OVERVIEW

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Biochemistry Key Stage 4
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The Biochemical Characterisation of Flap Endonuclease 1 (FEN1)

AGE
14-16
Year 10
Year 11

CURRICULUM
B
Biochemistry

OFQUAL
AO1
AO2
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Assessment Objectives

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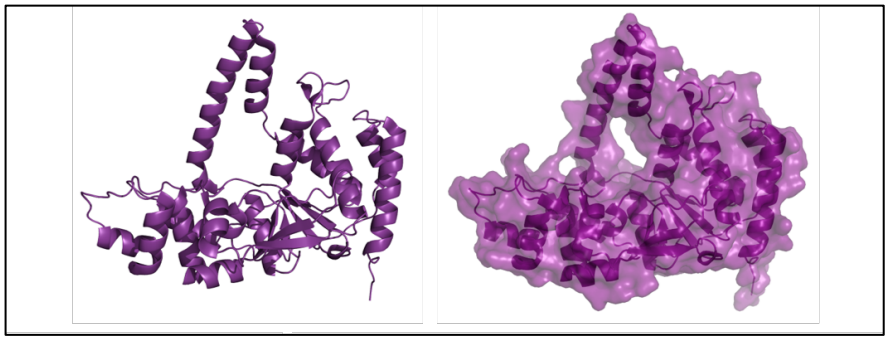
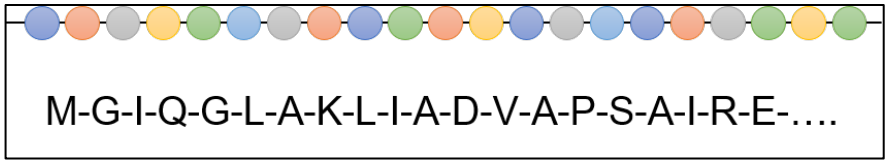
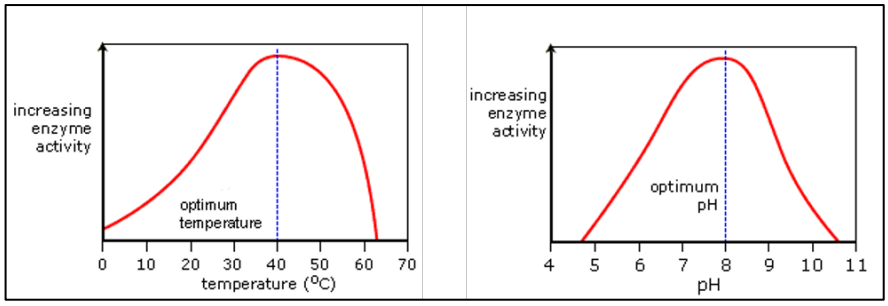




First Letter	Second Letter: T	Second Letter: C	Second Letter: A	Second Letter: G	Third Letter
T	TTT Phenylalanine (F)	TCT Serine (S)	TAT Tyrosine (Y)	TGT Cysteine (C)	T
	TTC Phenylalanine (F)	TCC Serine (S)	TAC Tyrosine (Y)	TGC Cysteine (C)	C
	TTA Leucine (L)	TCA Serine (S)	TAA STOP	TGA STOP	A
	TTG Leucine (L)	TCG Serine (S)	TAG STOP	TGG Tryptophan (W)	G
C	CTT Leucine (L)	CCT Proline (P)	CAT Histidine (H)	CGT Arginine (R)	T
	CTC Leucine (L)	CCC Proline (P)	CAC Histidine (H)	CGC Arginine (R)	C
	CTA Leucine (L)	CCA Proline (P)	CAA Glutamine (Q)	CGA Arginine (R)	A
	CTG Leucine (L)	CCG Proline (P)	CAG Glutamine (Q)	CGG Arginine (R)	G
A	ATT Isoleucine (I)	ACT Threonine (T)	AAT Asparagine (N)	AGT Serine (S)	T
	ATC Isoleucine (I)	ACC Threonine (T)	AAC Asparagine (N)	AGC Serine (S)	C
	ATA Isoleucine (I)	ACA Threonine (T)	AAA Lysine (K)	AGA Arginine (R)	A
	ATG Methionine (M)	ACG Threonine (T)	AAG Lysine (K)	AGG Arginine (R)	G
G	GTT Valine (V)	GCT Alanine (A)	GAT Aspartic Acid (D)	GGT Glycine (G)	T
	GTC Valine (V)	GCC Alanine (A)	GAC Aspartic Acid (D)	GGC Glycine (G)	C
	GTA Valine (V)	GCA Alanine (A)	GAA Glutamic Acid (E)	GGA Glycine (G)	A
	GTG Valine (V)	GCG Alanine (A)	GAG Glutamic Acid (E)	GGG Glycine (G)	G



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Proteins – From Building Blocks to Intricate Structures

Link to curriculum
Amino Acids, Proteins, Polymers

RESOURCE NOTES

The resource aims to show how the complicated final structure of proteins is built up in stages from the primary sequence of amino acids through 2 stages of folding to give the final shape. The three proteins shown are Flap Endonuclease 1 (FEN1) in purple, Green Fluorescent Protein (GFP) in green and human Insulin in blue. These proteins all have very different structures and very different roles. FEN1 is a protein crucial for DNA replication where it plays a role in lagging strand synthesis – this is the enzyme that I study. GFP was initially extracted from bioluminescent jellyfish and is now used in research where it is particularly useful for imaging given its bright green colour when stimulated by UV light (it is a barrel shaped protein). Insulin is a hormone with a crucial role in sugar metabolism. The images on the right show the surface of the protein when the side chain of the amino acids are taken into account, while those on the left just take into account the backbone of the protein, not the side chains. These images are all produced using x-ray crystallography by shining x-rays onto a crystal of protein molecules.

The primary sequence shown is the first 20 amino acids of human Flap Endonuclease 1 (FEN1) – it has 380 amino acids in its full sequence. The secondary structure shows a pair of alpha helices in blue and part of a beta sheet in green.

FURTHER READING

1. A further introduction to DNA replication. FEN1 is not mentioned specifically but is involved in lagging strand synthesis.
2. Wikipedia page on protein structure (warning given about the reliability of Wikipedia as an information source)
3. The paper which first described the crystal structure of human FEN1. Some bits of it are complicated and there is a lot of structural detail in it but it is an interesting structural paper.

ACTIVITY NOTES

Activity 1 notes

Requires students to fill in the 3-letter and single-letter codes from the table for the amino acid sequence given
The final word is CHEMISTRY
This activity could link further to protein synthesis and DNA codons

Activity 2 notes

Matching the words to the definition

Activity 3 notes

Students should use the answers to activity 2 to help them write a paragraph describing protein structure. The initial resource will also be helpful.

Primary structure – the sequence of amino acids which is individual for each protein
Secondary structure – regular shapes of alpha helices and beta sheets are formed in the amino acid chain
Tertiary structure – the global folding of the amino acid chain into a larger 3D shape
Quaternary structure – proteins (including FEN1 and insulin) have quaternary structures which may include metal ions around which they are organised or multiple amino chains.

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The Structure and Manipulation of DNA

Link to curriculum
DNA, Genetic Engineering, Polymers

RESOURCE NOTES

An X-ray crystal structure of DNA is used to introduce the 3D structure of this molecule. The structure is discussed further, with a mention of genes and DNA packaging into chromosomes before the structure of a DNA nucleotide is introduced along with the four DNA bases.

Genetic engineering is an important tool in biochemistry research, allowing researchers to manipulate the DNA of the organism or protein they are studying. While I use E. coli to overexpress the proteins I study, this can also be done in insect cells. The diagram shows the cutting of a circular DNA plasmid such as those found in bacteria using restriction enzymes and the ligation of a new piece of DNA into the gap using DNA ligase. These plasmids are also used to make the E. coli they are implanted into resistant to specific antibiotics such as ampicillin to ensure that when they are grown in the lab only those with the correct plasmids can grow in the media used (which is laced with antibiotic). Restriction enzymes cut DNA at specific sequences to produce blunt or sticky ends and are usually extracted from bacteria.

FURTHER READING

1. Wikipedia page on DNA structure (warning given about the reliability of Wikipedia as an information source)
2. Video giving details of protein synthesis in cells
3. This video shows the restrictions enzyme EcoR1 searching for the correct sequence in a circular DNA plasmid before cutting it open. The DNA is then ligated using DNA ligase to reform the circle.

ACTIVITY NOTES

Activity 1 notes

Pupils can fill in the sequence on the second DNA strand being sure to pair A with T and C with G

If pupils want to add in hydrogen bonds between the bases this can also be done A and T have 2 hydrogen bonds and C and G have 3.

Activity 2 notes

Allows pupils to think about how DNA codes for amino acids using codons. The first part involves pulling the required information from the table provided to give the sequence of the first 20 amino acids of human FEN1.

The effects of insertion, deletion and substitution of DNA bases is then explored using the sequence given. Students should be encouraged to think about how changing the DNA sequence in one of the 3 may mean a change in the resulting amino acid sequence but not necessarily if it is a substitution.

Activity 3 notes

Gives students a chance to practice some basic genetic engineering. The first sequence can be cut by ECORI and BAMHI, the second by ECORI and SMAI, and the third by BALI and ECORV.

The second of the 4 given plasmids does not have a corresponding insert.

RESOURCE 3

Biological Machines

Link to curriculum
Enzymes, Catalysts

RESOURCE NOTES

The resource explains the lock and key mechanism of enzyme catalysis and also touches on the effect of temperature and pH on the activity of enzymes. It also shows the structure of human flap endonuclease 1 and its preferred substrate (a double flap structure). The highlighted circle is a hole in the structure which the indicated strand of DNA is threaded through, placing the base to be cleaved into the active site. The lock and key model does not fully explain this process for FEN1. Instead what happens is when DNA is not present, the amino acid chain above and around the indicated hole (known as the arch) is flexible and not very tightly folded into alpha helices. This makes the hole larger and allows the indicated DNA strand (the 5′ flap) to thread through the hole. The amino acid chain of the arch then folds more tightly into alpha helix around the DNA. The DNA substrate is bent by the enzyme forming a 100° angle. What is not fully clear in the picture is that there is a groove in the surface of the enzyme in which the DNA double helix can sit, helping to place the bond to be cleaved into the active site.

FURTHER READING

1. (and 2.) Provide an introduction to enzymes – the first contains more detail than the second and includes details about enzyme kinetics as well as effects of temperature and pressure
2. See above
3. This research paper was published by my research group and investigates the changes which must take place in the shape of the DNA and protein to allow FEN1 to catalyse the hydrolysis of DNA. There is some very complicated science in this paper, but the introduction and discussion give a good explanation.

ACTIVITY NOTES

Activity 1 notes

This is designed to use think, pair, share but could also just be used as a discussion question or a question to answer individually.

Model answer: a catalyst is a molecule or compound which increases the rate of a reaction without being changed or used up in the reaction.

Activity 2 notes

High and low temperature and pH reduce the activity of an enzyme.

The optimum temperature and pH are those at which the enzyme has maximum efficiency. This tends to be 37°C and pH7 in the human body but can vary (Pepsin has pH2 as its optimum).

At extreme conditions enzymes can be denatured – this destroys their tertiary (and possibly secondary) structure.

Activity 3 notes

This is explained in the source explanation but should be written in the pupil's own words using the words given in their answer.

RESOURCE 4

Experimental kinetics

Link to curriculum
Kinetics, Rates of Reaction

RESOURCE NOTES

The graph in the resource is kinetic data from an experiment looking at the hydrolysis of DNA by Exonuclease 1, an enzyme which is closely related to FEN1. The amount of product in nM (nanomoles) produced at 6 timepoints during the reaction is plotted. The data is a good fit for the straight line and the gradient of this is the initial rate of the reaction (the maximum speed). Shown below in the table is some experimental data looking at the reaction of FEN1 and phosphorylated FEN1. Phosphorylation of proteins can be used as a way to control their activity (switching them on or off) and this experiment was done to see if it has this effect of FEN1.

FURTHER READING

1. This provides some more information about enzyme kinetics and the information that can be obtained
2. This is an extract from a review paper which provides information on the post translational modifications (PTMs) of Flap Endonuclease 1. The image labelled B is a summary of these PTMs with the explanation below it. There are many different PTMs of FEN1 reported and they are, on the whole, poorly understood. As well as changing the activity of an enzyme (increasing or decreasing it), PTMs can be used to signal to other molecules in the cell that it is time to degrade proteins or enzymes.

ACTIVITY NOTES

Activity 1 notes

This should produce two straight line (y=mx+c) graphs.

The best-fit line should not cross the y-axis on either graph as shown in the resource image.

Activity 2 notes

The gradient of the best fit line will give the rate of reaction in nM/min when 30pM enzyme is used.

The answers students get will very depending on the lines they have drawn in the first part of the activity.

If needed, the calculated gradients are 0.4237 for FEN1 and 0.384 for phosphorylated FEN1.

Activity 3 notes

It may be necessary to explain the relationship between pM and nM (picomoles and nanomoles) as students will have to convert between them.

Again, the answers calculated by the pupils will vary but the calculated values are 14.12 /min for FEN1 and 12.80 /min for phosphorylated FEN1.

Results this close mean that phosphorylation does not affect the activity of FEN1
It is hard to say how big a rate difference would be significant in this case as only one experiment has been done, not any repeats.

The current published result shows that phosphorylation of human FEN1 reduces its activity by 3-fold but my results disagree with this. I am currently trying to figure out why this might be.

Speeding Up or Slowing Down

Link to curriculum
Kinetics, Rates of Reaction

RESOURCE NOTES

This is real experimental data showing the rate of reaction of FEN1 with four different substrates which have slightly different structures. The four structures used in the experiment are shown in the table along with a description of their structure. The optimal structure for FEN1 is the double flap which is substrate A. The others are biologically relevant variations of this substrate which can still be cleaved but are not optimal.

FURTHER READING

1. An introduction to the kinetics of enzyme reactions
2. This provides a useful tool for learning about rates of chemical reactions which is aimed at GCSE students
3. Video demonstrating how the rate of a reaction between sodium thiosulphate and hydrochloric acid can be changed

ACTIVITY NOTES

Activity 1 notes

As the amount of substrate increases there is more present for the enzyme molecules to bind to. More reactions can happen in the mixture. This is true until all the enzyme molecules are bound to a substrate molecule at a particular time.

Activity 2 notes

At this point all the enzyme molecules have substrate bound at any point in time. The reaction speed cannot increase beyond this point.

Activity 3 notes

This question tries to link together the lock and key mechanism with the rate of reaction.

The non-optimal substrates are analogous to using the wrong key to unlock a door
The substrate order is A-B-C-D.

It is important to look at both the y and x axes to help work this out!

Activity 4 notes

Temperature, pressure, concentration of substrates, concentration of enzyme/catalyst, pressure, reaction volume etc.

RESOURCE 6

Electrophoresis in the Lab

Link to curriculum
Electrolysis

RESOURCE NOTES

Gel electrophoresis uses the principles of electrolysis by passing a current through a gel in a tank of buffer. It is a tool widely used in research to examine what has occurred in procedure or reactions involving DNA and proteins. The two most common types, SDS PAGE and agarose gel electrophoresis are discussed and some example gels are shown.

FURTHER READING

1. This video provides an introduction to electrolysis, the technique on which electrophoresis is based
2. Wikipedia page on gel electrophoresis (warning given about the reliability of Wikipedia as an information source)

ACTIVITY NOTES

Activity 1 notes

Electrolysis is the process by which ionic substances can be broken down into smaller parts by the application of electricity. Metals and gas may form at the positive and negative electrodes.

Activity 2 notes

Smaller fragments can pass more easily through the pores of the gel and therefore will move further down the gel than larger fragments which cannot move as easily through the pores.

Activity 3 notes

SDS PAGE gel 1 – protein purification has not worked well, there is not a clear band in the right hand lane. This means there are additional proteins in the sample, not just the desired one. If any experiments are done using the products from the right-hand lane the results would not be very trustworthy.

SDS PAGE gel 2 – phosphorylation is present possibly after 5 minutes and definitely after 10 minutes. Not all of the FEN1 is phosphorylated after 60 minutes as there are still 2 bands on the gel. The reaction is fairly slow, not going to completion in an hour and taking 10 minutes before phosphorylation can be seen on the gel.

Agarose gel 1 – 5 of the 7 reactions have worked, the two lanes towards the top of the gel have no PCR product band present.

Agarose gel 2 – Although the circular and linear DNA fragments are the same molecular weight, the circular DNA can move more easily through the pores of the gel than the elongated strand and therefore appears further down. Analogous to pushing through a crowd when you have to keep your arms in by your sides (the circular DNA) and when they are not allowed to touch your sides (the elongated strand).

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