PHOTOSYNTHESIS AND THE ORIGIN OF LIFE

HYMAN HARTMAN
IASB, 880 Spruce St., Berkeley, CA 94707, U.S.A.

(Received 30 August, 1996)

Abstract. The origin and evolution of photosynthesis is considered to be the key to the origin of life. This eliminates the need for a soup as the synthesis of the bioorganics are to come from the fixation of carbon dioxide and nitrogen. No soup then no RNA world or Protein world. Cyanobacteria have been formed by the horizontal transfer of green sulfur bacterial photoreaction center genes by means of a plasmid into a purple photosynthetic bacterium. The fixation of carbon dioxide is considered to have evolved from a reductive dicarboxylic acid cycle (Chloroflexus) which was then followed by a reductive tricarboxylic acid cycle (Chlorobium) and finally by the reductive pentose phosphate cycle (Calvin cycle). The origin of life is considered to have occurred in a hot spring on the outgassing early earth. The first organisms were self-replicating iron-rich clays which fixed carbon dioxide into oxalic and other dicarboxylic acids. This system of replicating clays and their metabolic phenotype then evolved into the sulfide rich region of the hotspring acquiring the ability to fix nitrogen. Finally phosphate was incorporated into the evolving system which allowed the synthesis of nucleotides and phospholipids. If biosynthesis recapitulates biopoesis, then the synthesis of amino acids preceded the synthesis of the purine and pyrimidine bases. Furthermore the polymerization of the amino acid thioesters into polypeptides preceded the directed polymerization of amino acid esters by polynucleotides. Thus the origin and evolution of the genetic code is a late development and records the takeover of the clay by RNA.

Introduction

The major premise which underlies the field of either the ‘RNA world’ or the ‘Proteinoid world’ is the prior existence of a soup of monomers consisting of sugars, pyrimidine and purine bases and amino acids. In the old conundrum as to which came first the chicken (protein) or the egg (RNA), the answer is the chicken soup (of monomers). The major premise of this paper is that the soup is a hypothetical construct.

In the absence of a soup the carbon entering the biosphere is in the form of carbon dioxide, the nitrogen is in the form of nitrogen gas, the hydrogen and oxygen enter the biosphere in the form of liquid water, the sulfur in the form of sulfide ion and the phosphate in the form of the phosphate ion.

The term, ‘biogenic elements’, which is applied to CHNOPS (carbon, hydrogen, nitrogen, oxygen, phosphorous, and sulfur) is misleading as the transition metals such as iron, manganese, cobalt, nickel, etc. are all ‘biogenic elements’ as are sodium, potassium, calcium and magnesium. The study of these elements, especially their behavior in liquid water on a terrestrial planet, is what can be truly called biogenic.

Origins of Life and Evolution of the Biosphere **28:** 515–521, 1998.

1. Photosynthesis

The early atmosphere of the earth is considered to have been neutral (nitrogen and carbon dioxide). The problem arises of how oxygenic photosynthesis could have evolved under these conditions. There were abundant reducing agents such as ferrous ion which made it unlikely that water would be used as an electron donor. It was thus suggested 'that atmospheric hydrogen peroxide played a key role in inducing oxygenic photosynthesis because as peroxide increased in a local environment, organisms would not only be faced with a loss of reductant, but they would also be pressed to develop the biochemical apparatus (e.g., catalase) that would be ultimately be needed to protect against the products of oxygenic photosynthesis. This scenario allows for the early evolution of oxygen photosynthesis while global conditions were still anaerobic' (McKay and Hartman, 1991). Oxygenic photosynthesis developed in the cyanobacteria.

The earliest bacterial fossils are found in the 3.5 billion year old stromatolites of Western Australia. These fossil bacteria resemble cyanobacteria (Awramik, 1992). One possible conclusion from the ancient stromatolites and the bacterial fossils is that oxygenic photosynthesis was being carried out by the cyanobacteria 3.5 billion years ago.

The cyanobacteria have two reaction centers; photosystem I and photosystem II. This is in contrast with the purple and the green photosynthetic bacteria which have only one reaction center. The purple bacteria have a pheophytin-quinone reaction center which is related to photosystem II. The green sulfur bacteria have a Fe-S reaction center which is related to photosystem I. 'The simplest scenario giving rise to the linked photosystems found in oxygen-evolving organisms is that some sort of genetic fusion event took place between two bacteria, one with a pheophytin-quinone reaction center and the other with an Fe-S reaction center. This produced a chimeric organism with two unlinked photosystems. Subsequently, the two photosystems were linked, and the oxygen evolving system added' (Blankenship, 1992).

An explanation for this fusion event is that there was a horizontal transfer of a Fe-S reaction center from a green sulfur bacterium by means of a plasmid into a purple non-sulfur photosynthetic bacterium. In other words the cyanobacterium is a purple bacterium into which a green sulfur reaction center has been horizontally transferred.

One way to test this hypothesis is to use the sequences of the protein thioredoxin from photosynthetic bacteria. Thioredoxins have recently been used to study the evolutionary histories of chloroplasts and bacteria. In that study, the photosynthetic bacterial thioredoxins clustered into three groups: (1) photosynthetic purple bacteria and close relatives such as *E. coli*; (2) the photosynthetic green sulfur bacterium *Chlorobium*; (3) the cyanobacteria. The groupings are similar to those generated from earlier 16s rRNA analyses (Hartman *et al.*, 1990). In chloroplasts which are closely related to the cyanobacteria, there are two thioredoxins; thioredoxin f and

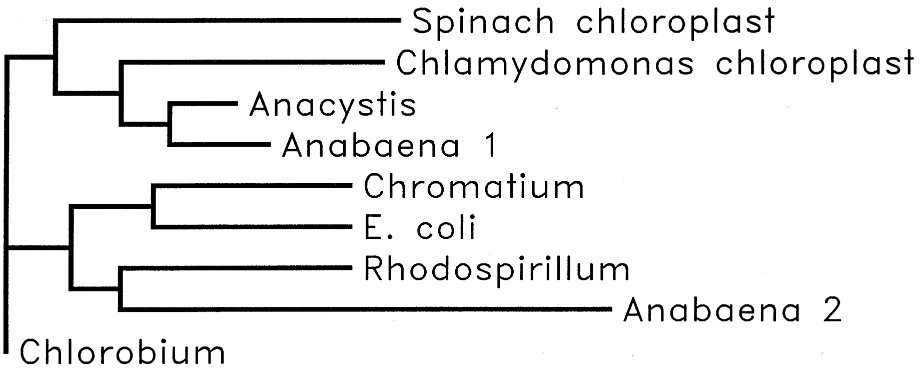

Figure 1. The sequences of the thioredoxins (thioredoxin m from chloroplasts and bacteria) and the alignments were as reported in Hartman *et al.*, 1990. The *Anabena* 2 sequence was obtained from Alam *et al.* (1989). The tree was generated by PAUP 3.0 computer program as described in Hartman *et al.*, 1990. Note that *Anabena* 1 is branching with *Anacystis* and the chloroplast thioredoxin m and *Anabena* 2 is branching with Rhodospirillum.

m. However the thioredoxin f came from the eukaryotic host cell. The need for two thioredoxins in the chloroplast raised the question as to whether there are two thioredoxins in the cyanobacteria. In *Anabena*, a cyanobacterium, there are two thioredoxins labeled *Anabena* thioredoxin 1 and *Anabena* thiredoxin 2.

The phylogenetic tree in which the thioredoxins from *Anabena* are added to the sequences of thioredoxins from the photosynthetic bacteria is shown in Figure 1. The *Anabena* thioredoxin 2 is branching with the thioredoxin of the purple photosynthetic bacterium Rhodospirillum while the *Anabena* thioredoxin 1 is branching with the thioredoxin of the cyanobacterium *Anacystis*.

This tree is most easily interpreted as a horizontal transfer of a reaction center with its thioredoxin moving from a green sulfur photosynthetic bacterium into a purple photosynthetic bacterium with its thioredoxin. The act of consolidation of these two centers in the purple bacterium caused the thioredoxin of the green sulfur to evolve away from its green sulfur roots.

Thus the cyanobacterium is a purple photosynthetic bacterium to which a green sulfur photosynthetic reaction center has been inserted.

If we are to probe more deeply into the origin of photosynthesis we have to look to the photosynthetic abilities of the green sulfur photosynthetic bacteria, the purple non-sulfur bacteria and the green gliding photosynthetic bacteria.

It should be noted here that there is a discrepancy between the evolutionary trees generated from the 16S rRNA and the evolutionary trees generated from the reaction centers especially in the case of the green gliding bacterium *Chloroflexus*. In this case according to the 16s rRNA analysis, *Chloroflexus* is the deepest branching organism within the photosynthetic eubacteria. However the reaction center sequences of *Chloroflexus* place it close to the later branching purple bacteria. ‘The significance of this disagreement between the evolutionary trees generated from the 16S rRNA and the reaction center sequences is yet unclear. The simplest explanation is that a lateral gene transfer may have moved genes necessary for photosynthesis into (or out of) an ancestor of *Chloroflexus*' (Blankenship, 1992).

In summary the cyanobacteria are considered to be the oldest fossil bacteria (3.5 billion years old). The 16s ribosomal RNA data have the green gliding bacteria (*Chloroflexus*) branching much earlier than the cyanobacteria. The thioredoxin sequence data implies that the cyanobacteria are a result of a horizon transfer of a green sulfur reaction center into a purple photosynthetic bacteria. All this implies that the origin and evolution of photosynthesis is much older than the fossil cyanobacteria and hence was present before 3.5 billion years.

The oldest sedimentary rocks (highly metamorphosed) for which there is evidence of photosynthesis are the Isua branded iron formations (3.8 billion years old). There is some evidence for carbon dioxide fixation in the Isua banded iron formations (Schidlowski, 1988). It was proposed that these banded iron formations were due to a microbial mat dominated by an anaerobic photosynthetic bacterium in which ferrous ion was the electron donor (Hartman, 1984). A search for such an anaerobic photosynthetic bacterium was begun. This recently led to the discovery by Ehrenreich and Widdel of the predicted anaerobic photosynthesis where ferrous ion was the electron donor. They then agreed with the previous hypothesis that: 'The existence of ferrous iron-oxidizing anoxygenic phototrophs may offer an explanation for the deposition of early banded-iron formations in an assumed anoxic biosphere in Archean times' (Ehrenreich and Widdel, 1994). The conclusion which can be drawn is that the early coupling of the geochemical iron cycle to the carbon cycle is of immense importance in considerations on the origin of life. This ia a very valuable clue to the origin of life.

All the previous discussion was based either on a fossil record or an evolutionay record stored in the nucleotide sequences of the 16s ribosomal RNA and in the amino acid sequences of the proteins (e.g. thioredoxin). What are we to do when these two records give out? Is there another record which we can study? The answer to these questions is clear. There is the metabolic record which is stored in the biosynthetic pathways of the photosynthetic bacteria.

The purple bacteria (and the cyanobacteria fix carbon dioxide by means of the reductive pentose phosphate cycle (Calvin cycle). The green sulfur bateria (*Chlorobium*) fix carbon dioxide by means of the reductive citric acid cycle (Buchanan, 1992). The green non-sulfur bacteria (*Chloroflexus*) fix carbon dioxide by means of the reductive glyoxalate cycle (Ivanovsky *et al*., 1993). Another carbon dioxide fixation cycle involving 3-hydroxypropionante has been found in *Chloroflexus* (Eisenreich *et al*., 1993). The evolution of carbon dioxide can be outlined as follows: First the reductive dicarboxylic acid cycle (e.g. the reductive glyoxalate cycle in *Chloroflexus*) which was then followed by the reductive tricarboxylic acid (the reductive citric acid cycle in *Chlorobium*). Finally the reductive pentose phosphate cycle appeared in the purple bacteria and was kept as the transfer of the photosystem of the green sulfur was introduced into a purple bacterium converting it into a cyanobacterium. When a new carbon dioxide fixation was evolved then the earlier form was still kept but as a back up. This evolutionary sequence of carbon dioxide fixations can be considered as record of the early stages of life.

2. The Origin of Life

The original living system was a set of replicating iron rich clays which evolved up the metabolic pathways. The major assumption is that life began as a photoautotroph in an atmosphere of carbon dioxide and nitrogen.

In 1975, I proposed that the origin of life was based on a primitive photosynthesis. The major ideas of that paper were that: (1) The derivation of the citric acid cycle came from the photoreduction of carbon dioxide where the reductant was ferrous ion; (2) the biosynthesis of the amino acids and sugars were offshoots of the citric acid cycle; (3) the first polymerizations would have been the synthesis of the fatty acids from acetyl-CoA; (4) the coenzymes came before the enzymes.

It was thus concluded that ‘the appearance of an early metabolism which driven by light (UV), fixating nitrogen and carbon dioxide, eventually evolved into the metabolism which we know today. From the view of the autotrophic origin of life, it is not surprising that the paleontological record should show the early appearance of blue-green algae (cyanobacteria). These organisms obtain their energy from sunlight, their carbon from carbon dioxide and their nitrogen from atmospheric nitrogen’ (Hartman, 1975).

In the paper entitled ‘Conjectures and reveries’ (Hartman, 1992) I reconstructed the origin and evolution of the energy conversions in photosynthesis. The major assumption of that paper is that life originated in an environment where there was no soup of organic molecules. The evolution of photosynthesis began with the photoreduction of carbon dioxide by the ferrous ions in self-replicating iron-rich clays. The next stage involved the entry of sulfur into the evolving clay system which led to the formation of acetyl thioesters and the polymerization of thioesters. This more complex iron-rich clay system now was able to fix nitrogen which led to the formation of pyrrole, flavin, nicotinamide, phycobilins, porphyrins and chlorophyll. Finally phosphate entered the evolving system and the formation of ATP became the energy currency of the evolving clay system. The chromophores evolved from ferrous ion through the quinones, caroteinoids, phycobilins to chlorophyll. The evolution of chromophores implies that photosynthesis began in the UV and evolved through the blue, yellow, orange into the red. The electron transport chain evolved from ferrous ion through the Fe_2S_2 and Fe_4S_4 cores to the hemes.

If we now correlate the carbon dioxide fixation with the four phases outlined above, we can reconstruct the fixation schemes as follows:

(1) The first phase was dominated by the photoreduction of carbon dioxide to form oxalate and with the aid of manganese the oxalate was reduced to glyoxalate.

The condensation of two glyoxalates and the subsequent decarboxylation lead to the formation of glycerate .

(2) The second phase was the entry of sulfur into the evolving clay system which led to the formation of Fe_2S_2 and Fe_4S_4 cores and acetyl thioesters. A reductive dicarboxylic cycle which involved acetyl thioester condensing with carbon dioxide to form pyruvate which condenses with carbon dioxide to form oxalacetate which in turn is reduced to form malate. The malate then splits to form acetyl thioester and glyoxalate (Ivanovsky *et al.*, 1993). This cycle was later expanded to form the reductive citric acid cycle. A variant of the reductive citric acid cycle would be driven by reduced ferredoxin analogs and thioesters in this phase.

(3) The third phase began with the ability to fix nitrogen. With the fixation of nitrogen into ammonia, the biosynthesis of the amino acids from the citric acid cycle becomes possible. The first polymerization of the thioesters of amino acids would result in the first polypeptides. These polypeptides were possibly made up of alternating hydrophilic and hydrophobic amino acids and formed the first membranes.

(4) In the final phase, phosphate entered the evolving system. This resulted in the fixation of carbon dioxide by the reductive pentose phosphate cycle (Calvin cycle). The formation of phospholipids now became possible which led to the fluid mosaic membrane. Finally the synthesis of nucleotides and the polymerization of the nucleotides into polynucleotides resulted in the origin of the genetic code.

Where on the primitive earth did life begin? The origin of life and photosynthesis would take place in a hot spring on the surface of the early earth. The water in a hot spring would be rich in ferrous ion and other transition metals (Cu, Co, Zn). The water would also contain magnesium ions, aluminum ions, silicate ions and gases such as carbon dioxide, nitrogen and hydrogen sulfide. After the sulfides had precipitated out, the excess ferrous ions would form iron-rich clays with the magnesium and silicate ions. These clays would then fix carbon dioxide into oxalic acid and other organic acids which can catalyze clay formation itself. These replicating iron-rich clays would evolve into the sulfide-rich region of the primordial hot spring acquiring nitrogen fixation in the process; finally phosphate is incorporated into the evolving system. The memory of the origin of life is still to be found in the photosynthesis carried out by the green bacteria, the purple bacteria and the cyanobacteria today. If we trace the evolution of photosynthesis we are in the process discovering that life is energy and the energy is sunlight. Life is also the chemistry of carbon dioxide, iron, sulfur, nitrogen and phosphate in water. But life is also memory. Today the memories are stored in DNA, at the Origin it was in iron-rich clays.

Since ‘biosynthesis recapitulates biopoesis’ (Granick, 1957), amino acids preceded the formation of the bases and polypeptides preceded polynucleotides. The reason for this is that all metabolism and the subsequent polymerization of amino acids and nucleotides took place in an evolving system of replicating and mutating iron-rich clays. The polynucleotides and polynucleotide directed polymerization of amino acids into polypeptides by means of a genetic code are a late entry into the evolving clay system.

It there was no soup and life began as a photoautotrophic iron rich clay on Earth, then when we sample the surface of Mars for fossils of early life. We should look not only for amino acids and other biochemicals but also for the ancient fossil minerals such as iron-rich clays and magnetite.

References

Alam, J., Curtis, S., Gleason, F. K., Gerami-Nejad, M. and Fuchs, J. A.: 1989, *Journal of Bacteriology* **171**, 162.
Awramik, S. M.: 1992, *Photosynthesis Research* **33**, 75.
Blankenship, R. E.: 1992, *Photosynthesis Research* **33**, 91.
Buchanan, B. B.: 1992, *Photosynthesis Research* **33**, 147.
Eherenreich, A. and Widdel, F.: 1994, *Applied and Environmental Microbiology* **60**, 4517.
Eisenreich, W., Strauss, G., Werz, U., Fuchs, G. and Bacher, A.: 1993, *Eur. J. Biochem.* **215**, 619.
Granick, S.: 1957, *Ann NY Acad Sci*, **69**, 292.
Hartman, H.: 1975, *J. Mol. Evol.* **4**, 359.
Hartman, H.: 1984 in *Microbial Mats; Stromatolites*, Y. Cohen *et al*. (eds.), Alan Liss Inc., New York.
Hartman, H.: 1992, *Photosynthesis Research* **33**, 171.
Hartman, H., Syvanen, M. and Buchanan, B. B.: 1990, *Mol. Biol. Evol.* **7**, 247.
Ivanovsky, R. N., Krasilnikova, E. N. and Fal, Y. I.: 1993, *Arch Microbial.* **159**, 257.
McKay, C. P. and Hartman, H.: 1991, *Origins Life Ecol. Biosphere* **21**, 157.
Schidlowski, M.: 1988, *Nature* **333**, 313.