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Chlorophyll and photosynthesis

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Photosynthesis is the prerequisite of all life on earth. Chlorophyll fulfils the requirements for photosynthesis: the absorption of visible light, the photochemical capabilities, a rich supply of redox levels and chemical stability. The biosynthetic pathway of chlorophyll can be read as the evolutionary history of photosynthesis. Our exegesis is that the primary porphyrins served for early photosynthesis. The porphyrins readily photo-oxidize organic compounds under the reducing, aqueous conditions of this early era. The formation of oxidized substances in a reducing atmosphere supplied the thermodynamic gradient necessary for organized life processes. Conversely, the closed-shell metalloporphyrins, notable magnesium porphyrins, are powerful photoreducing agents. When coupled to the ultimate electron source, water, oxygen was produced and the modern era of photosynthesis was born. At the same time, the efficiency and usefulness of the photopigments was increased by incorporating them into the organized cellular system of membranes. The clear gradient of ionic to hydrophophic structures along the biosynthetic pathway from porphyrins to chlorophyll supports this view. Experimental evidence on the photochemistry of porphyrins pigments in solution and in lipid bilayers form the basis for these arguments. In this way we can relate the structure of chlorophyll to its function in photosynthesis.

Photosynthesis transforms the energy of the sun into a chemical form useful to the cell. In so doing it furnishes the thermodynamic driving force necessary for all life on this earth. This process requires the absorption of solar photons and it seems that chlorophyll is well adapted for this function. I will attempt to rationalize the chemical structure of chlorophyll in terms of its function. First I will show that chlorophyll fulfils some basic requirements for a photosynthetic function. Then I will discuss a possible evolutionary sequence leading to the chlorophyll structure. These arguments will be supported by what is known of the biosynthetic pathway and of the photochemical properties of the porphyrin pigments.

Requirements for photosynthesis

The primary requirement of the photosynthetic apparatus is determined by the physics of solar radiation. Since the sun is essentially a black body at about 6000 K its quantum output is the broad band of Planck's radiation peaking near 600 nm. Absorption in this visible region requires a fairly large (*ca.* 1 nm) molecule, such as a porphyrin. The complexity of a large molecule usually leads to broad and multiple absorption bands which are a good match for the broad solar output. Although the fourfold symmetry of the porphyrins eases their formation from simpler molecules, this same symmetry renders their visible absorption bands rather weak. By decreasing the symmetry of the conjugated system the absorbtion is much increased. This is just what happens in the 1,2-dihydro or chlorin structure of chlorophyll. This reaction also moves the absorption bands further to the red. Bacteriochlorophyll, the opposed ring isomer of the second stage of this process, a tetrahydroporphyrin, absorbs in the far red. Further reduction

or reduction at the meso positions is not useful as the pigments are either chemically unstable or do not absorb at longer wavelengths. The pigments in the photosynthetic system are close enough together to interact electronically. This further broadens their absorption bands, but since the transition moments are approximately conserved, little increased absorption occurs. However, this closeness leads to rapid energy transfer, and thus a large optical cross section for absorption by the photo-reactive centre. This is obviously an advantage for photosynthesis in the ocean and under variable weather.

TABLE 1. REDUCING AGENTS FOR PHOTOEXCITED UROPORPHYRIN

(The quantum yield of urophlorin was determined at the optimal pH indicated in the second column. The excitation wavelength was usually 398 nm and the concentrations of substrate were 0.05–0.5 M. For details see Mauzerall (1962*a*, *b*).)

agent	pH	quantum yield
ethylenediamine tetraacetate	6	0.45
tetraethylethylenediamine	6	—
sparteine	5	0.20
δ-aminolaevulinate	> 7	—
thiazolidine-4-carboxylate	5.5	0.05
NADH	6	0.4
ascorbate	7	0.05
glutathione	> 6	—
thioglycollate	> 6	0.02
sulphite	5	—
dithionite	> 8 (dark reduction pH < 8)	

The second requirement for photosynthesis is that the pigment be photochemically active. In the old days, photoactivity was often associated with fluorescence. We now know that this correlation means that the excited singlet state lives long enough to spontaneously radiate. This roughly nanosecond lifetime is long enough for direct reactions in complexes or for formation of the long-lived triplet state which is the cause of most solution photochemistry. In fact, the early observations on porphyrins stressed their fluorescence and their photochemistry (Fischer & Stern 1940), even though the latter was usually a photo-oxidative self-destruction. The porphyrins and chlorophyll are naturally occurring photosensitizing agents (Giese 1971), and the early attempts to duplicate photosynthesis with chlorophyll preparations are well summarized by Rabinowitch (1945). More recent work has shown that these photoreactions can be very efficient. For example, the quantum yield of formation of urophlorin from uroporphyrin (URO) and EDTA is 0.45 (Mauzerall 1962*a*, see table 1). This is near the maximum value of 0.5 fixed by the disproportionation of the primary porphyrin radicals (Mauzerall & Feher 1964). Micromolar amounts of some acceptors are sufficient to react with all of the ZnURO triplet state (Carapellucci & Mauzerall 1975) in aqueous solution. The triplet state is itself formed in high yield from porphyrins (Gradyushko, Sevchenko, Solovyov & Tsvirko 1970) and chlorophyll (Bowers & Porter 1967). Thus the porphyrin structure has the required photochemical properties. These properties may be closely related to the energy band gap between the lowest excited singlet state and the ground state. This gap slows the radiationless transition to the ground state and thus increases the yield of fluorescence and intersystem crossing.

The third requirement for photosynthesis follows from the hypothesis, now known to be true in bacterial photosynthesis (Clayton 1973), that the primary photoreaction is one of charge transfer. The porphyrin pigments readily lose or gain one or more electrons. This occurs both

in the ground state as seen by polarographic (Stanienda & Bieble 1967) or redox (Fuhrhop & Mauzerall 1969) studies, and even more readily, in the excited state (Mauzerall 1975). The ground state oxidation potentials increase in a series of porphyrins in the same sequence as the decrease of reduction potentials (Fuhrhop 1974). The same holds for the excited states, as far as can be deduced (Mauzerall 1975) from the present qualitative data. Because the anions, produced by one or more electron addition to the ring, are powerful bases they add a proton from the usual solvents. Addition of the hydrogen to the meso positions produces a series of six reduction levels, culminating in the porphyrinogens (Mauzerall & Granick 1958). The protonated one (Mauzerall & Feher 1964) and two (Mauzerall 1962*b*) electron reduced species disproportionate to the porphyrin and further reduced levels. The equilibrium between the dihydro meso reduced porphyrin (phlorin) and the chlorin species is structure dependent (Woodward 1962). The structure of chlorophyll favors the chlorin form.

The fourth requirement for a practical photosynthetic system is the stability of the pigment and its redox conjugates. The stability of the porphyrins is legendary. The most often quoted proof of their stability is the occurrence of vanadyl porphyrins in ancient oil deposits (Dunning 1963). Essentially this is a test of the thermodynamic stability of the porphyrins, usually assigned to their 'aromatic stabilization or resonance energy'. However, even the non-conjugated porphyrinogens have been shown to be thermodynamically stable *vis à vis* linear polymers (Mauzerall 1960*a*). For a photosystem, chemical stability is as equally as important as thermodynamic stability. The rather drastic conditions used for some porphyrins reactions, e.g. devinylation of protoporphyrin in a resorcinol melt, or decarboxylation of uroporphyrin at 200 °C, attest to their chemical stability. The radical cations of metalloporphyrins are also very stable. They can be crystallized (Fuhrhop 1974), the ultimate tribute to the stability of a free radical. The anion free radicals are stable, but react rapidly with oxygen. In fact, the Achilles heel of porphyrins is their susceptability to oxidative attack. The present photosynthetic system takes pains to protect chlorophyll with the carotenes (Sistrom, Griffiths & Stonier 1956) and possibly by aggregation effects (Mauzerall 1975).

Evolution of biosynthetic pathway and photosynthesis

Having shown that the porphyrin pigments fulfil the necessary critera for a photosynthetic function, we must now show they fulfil a sufficient condition, namely that they can exist under natural conditions. The work of Shemin (1955) first indicated the incredibly simple biosynthetic path to the porphyrins. Essentially a porphyrin is an octamer of δ-aminolaevulinic acid which in turn is made of glycine and succinic acid or possibly, glutamic acid in plants (Beale & Castelfranco 1974) and this volume (p. 99). The constancy of this pathway in bacteria, plants, and animals is a most striking illustration of the evolutionary unity of all living things.

The delicate question of the isomers may be partially resolved by noting that the biologically occurring series III isomer of uroporphyrin is the most probable isomer and thus the most likely to be formed under pre-biotic conditions (Mauzerall 1960*b*). It is true that attempts to totally duplicate this biosynthesis *in vitro* have not been very successful. Ignoring this presumably temporary difficulty, we turn to the biosynthetic pathway of chlorophyll (Granick 1967) and this volume, p. 207), so well outlined by the work of Granick, Lascelles, Neuberger and others. Such a pathway has been characterized by Granick (1965) as a window looking back into evolution. In this view, the pathway represents molecular way stations, each with a function in

its evolutionary time. Random mutation and evolutionary selection combine to build the observed pathway. The coupling to function via selection ensures the path is not a random walk. Our exegesis of this passage stresses two aspects (Mauzerall 1973). First, that porphyrins were early photosynthetic pigments and second, that early photosynthesis occurred without benefit of much cellular organization, in the ocean. We arrive at the first statement by considering the function of photosynthesis: to generate the thermodynamic gradient used to drive the flow of energy and matter that stabilizes the active structure of the living cell. In the modern era this gradient feeds cellular respiration, and modern photosynthesis supplies the food and oxygen which constitute this gradient of free energy. However, it is believed that in the beginning the atmosphere was reducing (Granick 1965). A useful function of early photosynthesis would have been to oxidize the prevalent reduced organic compounds and emit hydrogen. The resulting oxidized organic compounds, containing reactive double bonds, carbonyl or imine groups would be very useful condiments to the prebiotic soup. Now photoexcited porphyrins first formed on the biosynthetic pathway will oxidize a variety of organic compounds (Mauzerall 1960*c*, 1962*b*) in anerobic neutral aqueous solution (table 1). For example, the products of the reaction with tertiary amines are carbonyl compounds and the secondary amine (Frisell, Chung & Mackenzie 1959; Mauzerall, unpublished observations). The reduced porphyrin, a phlorin, can disproportionate to porphyrin and more reduced porphyrins. These reduced porphyrins could emit hydrogen and reform porphyrins. In fact the actual primary products of the biosynthetic pathway are porphyrinogens, which could undergo this same reaction. It is possible that hydrogen emission could be enhanced by absorption of ultraviolet light present in this early era because of the low level of oxygen, and thus of the ozone shield. It is interesting that Gaffron & Rubin (1942) observed that some modern algae could be induced to emit and use hydrogen under anaerobic conditions. The structure of the ubiquitous vitamin B_{12}, clearly related to uroporphyrinogen III and the occurrence of a tetrahydrourohaem as the prosthetic group of a sulphite reductase in primitive anaerobic bacteria (Murphy, Siegel, Kamin & Rosenthal 1973) support our contention that uroporphyrin was prevalent in early biogenesis. Dr Granick (1965) has considered the development of a very primitive type of photosynthesis based on photoeffects in minerals. The iron sulphide model that he presented is similar to ferrodoxin. This is a striking example of chemical inference since the model was first postulated before ferrodoxin was discovered.

Returning to porphyrins, if a closed shell metal ion such as magnesium or zinc is chelated by the porphyrin, the preferred photochemical electron transfer reaction changes direction (Mauzerall 1975). The excited state of these metalloporphyrins is a strong reducing agent. This dramatic change can be explained by the redistribution of π-electrons in the macrocyclic ring (Fuhrhop & Mauzerall 1969). These metalloporphyrins are essentially porphyrin di-anions and so readily lose an electron while the porphyrin bases are neutral unsaturated systems and readily gain an electron. The transition metals such as Fe or Cu are also chelated by porphyrins, but the resulting complexes are relatively inactive photochemically (Mauzerall 1960*c*).

The reducing ability of the triplet state of zinc uroporphyrin is shown by the equality of the second-order rate constant, *ca.* 2×10^8 M^{-1} s^{-1}, for electron donation to either ferricyanide or nicotinamide dinucleotide (Carapellucci & Mauzerall 1975; Mauzerall 1975), despite the difference of about 1 V in their one electron redox potentials. We have used the electrostatic effects between the negatively charged zincuroporphyrin and acceptors of varying charge to measure the distance of electron transfer (Carapellucci & Mauzerall 1975; Mauzerall 1973). The results indicate

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that the electron from the triplet zinc uroporphyrin passes through an average of 1 nm of water on transferring to the donor. The mechanism is most likely that of quantum mechanical tunnelling (Mauzerall 1975). We believe this concept is capable of explaining the barrier to reverse electron transfer which makes photosynthesis possible (Mauzerall & Hong 1975). The barrier slows the wasteful reverse electron transfer sufficiently to allow secondary electron acceptors and donors to react and so couple the energy to biochemically useful products.

The reduction of organic compounds and the ultimate fixation of carbon dioxide by an electron from an excited metallochlorin is the function of modern photosynthesis. I believe the change from free porphyrin to metallo-porphyrin is the basis of the differentiation from early to modern photosynthesis. The occurrence of bacteriopheophytin in addition to bacteriochlorophyll in the reaction centres of photosynthetic bacteria (Strayley, Clayton, Parsons & Mauzerall 1973) may just possibly be a remnant of the older photosynthesis. The coupling of these reactions to the ultimate electron donor, water, and the consequent formation of oxygen opened the modern era. The mechanism of this most cataclysmic of evolution's discoveries remains a mystery to us.

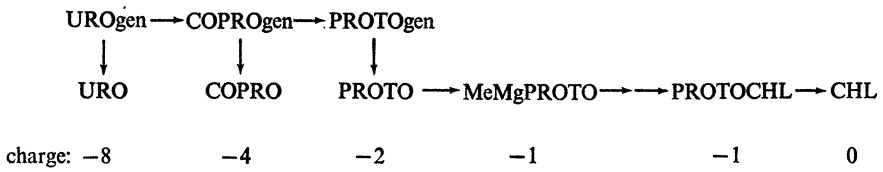

FIGURE 1. Schematic biosynthetic pathway of chlorophyll showing systematic decrease of ionic charge on the molecules.

Another glance at the biosynthetic pathway of chlorophyll reveals the monotonic drift from the highly ionic, water-soluble uroporphyrin (ogen) to the relatively nonpolar, lipid-soluble chlorophyll (Granick 1965; see figure 1). A constant characteristic of photosynthetic systems is their association with membranes. This organization of photosynthetic systems into subcellular organelles had at least two evolutionary driving forces. The first we connect to the rise of oxygen in the atmosphere. The photoreactions between porphyrins and organic compounds occur through the triplet state because of the time necessary for the components to meet by random diffusion in aqueous solution. But these triplet states are efficiently quenched by oxygen. If the pigment and the acceptor are held reasonably close together, however, the electron transfer can occur before the oxygen quenching occurs – in about 100 ns at the present level of oxygen. The basis of molecular organization in the cell is the lipid bilayer, and the primary photoact and subsequent electron transfers gain in efficiency by being localized in these membranes. This organization leads to the second advantage – the formation of electric fields across the membranes. This is a direct consequence of the anisotropy of the electron transfer in the membranes (Mauzerall & Hong 1975). The highly productive Mitchell hypothesis (1966) connects these electric fields to the formation of ATP. Thus both the efficiency and the productivity of elementary photoreactions were much improved by transferring the pigment and reactions from random locations in solution to organized locations in membranes. We have observed both the reaction in the presence of air and the formation of electric fields across the membranes in a simple model (Hong & Mauzerall 1974; Mauzerall & Hong 1975, see figure 2). A lipid-soluble metalloporphyrin or chlorophyll in the lipid bilayer reacts with an electron acceptor forced to remain in the aqueous phase. By the use of a 'tunable voltage clamp' measuring

technique (Hong & Mauzerall 1974) it was found that the electron transfer from the excited state of the pigment occurs in less than 100 ns, and that the voltage developed across the interface decays with a rate dependent on the concentration of electron donor at that interface. The rapidity and reversibility of these vectorial photochemical reactions made them an excellent probe of the interfacial region which is so critical to many biological phenomena.

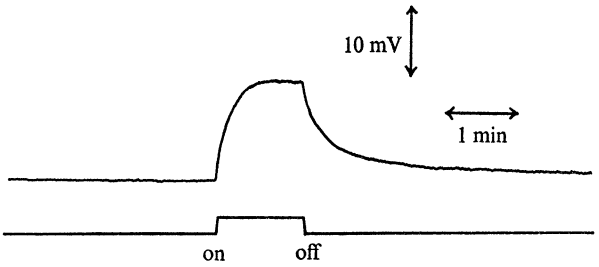

FIGURE 2. The open-circuit e.m.f. formed on specifically illuminating the lipid bilayer portion of a Ruden–Muller black lipid membrane. The egg lecithin membrane contained Mg meso amyl ester and separated solutions of 10 mM ferricyanide and ferrocyanide. For details see Hong & Mauzerall (1972).

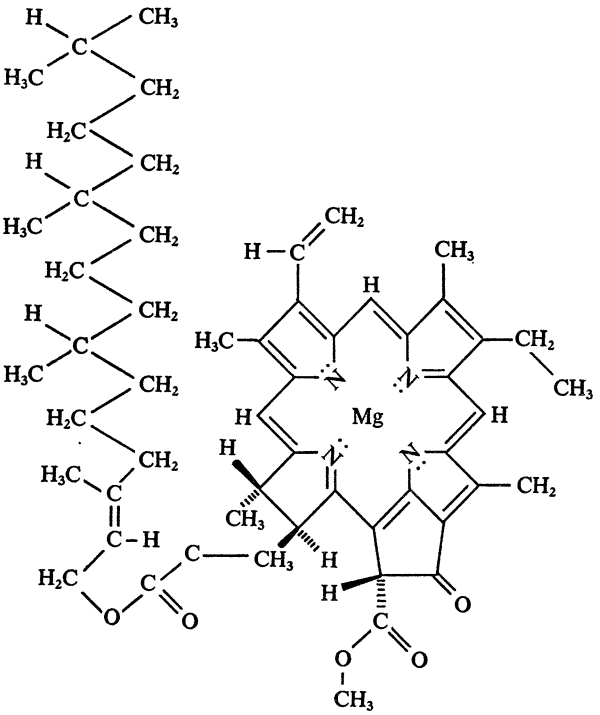

FIGURE 3. The structure of chlorophyll.

STRUCTURE OF CHLOROPHYLL

We can now look at the chemical structure of chlorophyll (figure 3) from the viewpoint we have now developed. The large size of the conjugated ring gives the ability to absorb visible light from the sun. The extra hydrogens of the chlorin structure enhance absorption in the red region of the spectrum. The convuluted structure of the porphyrin and the nitrogen atoms

provide a rich optical spectrum – that is, many energy levels in the visible region. It also provides a rich supply of redox levels so necessary for the photochemical reactions. The magnesium makes the excited state a powerful reducing agent. The basic conjugated ring furnishes the required thermodynamic and chemical stability of the molecule, and supplies the delicate detail which lead to high quantum yields in the photochemical reactions (Mauzerall 1975). The highly symmetric basic porphyrin ring is assembled by a uniquely simple biosynthetic pathway from small, common bio-molecules.

The side chains around the ring are the residues of the substituents originating in the remarkable biosynthesis. The chemical transformations appear to have a simple aim: to change the polar, ionic carboxyl groups to non-polar alkyl or less polar ester groups. The esterification with phytol reinforces the lipid solubility and anchors the chlorophyll in the lipoprotein complexes of the photosynthetic apparatus.

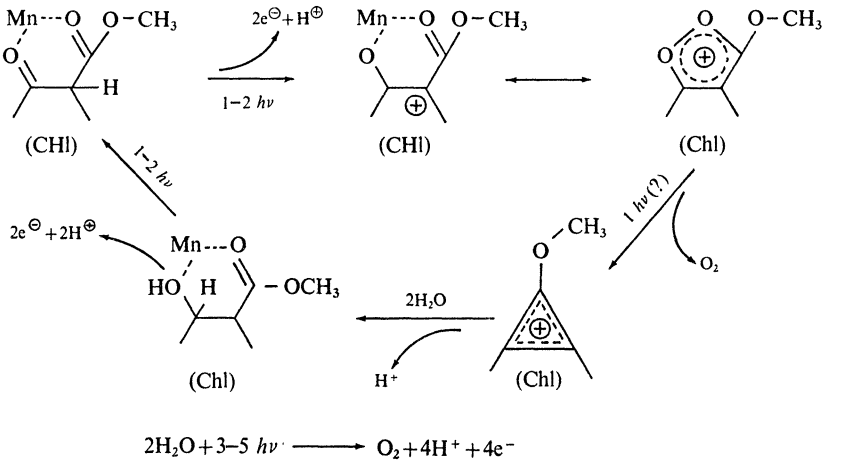

FIGURE 4. The dioxolium theory of photochemical formation of oxygen. Only the relevant β-keto-ester section of the chlorophyll molecule is shown. The oxidation of the hydroxy chlorophyll could occur in the dark given sufficient acceptor, e.g. NAD. Thus the quantum yield of oxygen production could vary with metabolic state of the cell.

The 'fifth ring' of chlorophyll contains the slightly polar carbonyl and ester groups and is the site of chemical oxidation of chlorophyll. It has always intrigued workers in this field. The deformation of the macrocyclic ring increases absorption in the red over that of a simple magnesium porphyrin. In a nice example of chemical symbiosis, the reduction of the adjacent pyrryl ring, forming the chlorin and strongly enhancing absorption in the red, also favours the formation of the fifth ring by reducing steric hinderance (Woodward 1962). A variety of speculations on the function of the fifth ring have been offered (Frank 1957; Rackow & Konig 1958; Vishniac & Rose 1958). Katz (p. 227) suggests that it and the magnesium atom are involved in an aggregated form of chlorophyll present in the photosynthetic unit. Our contribution is that it, together with manganese, may be involved in oxygen production. The theory (Mauzerall & Chivvis 1973) involves the hypothetical dioxolium ion (figure 4), the analogue of the known dithioiolium ion. Although our hypothesis can rationalize several observations on the photosynthetic formation of oxygen, we have as yet been unable to obtain any experimental support for this theory.

By means of the evolutionary viewpoint presented here, we are able to rationalize the structure–function relation of chlorophyll in physico-chemical terms. These attempts are some of the first steps toward understanding why life is possible on this earth. We are still quite far from making a tree.

It is a pleasure to acknowledge my debt to Dr S. Granick. His provocative questions and guiding comments are responsible for much of the synthesis I have presented here.

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