so the enzyme cannot work as a catalyst for the reaction. This will affect the rate of the reaction.

**Activity 7: The Effect of Temperature on Enzyme Experiment.**

Invertase breaks down sucrose into reducing sugar. To test for reducing sugar we can use clinistix which will turn from pink to purple if reducing sugar is present.

**Aim:** To investigate the effect of temperature on activity of invertase.

**Collect:**
- safety goggles
- 3 test tubes and rack
- 5 ml sucrose
- 2ml invertase
- 2 syringes
- stopwatch
- ice and hot water bath set at 60°C
- clinistix

**Method:**

1. Label three test tubes with A, B, C and your initials.
2. Collect sucrose and invertase \textit{which are already at the appropriate temperature} and measure 5ml of sucrose and 2ml of invertase into the correct test tube as shown below.

   ![Test Tubes](image)

   - A: cold (0°C)
   - B: warm (25°C)
   - C: hot (60°C)

3. Place test tube A into the ice bath, leave B at room temperature and place C in the water bath at 60°C and leave for 15 minutes.
4. Test each solution for the presence of reducing sugar using clinistix.
Results (record your results in the table below):

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Presence of reducing sugar (✓ / X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion: (What does your experiment show? Relate back to your aim.)

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Evaluation:
Name the variable which is being altered in this experiment.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

List the variables which must therefore be kept constant?

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

Explain why the sucrose and the invertase were adjusted to the correct temperature before being mixed.

________________________________________________________________________
________________________________________________________________________
________________________________________________________________________

What was done to make the results more reliable?

________________________________________________________________________

Write a word equation for this degradation enzyme reaction:

substrate ➔ enzyme ➔ product
**Key Questions**

Study the graph below and then answer the questions which follow.

1. Describe the enzyme activity at points A - B and B - C.
   
   A - B: _______________________________________________________
   
   B - C: _______________________________________________________

2. Describe what is happening to the enzyme activity at 50°C.
   
   _____________________________________________________________

3. Explain why there is no enzyme activity at 60°C.
   
   _____________________________________________________________

4. If the temperature was to drop from 60°C back to 30°C, explain what would happen to the rate of enzyme activity.
   
   _____________________________________________________________
   
   _____________________________________________________________
   
   _____________________________________________________________

5. The **optimum temperature** is the temperature at which the enzyme is most active. Using a cross (x), indicate on the line graph above, the optimum temperature for this enzyme.

Now complete the Enzymes Summary on the next page by filling in all the blanks.
ENZYMES

SUMMARY

factors affecting enzyme activity

Enzymes can be described as:

b_____________ c______________.

They are present in all living cells. The function of enzymes is to s_________ u__ chemical reactions, but they remain u_______________ by these reactions.

Enzymes are made of p__________

Enzymes are involved in two types of reactions:

degradation

Examples of degradation reactions are:

1. _____________ → _____________
2. _____________ → _____________
3. _____________ → _____________

s_________________

An example of a s___________ reaction is:

(substrate) → (enzyme) → (product)

Enzymes are involved in two types of reactions:

degradation

Examples of degradation reactions are:

1. _____________ → _____________
2. _____________ → _____________
3. _____________ → _____________

s_________________

An example of a s___________ reaction is:

(substrate) → (enzyme) → (product)

The conditions at which an enzyme is most active are known as the o______________ conditions.

Enzymes can be described as:

b_____________ c______________.

They are present in all living cells. The function of enzymes is to s_________ u__ chemical reactions, but they remain u_______________ by these reactions.

Enzymes are made of p__________

Enzymes are involved in two types of reactions:

degradation

Examples of degradation reactions are:

1. _____________ → _____________
2. _____________ → _____________
3. _____________ → _____________

s_________________

An example of a s___________ reaction is:

(substrate) → (enzyme) → (product)

Enzymes are involved in two types of reactions:

degradation

Examples of degradation reactions are:

1. _____________ → _____________
2. _____________ → _____________
3. _____________ → _____________

s_________________

An example of a s___________ reaction is:

(substrate) → (enzyme) → (product)
You will now carry out an investigation of the uses of enzymes in the food industry.

Your teacher will guide you through the planning of your investigation, which you will do **individually**.

You can then **work in small groups** to carry out the experimental part of the work.

Finally, you will work again **individually** to complete your final write up of the investigation.

**NOTE: The data that you collect may be used later as part of your assignment, completion of the assignment is part of your National 5 certificate.**

The rest of the space in this booklet can be used to record your findings.