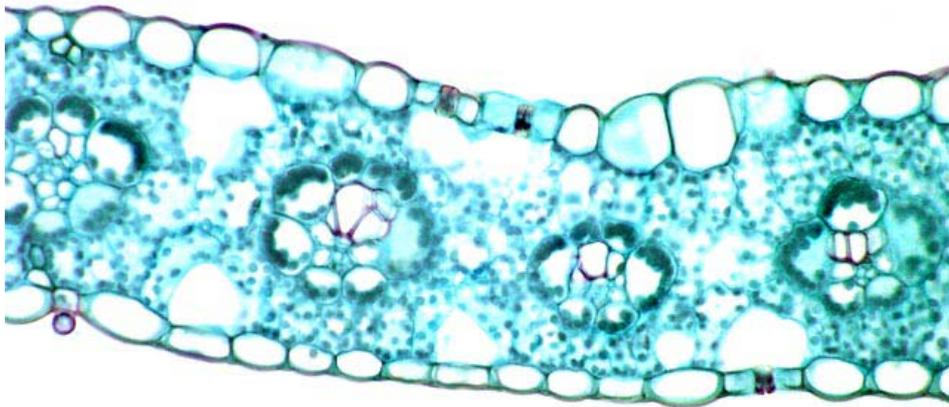


A level workbook

Photosynthesis



A2 level student guide

Brian Banks

Using the workbook

This workbook is designed to provide the student with notes, illustrations, questions and guided examples for the topic of photosynthesis at AQA A2 level.

The book is divided into several sections. Each section should take approximately 30 minutes to 1 hour to complete.

Each section contains different types of information.

Normal black text should be read thoroughly.

Text in red indicates KEY FACTS, which you must learn thoroughly prior to the examination. Additionally, red borders around illustrations also indicate that these illustrations are important to learn / understand.

Blue text indicates useful background information to help you understand what is going on in the key facts. Although you will be unlikely to be directly questioned about these things, they help you to develop a broader and more accurate understanding of photosynthesis, and will help you to relate the topic to other topics on the course.

Information in green is advanced information, which you will not have to answer questions on in the examination. However, this information will tell you more about the subject that you may find interesting and useful in a synoptic essay.

SYNOPTIC before information or questions indicates a tie in with other areas of the syllabus, which you should be familiar with for answering the synoptic questions in module 6 and module 8.

Syllabus entries are in pink. *These refer to the relevant part of the AQA A2 syllabus.*

Questions should be answered on separate sheets if you wish to hand them in for marking. The answers, in any case, are found at the back of the workbook.

Section 1. Photosynthesis – an overview

Why do living things need energy?

Life on earth differs from inorganic (non-living) material because it is maintained in a constantly different state to the surroundings. These differences in concentration of ions, pH, electrical state *etc* are the hallmarks of living cells. By encapsulating the active chemicals of life (mainly in the cytoplasm) inside a semi-permeable membrane, living cells can avoid their structure and chemical organisation falling back into a non-reactive (inorganic) state.

The law of Entropy states that any system, given time and left alone, will become more and more disorganised. Life and living things are constantly waging a battle with entropy, and attempting to keep a high level of organisation that allows them to maintain differences between themselves and their environment. Keeping these differences in state, and avoiding falling victim to entropy requires energy.

What energy sources are available on earth?

On earth, there are two forms of energy available for life to use. Of these, by far the most important is the energy in light radiation from the sun. **The second, only recently identified, is thermal energy from within the earth itself. There are only a few ecosystems known to use this energy source, e.g. hydrothermal vent communities.**

In fact, given the rarity of geothermal energy as the base energy source for an ecosystem, we can say that the vast majority of life on earth depends on sunlight for energy.

**Living things require energy to stay alive.
The main energy input to planet earth is from the sun.**

What living things can use this energy?

There are several groups of living things that can harness energy from the sun. These organisms are known as autotrophs – i.e. they produce their own food. There are different ways that they achieve the gathering of sunlight and the conversion of the sun's energy to chemical energy. However, the most common form of photosynthesis occurs in algae, higher plants and certain cyanobacteria.

**Organisms that make their own food are called autotrophs.
Autotrophic nutrition using sunlight energy is called photosynthesis.
Algae, higher plants and cyanobacteria carry out the main form of photosynthesis.
Animals that gain energy by eating other living things are called heterotrophs.**

How do photosynthetic organisms capture the sun's energy?

The sun produces a vast amount of energy in many different forms. The main form of energy from the sun is in the form of electromagnetic radiation, although it also produces vast quantities of subatomic charged particles into the space around it.

The electromagnetic radiation from the sun can be shown in a diagram:

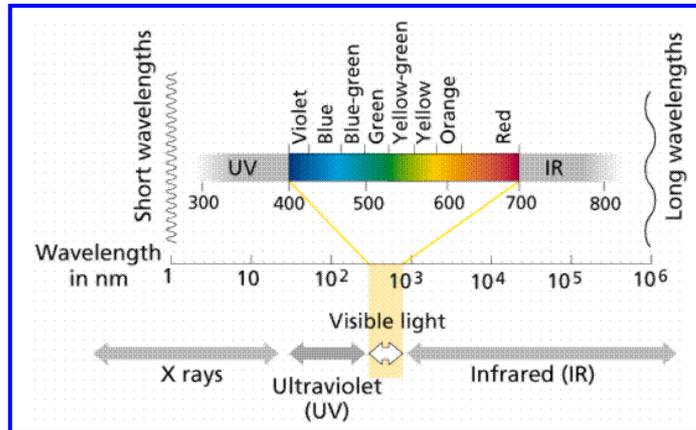


Fig. 1. The electromagnetic spectrum

Of the many different wavelengths of e-m radiation hitting the earth, very little passes through the atmosphere. X rays are absorbed in the Van Allen belt, high in the atmosphere. UV rays are also reduced by the gas ozone, although pollution from CFCs and other gases has damaged the ozone layer and permitted more biologically dangerous UV light to pass through to ground level. Infrared energy is trapped by the atmosphere – the so-called greenhouse effect – that keeps the temperature of the planet warm and stable.

Visible light passes readily through the atmosphere, and it is these wavelengths (between 400nm and 700nm) that photosynthetic organisms use.

The sun produces many wavelengths of electromagnetic radiation, but only visible radiation is used for photosynthesis.

As fig. 1 shows, although visible light appears to be white, it is made up of many different wavelengths of radiation, each of a different colour. When sunlight strikes an object, it can either:

- (i) Pass straight through it (transmission)
- (ii) Reflect off it
- (iii) Be absorbed by it

In reality, most objects permit a little of all three to happen. However, not all the different colours of light will behave the same when striking an object.

If the object appears to be coloured, it is because the white light striking it is being absorbed / reflected differently for each wavelength. E.g:

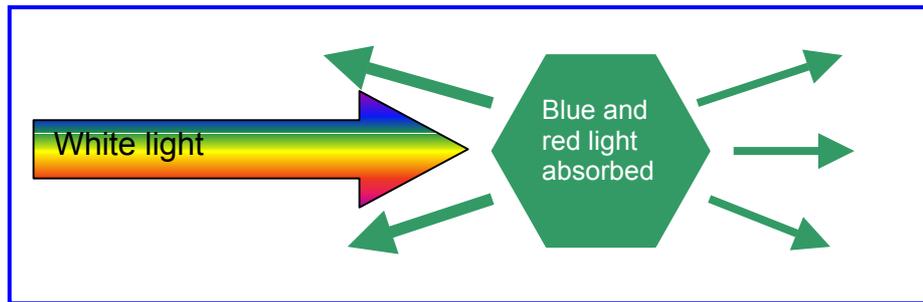


Fig 2. When white light strikes this green object, blue and red wavelengths are absorbed more than the green wavelengths, which are either reflected off or transmitted through the object. This is why the object appears coloured to the eye.

Photosynthetic organisms contain a variety of coloured pigments, normally tightly organised on membranes within chloroplasts. Of these, chlorophyll is the most important. When white light hits these pigments, selective wavelengths of light are absorbed. E.g:

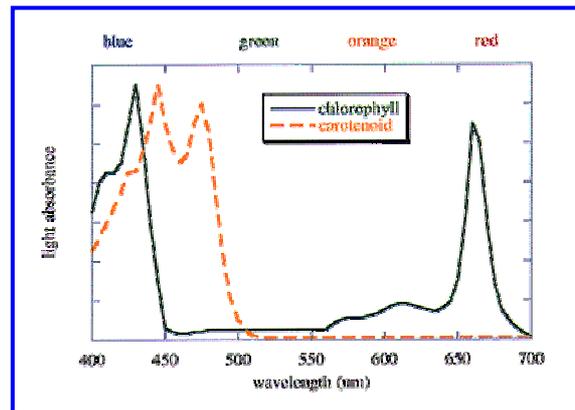


Fig 3. The absorption spectrum of a typical green plant. White light hitting a leaf is absorbed strongly in the red and blue wavelengths, allowing green light to be reflected and transmitted. This is why plants are green.

Coloured pigments absorb different wavelengths of white light.
Plants contain coloured pigments that absorb red and blue light strongly.
The main pigment used for this is chlorophyll.
Photosynthesis cannot occur without chlorophyll.

What do photosynthetic organisms do with the absorbed light energy?

SYNOPTIC – (ectothermic animals) When radiation is absorbed, it is typically converted into and lost as heat. This is why you feel warmer on a sunny day if you wear black clothing – the radiation is absorbed by the black material, and released as heat. Conversely, white clothing reflects most wavelengths of light, making you feel cooler.

For photosynthetic organisms, losing the sunlight they absorb as heat is mostly useless. They must convert the energy into a form that they can store and use later to drive cellular reactions.

Photosynthetic organisms convert light energy into chemical energy. This chemical energy can be stored or used to drive cellular reactions. Little of the absorbed sunlight is lost as heat.

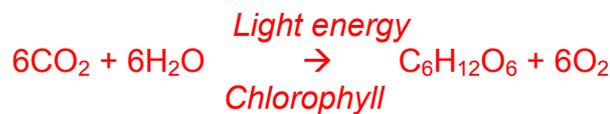
In what form do photosynthetic organisms store energy?

SYNOPTIC mod 1 - In the process of photosynthesis, the main energy-containing chemical produced is glucose. However, the energy from the sun can also be used to manufacture other chemicals using the photosynthetic reactions to build 'carbon-backbones' for useful molecules such as fats, oils and amino acids. Therefore, photosynthesis is not merely a way of storing energy for later metabolism – it is also a vital driving force for the manufacture of many necessary molecules in the photosynthetic organism.

SYNOPTIC mod 1/5 -The main energy-rich chemical produced through photosynthesis is glucose. The process also makes other useful chemicals such as amino acids, fats and oils.

The overall chemical reaction of photosynthesis

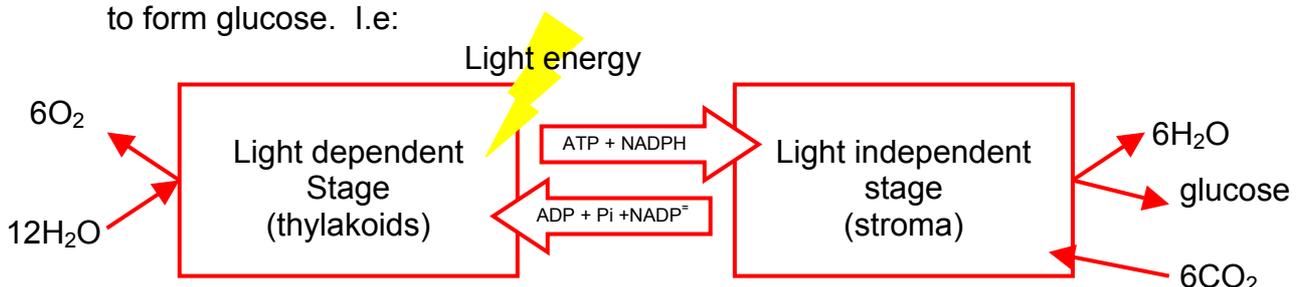
The entire process of photosynthesis can be described by the equation:



Carbon dioxide from air is absorbed through the pores (stomata) of the leaves. Water is absorbed from the soil through the vascular system (xylem vessels) of the plant. Glucose is produced, and quickly polymerised to form starch, which can be stored. Oxygen is released, and leaves through the stomata. The process requires energy from light, and only occurs in parts of the plant containing chlorophyll (green parts of the plant.)

Is this equation not a bit simplistic?

In fact, the actual process of photosynthesis is the result of two related stages. The light dependent phase uses electron transport driven by the light energy to produce ATP and NADPH. This stage requires water to function. The second stage, or the light independent stage ('Calvin cycle', 'Calvin-Benson cycle', 'Dark reaction') uses the ATP and the NADPH to 'fix' carbon from CO₂ to form glucose. I.e:



Questions

1. What are the two main sources of energy on earth that can drive ecosystems?
2. What are the technical terms for organisms that make their own food, and organisms that obtain food through eating other living things?
3. Of the electro-magnetic radiation from the sun, which types reach the earth's surface?
4. Why are plants green?
5. What is the overall equation for photosynthesis?
6. Why can plants absorb sunlight?
7. What normally happens to absorbed sunlight?
8. In terms of energy, plants change light energy into what type of energy?
9. What products are made from photosynthesis?
10. What raw materials are required?
11. What is the waste product from photosynthesis?
12. What do the two stages of photosynthesis do?

Section 2. Leaf and chloroplast structure

Green plants are successful at photosynthesis because of the way they are designed.

How are leaves designed for their job?

The main photosynthetic organ in most plants is the leaf. By producing numerous flat, broad leaves, plants can increase

- The amount of light that can be captured (large surface area)
- The rate of diffusion of gases into and out of the leaf (flat)
- The rate that water moves from soil to the leaf (many veins and xylem vessels in a leaf)
- The rate of movement of water vapour within the leaf (large spaces within leaf tissue)
- The probability of chloroplasts being struck by light (large number of chloroplasts, and the stacked organisation of each chloroplast)

Consider the diagram of a leaf below:

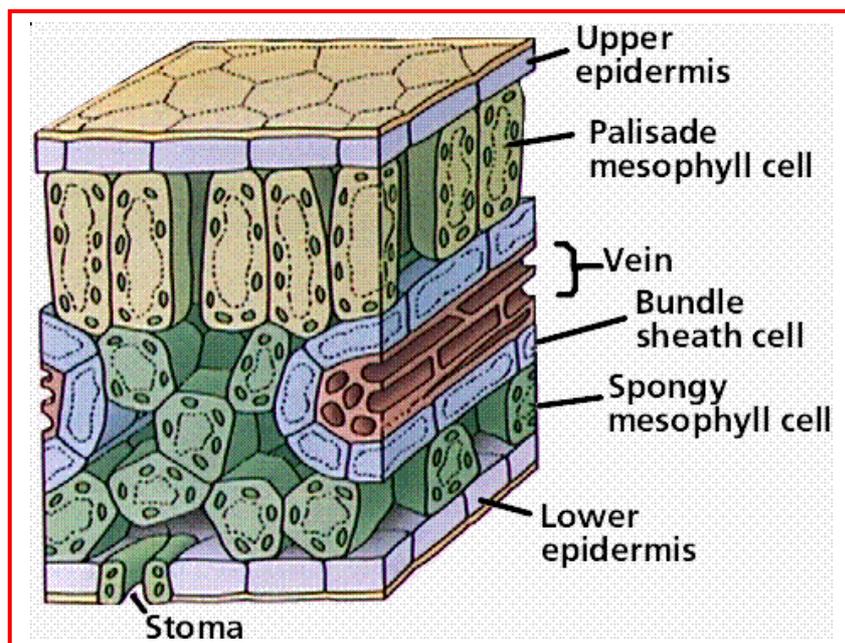


Fig. 4. The structure of a typical C₃ green leaf. Note the arrangement of chloroplasts in the palisade mesophyll layer, intended to maximise the chance of 'catching' light as it passes through the leaf. Note also the large air spaces in the leaf which allow diffusion of gases and the movement of water through the organ. The water is also supplied through the vessels of the vein (xylem) which also contain phloem tubes, that transport photosynthetic products away from the leaf in the sap. The stoma can open or close to regulate the activity of the leaf, and the water loss through evaporation.

? C₃ is the main form of photosynthesis in green plants. Some plants, however, have different chemical reactions to suit their environment, e.g. C₄ and CAM photosynthesis.

Leaves are the main photosynthetic organs of the plant.

Their large surface area captures much light, and their thin shape allows quick diffusion of gases and water within the leaf.

The arrangement of chloroplasts in the leaf also maximises light harvesting potential.

The main tissue involved in photosynthesis is the palisade mesophyll tissue.

SYNOPTIC mod 6 - Leaves are connected to the rest of the plant by organised transport systems.

SYNOPTIC mod 6 - Xylem vessels conduct water from the soil to the leaves by transpiration.

The products of photosynthesis are moved away from the leaf and around the plant by the phloem tubes, next to the xylem vessels in the veins.

Plants can move their leaves to position them best to collect sunlight. You can see this in houseplants if you turn the plant away from the window and leave it for several days.

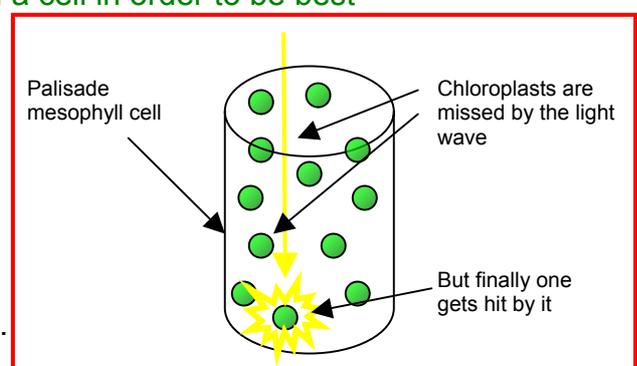
How are chloroplasts arranged in the leaf cells?

Leaf cells can contain many chloroplasts. Since the chloroplasts are the site of photosynthesis, their position within the cells is important – they must be arranged in the best way to gather as much light as possible.

Plants in shade often produce more chloroplasts than well-lit plants. This can be seen by the dark colour of the leaves, for example the dark green colour of jungle plants that are permanently shaded by the forest canopy. Holly is another good example, as it is often found in shade and has a rich dark colour indicative of high amounts of chlorophyll.

SYNOPTIC – an example of cytoskeleton / microtubule use. It has been shown that chloroplasts can migrate around a cell in order to be best positioned for light capture.

Looking at the diagram on the right, you can see that a beam of light passing through a palisade cell is likely to hit at least one chloroplast because of the way that they are organised. This adds to the efficiency of light capture by the cell, and is the reason for their elongated design.



'Palisade' is the term used for a fence made of logs, each side by side. This is where the name 'palisade mesophyll' comes from – the cells look like the logs in a palisade fence.

The arrangement of palisade cells in the leaf maximises the chance of light rays hitting the chloroplast by forming a relatively thick block of tightly packed light capturing cells.

The arrangement of chloroplasts in the cells also increases the chance of light hitting them, by being arranged in an 'overlapping' way.

Leaves on a plant will arrange themselves to maximise light capture. Each leaf on a branch will be positioned to avoid shading another, and to maximise the light capturing surface area of the plant.

This movement is a form of phototropism, and shows that plants (as with all living things) respond to their environment.

How are chloroplasts adapted for light capture?

A chloroplast diagram is shown below:

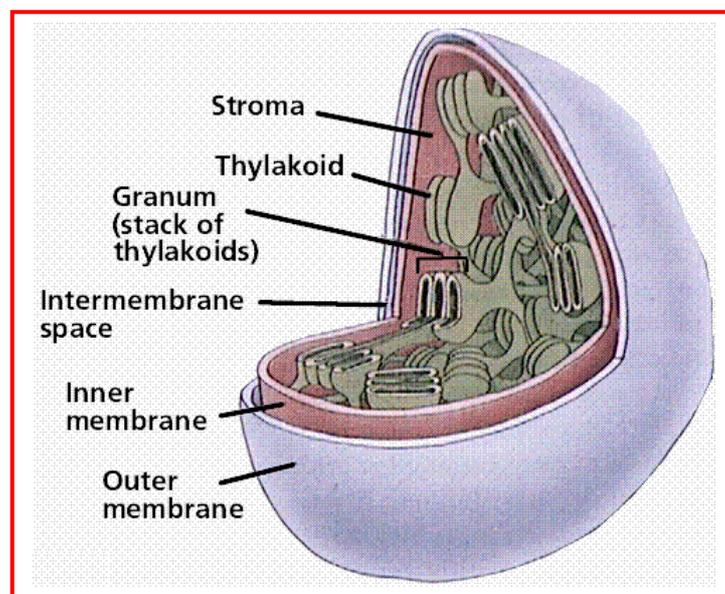


Fig. 5. 3D representation of a chloroplast.

SYLLABUS – 14.6. Chloroplasts. The role of chloroplasts in photosynthesis

Chloroplasts are highly structured; membrane bound organelles found in higher plants. They occur in many shapes and sizes, but all contain the same basic apparatus.

Chloroplasts are thought to be the remnants of a symbiosis between primitive eukaryotic cells and a photosynthetic bacterium. They contain their own circular DNA plasmid (*SYNOPTIC – eukaryotic and prokaryotic cells*) and possess two membranes. This double membrane, similar to that of a mitochondrion, indicates that the chloroplast used to be a single-membrane structure which obtained the second membrane on entry to the eukaryotic cell (*SYNOPTIC – phagocytosis*). In return for producing glucose and other metabolites from sunlight, the primitive prokaryote would have been protected

in the eukaryotic cell and would have had a ready supply of raw materials for photosynthesis.

The chloroplast is composed of two major regions – the stroma (liquid matrix containing the enzymatic apparatus of the light independent reaction (Calvin-Benson cycle), and the thylakoids, possessing the photosystems, pigments, enzymes and electron transfer elements of the light dependent reactions.

The photosystems are arranged neatly on the thylakoid membrane. This is important, as the geographical proximity of the photosystems to one another and to their associated electron carriers and enzymes are vital to the process of photosynthesis allowing smooth transfer of energy with minimum waste.

If you blend chloroplasts thoroughly in a high-speed blender, the membrane organisation is disrupted. Photosynthesis no longer occurs, which is an indication of the degree by which geographical proximity is important for photosynthesis. In fact, plant extracts treated this way will fluoresce – which is the manner that excited electrons release their energy in the absence of electron carriers.

Here is an illustration of the arrangement of photosystems and associated enzymes / electron carriers in the membrane of a thylakoid.

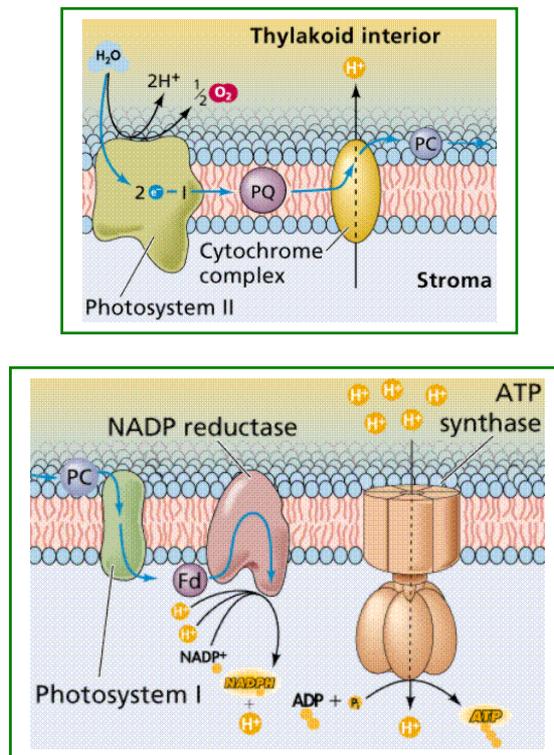


Fig 6. Artists impression of the arrangement of photosystems and associated electron carriers / enzymes in the membrane of a thylakoid. Note the close geographical arrangement of the elements, which controls the loss of energy from the electrons, coupling their movement to energy-harvesting enzymes / H^+ pumps. Note also that the net effect of PSII is to split water (hydrolysis) and pump protons for later ATP synthesis, and that the effect of PSI is to directly use excited electrons to reduce NADP

The thylakoids themselves within the chloroplast are arranged in little "stacks" called grana. This 'stack of coins' layout also increases the light capturing ability of the chloroplast.

SYNOPTIC – module 6. The 'stack of coins' layout of thylakoids is similar to the arrangement of light detection membranes in rod and cone cells in the retina.

SYNOPTIC – all modules. The thylakoid layout maximises surface area for light capture.

Showing this stacking as a simple diagram (right) we can see that photosystems are more likely to be activated by light because of their arrangement.

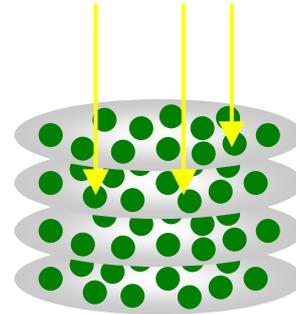


Fig. 7. Grana increase the chance of light hitting a photosystem.

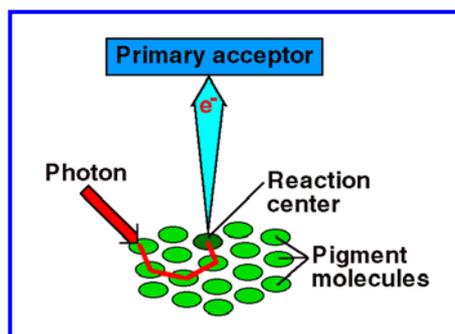
Chloroplasts are designed for photosynthesis by holding all the necessary light capturing apparatus, electron transfer molecules and enzymes in close proximity on the thylakoid membranes.

The nearby stroma contains the enzymes and reactants for the light-independent stage of photosynthesis, maximising the rate by which ATP and reduced NADP (NADPH) are used to fix carbon into carbohydrates.

The arrangement of thylakoids into stacks or grana increases the chance of light hitting a photosystem.

What is a photosystem, and how does it increase the light harvesting ability of a chloroplast?

A photosystem is simply a tightly structured arrangement of light-capturing pigment molecules, surrounding a molecule of chlorophyll a (the antenna molecule or reaction centre). Energy from light hitting any pigment in the photosystem is channelled toward the centre chlorophyll a molecule, which loses 2 electrons to electron transfer as a result. In this way, photosystems 'funnel' light energy. I.e.



There are two photosystems, called PSI and PSII. Each has slightly different combinations of pigments, and fulfil different roles in the light reaction.

Photosystems act as 'funnels' collecting light energy and passing it down to the reaction centre molecule, which loses two electrons into the electron transfer chain.

This loss of electrons drives the light dependent reaction.

Chlorophyll is able to lose electrons because it contains magnesium. This makes magnesium an important mineral ion for all plants.

Questions

1. List **three** ways that leaves are adapted for their job.
2. Draw a simple, two-dimensional diagram of the cellular structure of a typical leaf. Label the diagram.
3. What is the job of the plant vascular system in photosynthesis? (The vascular system is the phloem and xylem tubing in the veins of the plant.)
4. How is the arrangement of chloroplasts in leaf cells designed to maximise light capture?
5. In what way do chloroplasts indicate the existence of a cell cytoskeleton?
6. Draw a simple diagram of the internal structures in a chloroplast.
7. On the diagram (6) indicate clearly where the two stages of photosynthesis occur.
8. What is a photosystem?
9. How is the arrangement of enzymes, electron carriers and photosystems designed to maximise energy capture from light?
10. With regard to the proximity of the stroma to the thylakoids, why does the light independent reaction happen so quickly after the light dependent reaction?
11. How is the arrangement of thylakoids into grana (stacks) designed to maximise light capture?

Section 3 – the light dependent reactions

The light dependent reactions of photosynthesis all occur in the membranes of the thylakoids. They can be shown in a single diagram, sometimes called the 'Z-scheme'.

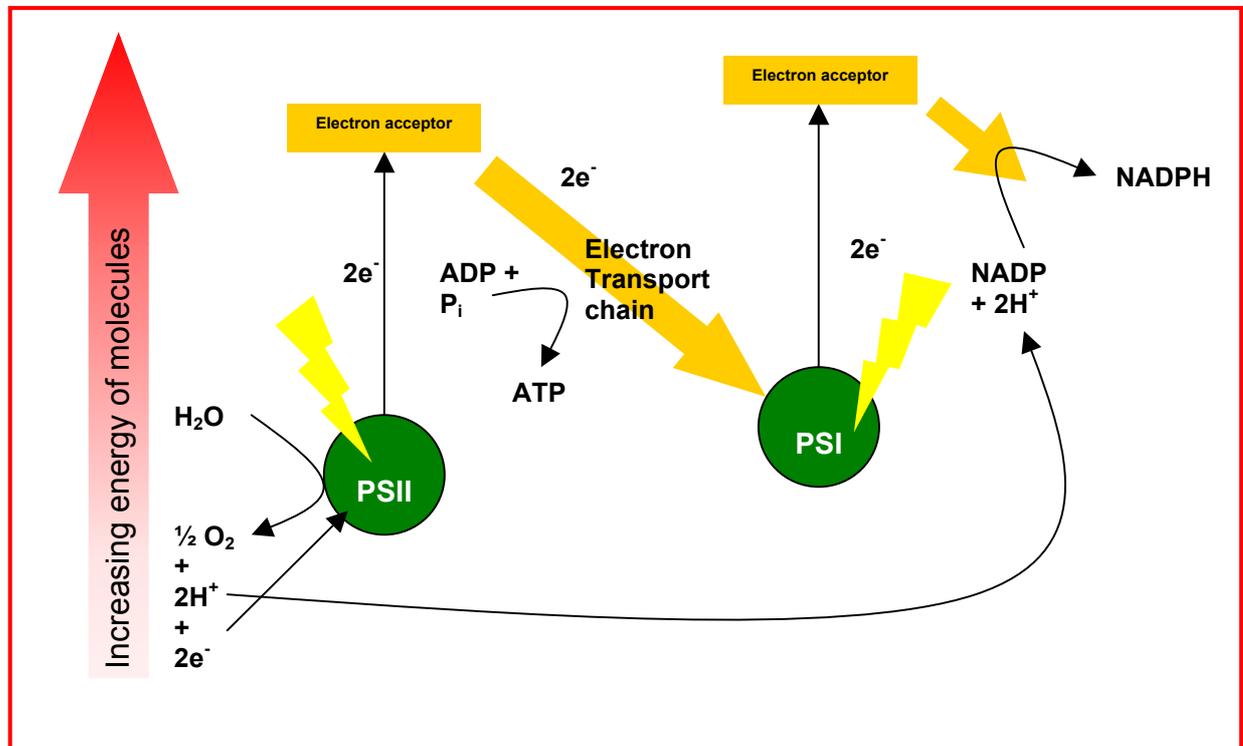


Fig. 8. The 'Z-scheme' of the light reactions. Note the simultaneous activation of the photosystems, the increasing energy of the molecules, the harvesting of ATP during electron transfer between PSII and PSI through the electron transfer chain, and the formation of NADPH from the electrons shifted from PSI. Note also the important photolysis of water to form oxygen gas, protons and electrons as a result of the light activation of PSII. The electron transfer chain is a sequence of mediated redox reactions.

How does light activate the light dependent reactions?

Syllabus – 14.6. "The biochemistry of photosynthesis. The light independent and light-dependent reactions in a typical C₃ plant.

These processes should be considered only in such detail as to show that:

- (i) in the light-dependent reactions.
 - electrons in chlorophyll are excited by light energy"

Chlorophyll molecules contain several important structures:

- A long organic tail for binding the molecule to the structural membrane of the photosystem.
- A porphyrin ring – characteristic of pigmented organic molecules. These rings absorb light well. Another molecule with a porphyrin ring is haemoglobin, found in blood.

- A central magnesium ion 'chelated' (a form of complex chemical bonding) to the centre of the porphyrin ring. This metal ion provides the 'loose' electrons for excitation by light.

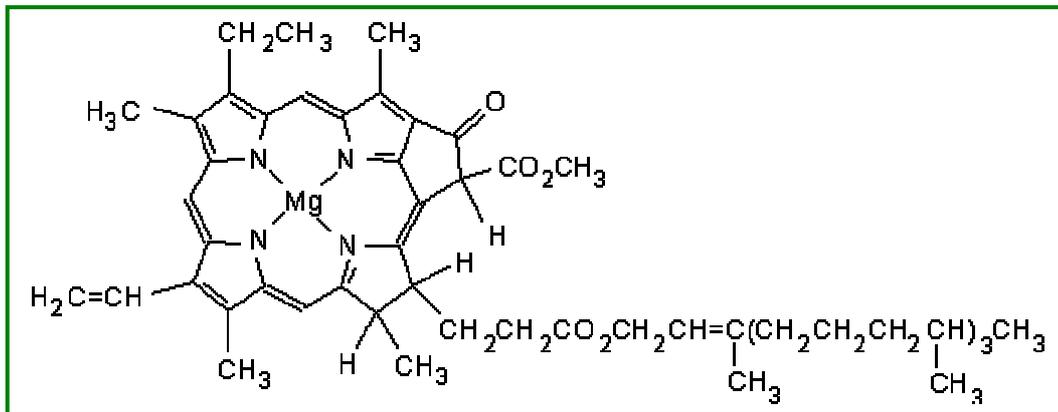
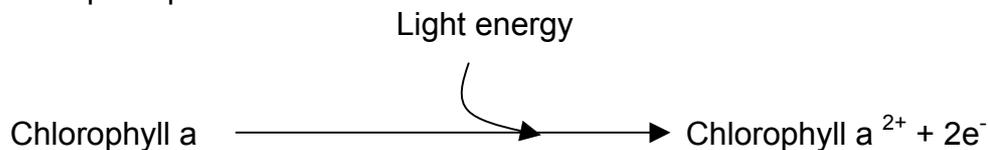


Fig. 9. Chemical structure of chlorophyll a. Note the porphyrin ring, the central Mg ion, and the long organic chain used to position and orientate the porphyrin ring for light capture.

When light energy arrives at a molecule of chlorophyll a, 2 electrons absorb the energy and move into a much higher (and increased energy) orbit around the chlorophyll molecule. This brings the high-energy electrons into close proximity to an electron acceptor, which captures the electrons leaving a 'positive hole' in the chlorophyll molecule where the electrons once were. As a simple equation:



The chlorophylls in both photosystems are excited by light energy at the same time. Both lose 2 high-energy electrons as a result. (See 'Z-scheme' on page 15).

Electron acceptors located close to each photosystem capture these electrons.

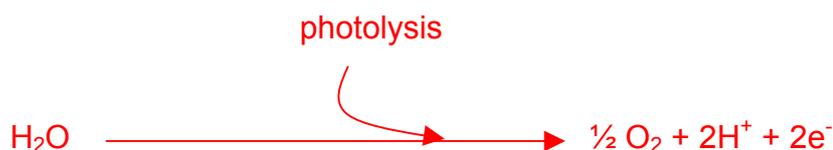
Photolysis – the role of water

Syllabus – photolysis of water produces protons and electrons.

The loss of 2 electrons by the chlorophylls in the photosystems bleaches the chlorophyll molecule. In this state, it can no longer absorb light energy effectively. Therefore, the electrons lost to the electron transfer chain must be replaced. Otherwise photosynthesis would grind to a halt.

Photolysis (the light-mediated splitting of water molecules) occurs as photosystem II is excited by light.

When PSII is activated by light, water molecules are split to form oxygen gas, protons (H^+ ions) and electrons.



This reaction provides protons for the reduction of NADP. It also provides 2 electrons to 'recharge' the chlorophyll molecule in PSII. Oxygen gas is released from this reaction.

Syllabus – oxygen is a valuable waste product of photolysis

Diatomic oxygen gas (O_2) is released as a result of photolysis. This is an important waste product, as it is thought to be photosynthetic activity that changed the earth's early CO_2 rich atmosphere into the O_2 rich atmosphere we have today.

A high oxygen atmosphere allowed the development of efficient respiration mechanisms in early cells, which enabled faster growth and development which would have been a key event in accelerating the evolution of animal life on earth.

Our atmospheric O_2 content is almost perfect for life. If the O_2 level was to increase significantly, combustion would occur more readily causing many more natural fires. Additionally, a highly oxidative environment is damaging to life. Therefore the balance of O_2 and CO_2 in our atmosphere is almost optimal.

The process of photolysis is still not fully understood. It is seen as one of the 'Holy Grails' of biochemistry. To understand it fully and harness the phenomenon commercially would provide a method of producing clean, high-energy hydrogen fuel from sunlight and water. Whoever finally cracks this process will (a) be famous and (b) become very, very rich!

SYNOPTIC – respiration. There is a close relationship between the activities of respiration and photosynthesis in living things. These two activities counteract each other in many ways, and a balance of the processes are necessary to maintain the favourable O_2/CO_2 ratio in the atmosphere.

SYNOPTIC – deforestation and human impact. Widespread deforestation leads to reduced ability of plants to remove CO_2 from the atmosphere. This is thought to be a major contributing factor to global warming. The burning of fossil fuels by industry also uses oxygen from our current atmosphere, and releases carbon dioxide from a prehistoric atmosphere (when the organisms that became the coal / oil / gas lived). This causes a net imbalance in the gases, and raises the overall CO_2 level.

SYNOPTIC – biomass as a renewable, clean fuel. Burning wood and plant material grown today is thought to be 'carbon-neutral'. Since the CO_2 used by the plants to grow was removed from the atmosphere only a few years ago, burning the plants for fuel merely reintroduces the CO_2 back to the air.

How do plants harvest the energy in the excited electrons to form relatively stable and transferable high-energy molecules?

SYLLABUS – energy from these excited electrons generates ATP and reduced NADP

From the 'Z-scheme' on page 16, it can be seen that the high-energy electrons are passed down one of two electron transport chains. It is during this transfer (*via* a series of oxidation-reduction reactions) that their energy is lost. By coupling the redox reactions in the electron transfer chain to enzyme reactions, it is possible to use their energy to drive the formation of energy-rich chemicals.

The light dependent reaction harvests the energy from high-energy electrons in the form of chemical energy.

The two energy-rich products of the reaction are ATP and NADPH (reduced NADP)

The syllabus refers to NADPH as reduced NADP. Almost no other source does this!

Light energy can be seen as 'packets' of energy called photons. It takes two photons of light to excite one chlorophyll molecule.

ATP production (photophosphorylation)

The electrons from photosystem II are passed through an electron transfer chain which terminates at photosystem I. Therefore the final destination for the 2 electrons from PSII is the 'positive hole' in PSI – this 'resets' the chlorophyll molecule in PSI.

Along the way the energy lost by the electrons during travel from electron acceptor to electron acceptor is used to drive the formation of ATP – the universal energy currency in cells.

Electrons from PSII travel through an electron transfer chain towards PSI.

In PSI they replace the 2 electrons lost by that photosystem.

Along the way, they lose energy that is coupled to the formation of ATP

In reality, the energy lost by the PSII electrons is used to drive the pumping of protons to the interior of the thylakoid. Since the membrane is impermeable to protons, a 'electro-chemical' gradient between the inner of the thylakoid and the stroma is developed. The only way this gradient can be resolved is by the protons passing through the ADP synthetase complex (see page 11, fig. 6).

As protons flow through this complex molecule, ATP is produced.

This is Mitchell's chemiosmotic theory, and has been shown to be strongly supported by the available evidence. It is the same mechanism by which ATP is produced from NADH and FADH₂ in oxidative phosphorylation (respiration.)

Formation of NADPH (reduced NADP)

The electrons from PSI also pass through a short electron transfer chain. At the end of the chain they are used, along with the two protons from water, to reduce NADP (an energy-carrying molecule.) The resulting molecule (reduced NADP) is often referred to as NADPH.

The electrons from PSI combine with NADP and protons from water to form reduced NADP – a high energy chemical.

The fate of ATP and reduced NADP.

Both ATP and reduced NADP are formed on the stroma side of the thylakoid membrane. Therefore they are immediately available to the light independent reaction.

They are then used to drive the enzymatic formation of glucose in the light independent stage of photosynthesis.

ATP and reduced NADP are formed in the stroma.

They are then used to drive the energetically unfavourable reactions of the light independent stage (fixation of carbon dioxide to yield carbohydrate.)

In this way the energy from light has been converted to a form useful for the driving of chemical reactions to produce stored energy in carbohydrates.

Questions

1. Where does the light dependent stage of photosynthesis occur in the chloroplast?
2. Draw the 'Z-scheme' of these reactions.
3. How does the chlorophyll use light energy?
4. What structural component does chlorophyll have that allows it to capture light?
5. In what ways is chlorophyll similar to haemoglobin?
6. What inorganic ion is essential for chlorophyll production?
7. How are the chlorophyll molecules in PSI and PSII 'reset' – i.e. how are the electron 'holes' in the molecules filled?
8. What is the significance for life on earth of photolysis?
9. What is Mitchell's chemiosmotic theory, and how does it explain ATP formation?
10. What two high energy compounds are made in the light dependent reaction?
11. How are these chemicals (question 10) used to make carbohydrates?

Section 4 – the light independent reactions

In 1961, Melvin Calvin and Andy Benson were awarded the Nobel Prize for unravelling the method by which carbon, in the form of carbon dioxide, was incorporated into plants to form glucose. The reaction is now known as either 'the light independent reaction', 'the Calvin cycle', 'the Calvin-Benson cycle' or, inappropriately, 'the dark reaction.' This set of reactions is the cornerstone of all life on earth. Simply, without them organic carbon-based life would not exist.

The light independent reactions of photosynthesis were essential for the development of life on this planet.



Fig.10. Melvin Calvin with some of his apparatus used to unravel the light independent stage of photosynthesis.

One for the pub quiz...

The most common protein in the world is the snappily named "Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase" or "RUBISCO". It is the major enzyme in the light independent stage of photosynthesis, which takes in CO_2 and combines it with Ribulose-1,5-Bisphosphate (RuBP) to form a 6 carbon intermediate which quickly splits to form 2 molecules of glycerate 3-phosphate (GP), a three carbon molecule. ATP and reduced NADP convert this into triose phosphate from which:

- RuBP is regenerated
- 1 molecule of glucose is formed for each 6 molecules of CO_2

This is the essence of the light independent stage of photosynthesis.

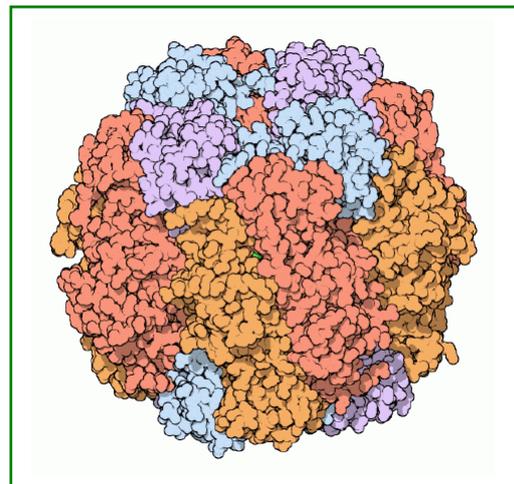


Fig. 11. RUBISCO – the most common enzyme / protein on planet Earth

SYLLABUS. "(ii) in the light-independent reactions:

- ribulose biphosphate (RuBP) acts as a carbon dioxide acceptor leaving to the formation of two molecules of glycerate 3-phosphate (GP).
- ATP and reduced NADP are required for the reduction of GP to triose phosphate.
- RuBP is regenerated in the Calvin cycle.
- Triose phosphate is converted to useful carbohydrates, amino acids and lipids"

What is the sequence of reactions in the Calvin cycle?

The Calvin cycle is best viewed as a diagram, from the point of view of the overall equation (using 6CO_2).

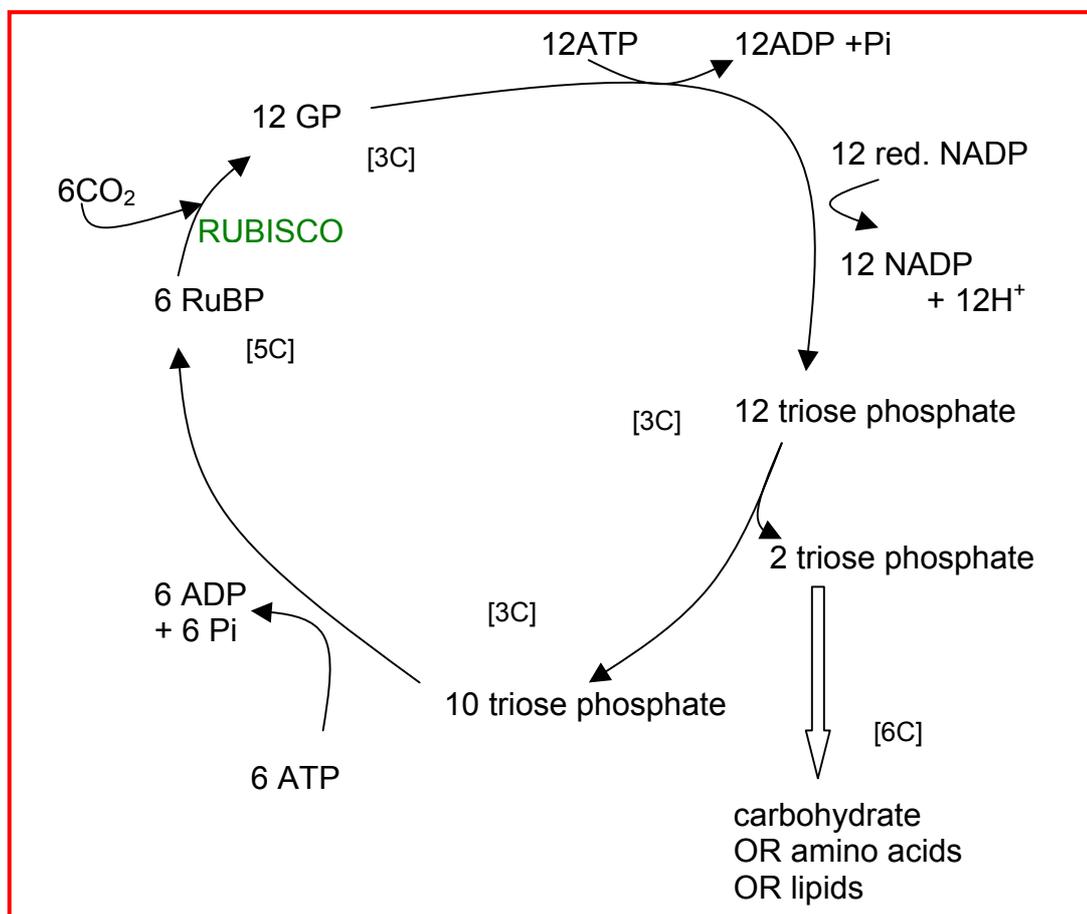


Fig. 12. The Calvin-Benson cycle. Note that 2 molecules of triose phosphate are 'harvested' from the reaction every 6 turns of the cycle. By viewing the sequence of events from the point of view of 6CO_2 molecules (as above), the cycle shown above describes the events after 6 turns of the cycle.

The enzyme RUBISCO, in spite of being the most common enzyme in the world, is very inefficient for an enzyme. It can add approximately 3CO_2 to 3 molecules of RuBP each second, which is very slow for an enzyme. To make up for this, plants produce vast quantities of RUBISCO, with it composing 50% of the protein in a chloroplast. Additionally, it is not a very specific enzyme

(SYNOPTIC – module 1) and frequently combines RuBP with oxygen rather than CO₂ because of a relatively non-specific active site. This leads to a useless oxygenated intermediate, and pathways exist to break this useless intermediate back down to form RuBP.

For this reason, high O₂ concentrations are unhelpful to photosynthesis.

The Calvin cycle combines CO₂ with a 5 carbon RuBP molecule to form 2 molecules of (3C) GP.

Energy from ATP and high-energy electrons from reduced NADP are necessary to drive the conversion of GP to triose phosphate. SYNOPTIC – triose phosphate is an intermediate of respiration

Two of these triose phosphates are harvested to form useful carbohydrates (around 80%) or amino acids / lipids as required.

The remaining 10 triose phosphates (containing 15 carbons in total) are used to regenerate 3 molecules of RuBP, which allows the continued activity of the cycle.

If levels of RuBP are low, the harvesting of carbohydrate can be inhibited to use the CO₂ to increase RuBP levels.

How was the cycle determined?

Melvin Calvin and Andy Benson (1940s) used radioactive carbon dioxide, containing C¹⁴ atoms, to grow algae under controlled conditions. By stopping algae growth second by second, and by identifying which compounds were radioactive in the algae, they were able to work out the order in which new compounds were formed using the C¹⁴.

E.g. At the start of the experiment, only CO₂ was radioactive. After 5 seconds, radioactive GP began to appear. This had become radioactive after the combination of radioactive CO₂ with RuBP. After 10 seconds, radioactive triose phosphate also appeared, which indicated that GP was being converted to TP (triose phosphate). After 15 seconds, radioactive glucose was also seen along with ribulose phosphate (the precursor of RuBP.) Finally, radioactive RuBP was identified, showing that the reactions were part of a cycle.

Further studies using the radioactive intermediates (such as radioactive RuBP) helped to tie all the pieces of the puzzle together to finally determine the overall cycle.

Recently, protein X-ray crystallography, nuclear magnetic resonance, genetic technology and protein sequencing have all been used to further our knowledge of this cycle.

Questions

1. Where does the Calvin cycle occur?
2. What is the most common enzyme in the world?
3. Describe two ways in which this enzyme (question 2) is inefficient compared to other enzymes.
4. In terms of the numbers of carbon atoms at each stage in 6 turns of the Calvin cycle, show that the cycle balances out.
5. According to the syllabus, which stage of this cycle requires energy?
6. According to the actual cycle, what other stage uses energy?
7. What are the products of 6 turns of the Calvin cycle?
8. Describe briefly how the cycle was determined in the 1940s.
9. Just *how* long does it take to get a Nobel Prize????