Pearson Edexcel Level 3 GCE

Wednesday 21 June 2023

Morning (Time: 2 hours)

Biology A (Salters Nuffield)

Advanced
PAPER 3: General and Practical Applications in Biology

Scientific article for use with Question 8
Do not return the insert with the question paper.

Turn over ▶

P71908A

Pearson

Scientific article for use with Question 8

'Green Planet': **Radiation and the Evolution of Photosynthesis**

1. The world is so dominated by the green machinery of photosynthesis that it is easy to miss the wood for the trees – to overlook the conundrum at its heart. Photosynthesis uses light to split water, a trick that we have seen is neither easy nor safe: it amounts to the same thing as irradiation. A catalyst such as chlorophyll gives ordinary sunlight the destructive potency of x-rays. The waste product is oxygen, a toxic gas in its own right. So why split a molecule as robust as water to produce toxic waste if you can split something else much more easily, such as hydrogen sulphide or dissolved iron salts, to generate a less toxic product?

2. The immediate answer is easy. For living organisms, the pickings from water-splitting photosynthesis are much richer than those from hydrothermal activity, the major source of hydrogen sulphide and iron salts. Today, the total organic carbon production deriving from hydro-thermal sources is estimated to be about 200 million tonnes (metric tons) each year. In contrast, the amount of carbon turned into sugars by photosynthesis by plants, algae and cyanobacteria is thought to be a million million tonnes a year – a 5000-fold difference. From a biological point of view, if you can split water, you can split anything. Such a powerful weapon must be caged in some way lest it run amok and attack other molecules in the cell. If, when the water-splitting device first evolved, it was not yet properly caged, as we might postulate for a blindly groping first step, then it is hard to see what advantage it could offer.

3. Why, and how, then, did oxygenic photosynthesis evolve? There is good circumstantial evidence that oxidative stress, produced by solar radiation as on Mars, lies behind the evolution of photosynthesis on the Earth. The details are fascinating but also reveal just how deeply rooted is our resistance to oxygen toxicity: part and parcel, it seems, of the earliest known life on Earth. The earliest known bacteria did not produce oxygen by photosynthesis, but they could breathe oxygen – in other words they could apparently generate energy from oxygen-requiring respiration before there was any free oxygen in the air. To understand how this could be, and why it is relevant to our health today, we need to look first at how photosynthesis works, and how it came to evolve.

4. Of the different types of photosynthesis carried out by living organisms, only the familiar oxygenic form practised by plants, algae and cyanobacteria generates oxygen. All other forms (collectively known as anoxygenic photosynthesis) do not produce oxygen and pre-date the more sophisticated oxygenic form. Despite our anthropocentric interest in oxygen, plants are not much concerned with the gas – what they need from photosynthesis is energy and hydrogen atoms. The different forms of photosynthesis are united only in that they all use light energy to make chemical energy (in the form of ATP) needed to cobble hydrogen onto carbon dioxide to form sugars. They differ in the source of the hydrogen, which might come from water, hydrogen sulphide or iron salts, or indeed any other chemical with hydrogen attached.

5. Overall, plant photosynthesis converts carbon dioxide (CO_2) from the air into simple organic molecules such as sugars (general formula CH_2O). These are subsequently burnt by the plant in its mitochondria (see Chapter 3) to produce more ATP, and also converted into the wealth of carbohydrates, lipids, proteins and nucleic acids that make up life. We met the enzyme that cobbles hydrogen onto carbon dioxide in Chapter 5 – RUBISCO, the most abundant enzyme on the planet. But RUBISCO needs to be spoon-fed with its raw materials – hydrogen and carbon dioxide. Carbon dioxide comes from the air, or is dissolved in the oceans, so that is easy. Hydrogen, on the other hand, is not readily available – it reacts quickly (especially with oxygen to form water) and is so light that it can evaporate away into outer space. Hydrogen therefore needs a dedicated supply system of its own. This is, in fact, the key to photosynthesis, but for many years the lock resisted picking. Ironically, the mechanism only became clear when researchers finally understood where the oxygen waste came from.

6. At first, scientists guessed that the oxygen came from carbon dioxide – a perfectly reasonable and intuitive assumption, but quite wrong as it turned out. The fallacy was first exposed in 1931, when Cornelis van Niel showed that a strain of photosynthetic bacteria used carbon dioxide and hydrogen sulphide (H_2S) to produce carbohydrate and sulphur in the presence of light – but did not give off oxygen:

$$CO_2 + 2H_2S \rightarrow (CH_2O) + H_2O + 2S$$

7. The chemical similarity between H_2S and H_2O led him to propose that in plants the oxygen might come not from carbon dioxide at all, but from water, and that the central trick of photosynthesis might be the same in both cases. The validity of this hypothesis was confirmed in 1937 by Robert Hill, who found that, if provided with iron ferricyanide (which does not contain oxygen) as an alternative to carbon dioxide, plants could continue to produce oxygen even if they could not actually grow.

8. In oxygenic photosynthesis, then, hydrogen atoms (or rather, the protons (H^+) and electrons (e^-) that constitute hydrogen atoms) are extracted from water, leaving the 'husk' – the oxygen – to be jettisoned into the air. The only advantage of water is its great abundance, for it is not easy to split in this way. The energy required to extract protons and electrons from water is much higher (nearly half as much again) than that needed to split hydrogen sulphide. Controlling this additional energy requires special 'high-voltage' molecular machinery, which had to evolve from the 'low-voltage' photosynthetic machinery previously used to split hydrogen sulphide. To understand how and why this voltage jump was made, we need to look at the structure and function of the machinery in a little more detail.

9. The interaction of light with any molecule always takes place at the level of the photon. In photosynthesis, chlorophyll is the molecule that absorbs photons. It cannot absorb any photon – it is constrained by the structure of its bonds to absorb photons with very particular quantities of energy. Plant chlorophyll absorbs photons of red light, with a wavelength of 680 nanometres. In contrast, the anoxygenic purple photosynthetic bacterium *Rhodobacter sphaeroides* has a different type of chlorophyll, which absorbs less-energetic infrared rays with a wavelength of 870 nanometres.

10. When chlorophyll absorbs a photon, its internal bonds are energized. The energetic vibrations force an electron from the molecule, leaving the chlorophyll short of one electron. Loss of an electron creates an unstable, reactive form of chlorophyll. However, the newly reactive molecule cannot simply take back its missing electron. That is snatched by a neighbouring protein and is whipped off down a chain of linked proteins, putting it beyond reach, like a rugby ball being passed across the field by a line of players. On the way, its energy is used to power the synthesis of ATP in a manner exactly analogous to that in mitochondrial respiration. The theft of an electron is half way to stealing an entire hydrogen atom, as hydrogen consists of a single proton and a single electron. Little extra work is needed to steal the proton. Electrostatic rearrangements draw a positively charged proton (from water in the case of oxygenic photosynthesis) after the negatively charged electron. The proton and then electron are eventually reunited by RUBISCO as a hydrogen atom in a sugar molecule.

11. What happens to the chlorophyll? Having lost an electron, it becomes far more reactive, and will snatch an electron from the nearest suitable source. The source of suitable electrons includes any plentiful sacrificial chemical, such as water, hydrogen sulphide or iron. Devouring an electron settles the chlorophyll back into its normal equable state, at least until another photon sets the whole cycle in motion again.

12. Which electron donor is used in photosynthesis – hydrogen sulphide, iron or water – ultimately depends on the energy of the photons that are absorbed by the chlorophyll. In the case of purple bacteria, their chlorophyll can only absorb low-energy infrared rays. This provides enough energy to extract electrons from hydrogen sulphide and iron, but not from water. To extract electrons from water requires extra energy, which must be acquired from higher-energy photons. To do this requires a change in the structure of chlorophyll, so it can absorb red-light photons instead of infrared light.

13. According to Robert Blankenship of Arizona State University and Hyman Hartman of the Institute for Advanced Studies in Biology at Berkeley, California, tiny changes in the structure of bacterial chlorophylls can lead to large shifts in their absorption properties. Two small changes to the structure of bacteriochlorophyll *a* (which absorbs at 870 nm) are all that it takes to generate chlorophyll *d*, which absorbs at 716 nanometres. In 1996, an article in *Nature* by Hideaki Miyashita and colleagues of the Marine Biotechnology Institute in Kamaishi, Japan, reported that chlorophyll *d* is the main photosynthetic pigment in a bacterium called *Acaryochloris marina*, which splits water to generate oxygen. Thus, an intermediate between bacteriochlorophyll and plant chlorophyll is not only plausible: it actually exists. From chlorophyll *d* another trifling change is all that is required to produce chlorophyll *a*, the principal pigment in plants, algae and cyanobacteria, which absorbs light at 680 nanometres.

14. Technically, then, the evolutionary steps required to get from bacteriochlorophyll to plant chlorophyll are simply achieved. The question remains, why? A chlorophyll that absorbs light at 680 nanometres is less good at absorbing light at 870 nanometres. It is therefore less efficient at splitting hydrogen sulphide, and so bacteria carrying it are at a competitive disadvantage compared with the bacteria that kept their original chlorophyll. Even worse, switching chlorophylls to split water poses the problem of what to do with the toxic oxygen waste, as well as any leaking free-radical intermediates – the same as those produced by radiation. Without foresight, how did life manage to cope with its dangerous new invention?

15. Chlorophyll extracts electrons from water one at a time. To generate oxygen from water, it must absorb four photons and lose four electrons in succession, each time drawing an electron from one of two water molecules. The overall water-splitting reaction is:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$
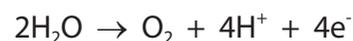
Only in the final stage is oxygen released. The rate at which chlorophyll extracts electrons depends on how quickly the photons are absorbed. As the successive steps cannot take place instantly, a series of potentially reactive free-radical intermediates must be produced, if only transiently.

16. If photosynthesis is to work at all, the reactive intermediates from water must be sealed inside a structure that immobilizes them, preventing them from escaping before oxygen is released. Needless to say, they are sealed in such a cage, this is how photosynthesis works. The cage is made of proteins and is called the oxygen-evolving complex (or sometimes the water-splitting enzyme). Water is bound tightly inside the protein cage while the electrons are extracted one at a time. But this is no ordinary cage. Its structure conceals a secret that is much older than the hills, which transports us back to the time before oxygenic photosynthesis evolved, to a time more than 2.7 billion years ago, before there was any oxygen in the atmosphere. This structure is the key to life on Earth, for without it the Earth would have remained as sterile as Mars.

17. The structure of the oxygen-evolving complex is very similar to that of an antioxidant enzyme called catalase. In fact, the oxygen-evolving complex looks as if it evolved from two catalase enzymes lashed together. If so, then catalase must have evolved *before* the oxygen-evolving complex. If so, the chronology must be as follows. Catalase evolved on the early Earth, in an atmosphere devoid of oxygen. One day, two catalase molecules became bound together to form a cage that enabled the safe splitting of water: the oxygen-evolving complex. This cage allowed the evolution of oxygenic photosynthesis. As a result, the atmosphere filled with oxygen. Life was put under serious oxidative stress. Luckily it could cope: it already had at least one antioxidant enzyme that could protect it – catalase. How convenient! But wait a moment. If catalase came before photosynthesis, then even if there was no atmospheric oxygen, there must have been oxidative stress. Is this plausible? To answer this question, we must take a look at how catalase works.

18. There are several different types of catalase. Most animal cells have a form that has four haem molecules embedded in its core. In contrast, some microbes have a different sort of catalase, which contains manganese instead of haem at its core. Despite their different structures, both enzymes are equally fast, and are correctly called catalase, in the sense that they work in the same way – they both catalyse the reaction of two molecules of hydrogen peroxide with each other to form oxygen and water:

$$2H_2O_2 \rightarrow 2H_2O + O_2$$
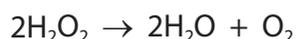
This simple reaction mechanism reveals a great deal about conditions on the Earth 3.5 billion years ago. It is the exact equivalent of the natural reaction between two molecules of hydrogen peroxide, but is speeded up 100 million times by the enzyme. The need for two molecules of hydrogen peroxide means that catalase is extremely effective at removing hydrogen peroxide when concentrations are high, when it is easy to bring two molecules together. It works less well at low concentrations of hydrogen peroxide, when it is harder to find two molecules close together. Catalase is thus swift to remove high concentrations of hydrogen peroxide, but is poor at mopping up trace amounts or at maintaining a stable low-level equilibrium.

19. Today, most aerobic organisms have a second group of enzymes, known as the peroxidases, which can dispose of trace amounts of hydrogen peroxide. These enzymes work better at low levels of hydrogen peroxide because they act in a fundamentally different way. Rather than bringing two molecules of hydrogen peroxide together, they use antioxidants such as vitamin C to convert a single molecule of hydrogen peroxide into two molecules of water, without generating any oxygen. Most aerobic cells have both sets of enzymes, and break down hydrogen peroxide using both mechanisms. Catalase is used for bulk removal, peroxidase for subtle adjustments.

20. Catalase would presumably have been present in the photosynthetic bacteria that generated energy by splitting hydrogen sulphide or iron salts in the era before oxygenic photosynthesis. In fact, hydrogen peroxide has some parallels with these early photosynthetic fuels. To remove electrons from hydrogen peroxide requires a similar input of energy to that required to remove electrons from hydrogen sulphide, and so could have been achieved using the same bacteriochlorophyll. Hydrogen peroxide would therefore have been a good source of hydrogen for photosynthesis. And, while far less plentiful than hydrogen sulphide and iron salts, it was nonetheless formed most readily in the surface waters, closest to the full power of the Sun. If this scenario is true, then catalase could have doubled as a photosynthetic enzyme. Because splitting hydrogen peroxide generates oxygen, this recruitment of catalase to photosynthesis also bridges the evolutionary gap between anoxygenic and oxygenic photosynthesis.

21. If catalase was acting as a photosynthetic enzyme, then it would be natural for a number of catalase molecules to cluster around the photosynthetic apparatus. In these circumstances, it would be simple for two catalase molecules to became associated as a complex: the prototype oxygen-evolving complex. At first it would have continued to use hydrogen peroxide as an electron donor, but given the right energy input, this complex could split water. We know that three small changes in the structure of bacteriochlorophyll can transform its properties, enabling it to absorb high-energy light at a wavelength of 680 nm. We now have a prototype oxygen-evolving complex (the nutcracker that can physically split water) and a chlorophyll that can provide enough energy for it to do so (or the hand that presses the nutcracker). Thus, with no foresight and no disadvantageous steps, we have taken a path leading from anoxygenic photosynthesis to oxygenic photosynthesis.

Adapted from: Nick Lane. 'Green Planet'. In *Oxygen*. Oxford, Oxford University Press, 2002. pp131–146.

BLANK PAGE

BLANK PAGE