THE FUNCTIONS OF CHLOROPHYLLS IN PHOTOSYNTHESIS

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Contents

Summary

In recent years, considerable progress has been made in the elucidation of the mechanism of natural photosynthesis. The authors critically review the results obtained by X-ray crystallography on the photosynthetic reaction centers of non-oxygenic photosynthetic purple bacteria and those of oxygenic photosynthetic organisms such as higher plants, algae, and cyanobacteria. The present state of knowledge concerning the mechanism of the photosynthesis of oxygenic organisms is reviewed with special reference to the structure and function of the water-splitting enzyme. The history of the "chlorophyll special-pair" model is presented in detail and some alternative proposals are considered, including the chlorophyll enol derivatives and C-$13^2(S)$-epimers, for the structure of the reaction-center chlorophyll. The crystallographic structures of some bacterial and plant light-harvesting antenna complexes are then examined, focusing particularly on the intermolecular distances and orientations of the photosynthetic pigments. These parameters are considered to play a crucial role in determining the rate and efficiency of energy transfer, which are astonishingly high in natural photosynthetic

systems. The structures and functions of photosynthetic carotenoids are noted. Finally, some opportunities offered by chlorophyll and photosynthesis research are briefly discussed.

1. Introduction

1.1. Importance of Photosynthesis for Life on Earth

Life on Earth is based on the energy of solar radiation, which is captured by higher plants, algae, and photosynthetic bacteria. These organisms contain photosynthetic pigments such as chlorophylls, phycobilins, and carotenoids, which absorb light in a wide range of wavelengths, covering the whole visible region and extending even to the near infrared region (Figure 1).
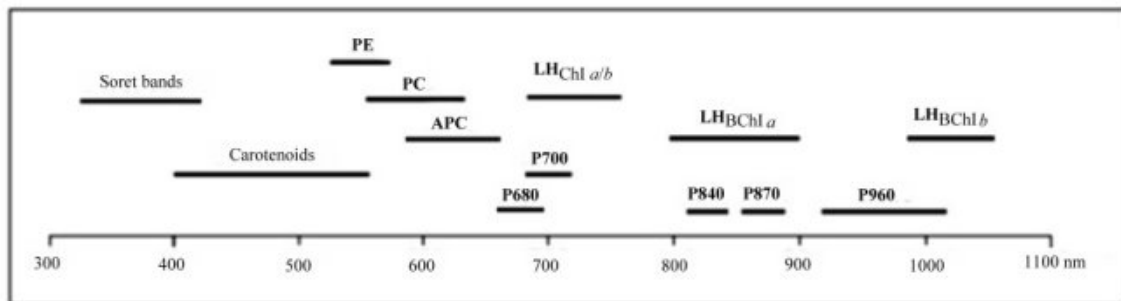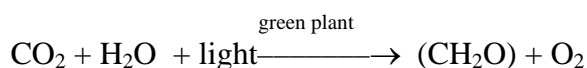

Figure 1. Light absorption of photosynthetic pigments

By means of the so-called antenna system, the photosynthetic organisms can harvest light quanta efficiently and funnel the excitation energy to the reaction centers, where the captured light energy is converted with a high quantum yield into chemical energy. Finally, the energy is stored in the form of carbohydrates and other hydrogen-containing organic compounds. It has been estimated that photosynthesis produces annually about 5×10^{10} tons of organic carbon, which means liberation of 13×10^{10} tons of oxygen into the air and fixation of about 20×10^{11} tons of carbon dioxide (CO_2) from the air and the oceans. Non-photosynthetic organisms in turn utilize hydrogen-containing compounds as their fuel, obtaining the energy for their life processes by oxidizing the compounds in the cell respiration process, which yields CO_2, ATP and H_2O. It is also noteworthy that the fossil fuels (earth oil, gas, coal, and peat) were produced by photosynthesis long ago. As only a relatively small fraction of solar radiation hits the surface of the Earth, evolution had to discover efficient mechanisms for capturing solar energy and converting it into an adequately stable form of chemical energy. The mechanisms created by evolution are so complicated that they have remained until now as one of the most challenging puzzles in science. Even the elucidation of the total reaction of plant photosynthesis took about a century.

1.2. Discovering the Total Reaction of Plant Photosynthesis

The total reaction of plant photosynthesis is often presented in the simple form:

$$CO_2 + H_2O + light \xrightarrow{\text{green plant}} (CH_2O) + O_2$$

Here (CH_2O) represents organic matter. Multiplying both sides by 6 gives one mole of glucose. Joseph Priestley (1733–1804) discovered that oxygen is evolved in this reaction. He observed that the air in a glass jar, debilitated by the burning of candles, could be "restored" by introducing a small plant into the jar. Jan Ingenhousz (1730–1799) realized that sunlight was necessary for the photosynthetic activity of plants. About at the same time, Jean Senebier (1742–1809) proved the participation of "fixed air," that is, carbon dioxide, in the photosynthetic process. Some years later, Théodore de Saussure (1767–1845) verified that the sum of the masses of organic matter and oxygen produced was greater than the mass of carbon dioxide consumed. He concluded from this that water participates in photosynthesis. Finally, Julius Robert Mayer (1814–1878) realized that plants store the energy of sunlight in the form of chemical energy. Mayer saw in the photosynthetic process an important illustration of the law of conservation of energy.

1.3. General Principles of the Mechanism of Photosynthesis

The total reaction of photosynthesis tells us virtually nothing about the mechanism of the process, which has turned out to be extremely complicated in the case of oxygenic photosynthetic organisms (green plants, algae, and cyanobacteria). The photosynthetic apparatus of the oxygenic organisms is located in the thylakoid membranes of chloroplasts, which contain photosystem I (PS I) and photosystem II (PS II), operating in series. Each photosystem consists of a reaction-center (RC) complex surrounded by an antenna system (AS). The PS II extracts electrons and protons from water and pushes the electrons to PS I. The photosynthetic apparatus of the nonoxygenic photosynthetic bacteria (green bacteria and purple bacteria) is much simpler, consisting of only one RC, surrounded by the AS. These photosynthetic organisms are unable to extract reducing equivalents (H·) from water. Instead of water, they use other hydrogen-containing organic substrates, such as hydrogen sulfide.

Traditionally the overall photosynthetic process is divided into two stages, referred to as light reactions (also called the primary events) and dark reactions. The light reactions produce NADPH and ATP, which are then used in the dark reactions to reduce and fix carbon dioxide in the Calvin cycle, the key reaction of which is catalyzed by ribulose-1,5-bisphosphate carboxylase, the most abundant protein on Earth. The cyclic tetrapyrrole pigments, called chlorophylls, play a crucial role in the light reactions, but do not participate in the dark reactions. Apparently, there has been a common misunderstanding that the chlorophylls function only in light harvesting. But they do much more. They also "funnel" the excitation energy into the RCs, acting there as important redox pigments. It has been very difficult to understand how the same molecule can do all this. Ultimately, there must be a relationship between the functions and the chemical properties of the chlorophylls. Any attempt to identify the relationship inevitably demands a thorough knowledge of the structures and chemical properties of the photosynthetic pigments.

2. Structures, Properties and Natural Occurrence of Chlorophylls

When used as a group name, chlorophyll refers to a number of structurally closely related cyclic tetrapyrroles, whose parent compounds are called porphyrin, chlorin, or bacteriochlorin, depending on the reduction degree of the macrocycle (Figure 2).

In addition to the variable degree of π-electrons in the macrocycle, the members of the chlorophyll group differ from one another by the nature of peripheral substituents (Figure 3).
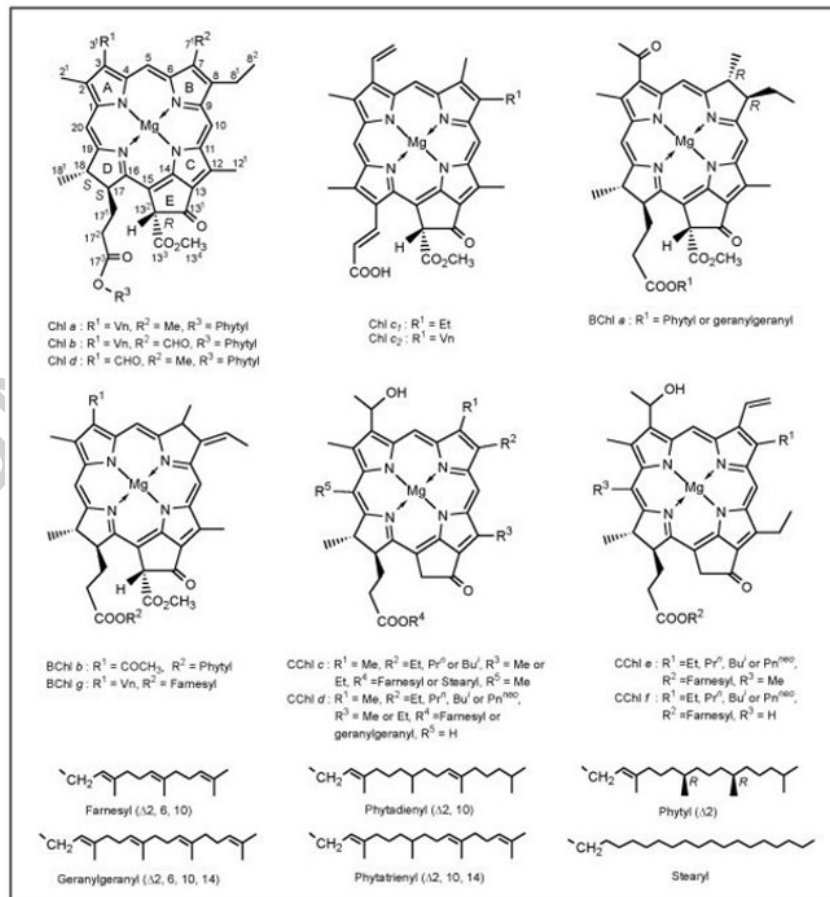

Figure 2. Parent compounds of cyclic Tetrapyrroles

Figure 3. Structures and names of the members of the chlorophyll group

In the naming, we have adopted a new practice by replacing the former "*Chlorobium* chlorophylls" with the names "chlorosome chlorophylls (CChl) *c*, *d,* and *e*." This is because these compounds are all derivatives of chlorophyll *a*, which occur only in the accessory light-harvesting units, called "chlorosomes." These can be found in the photosynthetic bacterial species, belonging to the families Chlorobiaceae and Chloroflexaceae (suborder: Chlorobiineae, order: Rhodospirillales). The German microbiologists H.G. Trüper and N. Pfennig renamed the C*hlorobium* chlorophylls as bacteriochlorophylls (BChl) *c*, *d*, and *e*, on the basis of their occurrence in certain photosynthetic bacteria. Such names have caused a lot of confusion as to the chemical structure of these compounds, because the names suggest that we are dealing with derivatives of bacteriochlorin according to the chemical systematics of Figure 2. Besides, if every member of the chlorophyll group were named according to the species where it occurs, there would be thousands of various chlorophylls! Nevertheless, a similar naming problem also concerns chlorophylls c_1, c_2 and c_3, which actually are fully delocalized porphyrins according to the chemical classification of Figure 2. The occurrence of various chlorophylls in nature is shown in Table 1.

Organism	Chl			
	a	*b*	*c*	*d*
Higher plants, ferns and mosses	+	+	-	-
Algae				
Chlorophyta	+	+	-	-
Chrysophyta				
Xanthophyceae	+	-	-	-
Chrysophyceae	+	-	+	-
Bacillariophyceae	+	-	+	-
Euglenophyta	+	+	-	-
Pyrrophyta				
Cryptophyceae	+	-	+	-
Dinophyceae	+	-	+	-
Phaeophyta	+	-	+	-
Rhodophyta	+	-	-	+
Cyanophyta	+	-	-	-

	BChl				CChl		
	a	*b*	*g*		*c*	*d*	*e*
Bacteria							
Chromatiaceae	+	+	-		-	-	-
Rhodospirillaceae	+	+	-		-	-	-
Chlorobiaceae	+	-	-		+	+	+
Chloroflexaceae	+	-	-		+	-	-
Heliobacterium chlorum	+	-	+		-	-	-

Table 1. Occurrence of various chlorophylls in nature

Irrespective of the number of reduced peripheral carbon-carbon bonds, all chlorophylls contain the same π-electron delocalization pathway, shown in the thicker lines in Figure 2. This delocalization pathway represents an aromatic 18 π-electron [18]-diazaannulene system: that is, 18 π-electrons are delocalized over 16 carbon and two nitrogen atoms. Such an electron system is of fundamental importance for the functioning of chlorophylls, both as light-harvesting pigments in the antennas and as redox-active pigments in the reaction centers. The π-system determines the specific light-absorption and redox properties of the chlorophyll molecule. Relatively low-energy visible light is adequate to cause π–π* transitions, resulting in excited singlet states of the molecule. The delocalized π-system of chlorophylls is also unique in that it can easily release or take up an electron, thus forming a π-cation radical or a π-anion radical, respectively. In each case, there is an unpaired electron delocalized in the macrocyclic π-system. Clearly, such a property is relevant to the functioning of chlorophylls as redox-active pigments in the photosynthetic reaction centers.

Another salient feature in the structures of chlorophylls is the presence of a magnesium ion, coordinated to the nitrogens of the four pyrrolic subrings. Such a magnesium is electrophilic, that is, electron-deficient, and therefore tends to bind various ligands (nucleophiles) from the surroundings, such as H_2O or a nucleophilic amino acid side-chain. The chlorophylls also contain polar functional groups such as carbonyls (or hydroxyls in the chlorosome chlorophylls), which can act as ligands for the central magnesium of a neighboring chlorophyll molecule or form hydrogen bonds with appropriate partners in the surroundings. The unique electron donor–acceptor (EDA) properties of chlorophylls play an essential role in the organization of chlorophyll molecules *in vitro* and *in vivo*. Trough variation of the chlorophyll organization, the light absorption, energy transfer, and redox properties of the molecules can be modulated.

Further, there is a close relationship between the stereochemistry and the EDA-properties of the chlorophyll molecule. It is important to realize that the chlorophylls are chiral molecules with one or more asymmetric centers in their structures (Figure 3). Obviously, the chirality must be of importance for the organization and functions of these molecules in the photosynthetic membranes. For example, Chl *a* has three asymmetric carbons in its macrocycle, with the absolute configurations $13^2(R)$, $17(S)$ and $18(S)$, and there are two more asymmetric centers in its phytyl (P) group with the configurations $P7(R)$ and $P11(R)$. One of the authors of this article (Paavo Hynninen) has shown that the change in configuration only at $C-13^2$ results in unexpected conformational alterations in the whole molecule. These alterations were attributed to the steric crowding (repulsion) arising between the 13^2-methoxycarbonyl group and the bulky C-17 propionic acid phytyl ester moiety, when these substituents become oriented on the same side of the macrocyclic ring plane (Chl *a*' = $13^2(S)$–Chl *a*, see Figure 4). The steric crowding weakens the electron-pair donor properties (nucleophilicity) of the three carbonyl groups in the lower periphery of the chlorophyll $13^2(S)$-epimer, which therefore has prominently different coordination and hydrogen-bonding properties as compared with the $13^2(R)$-chlorophyll.
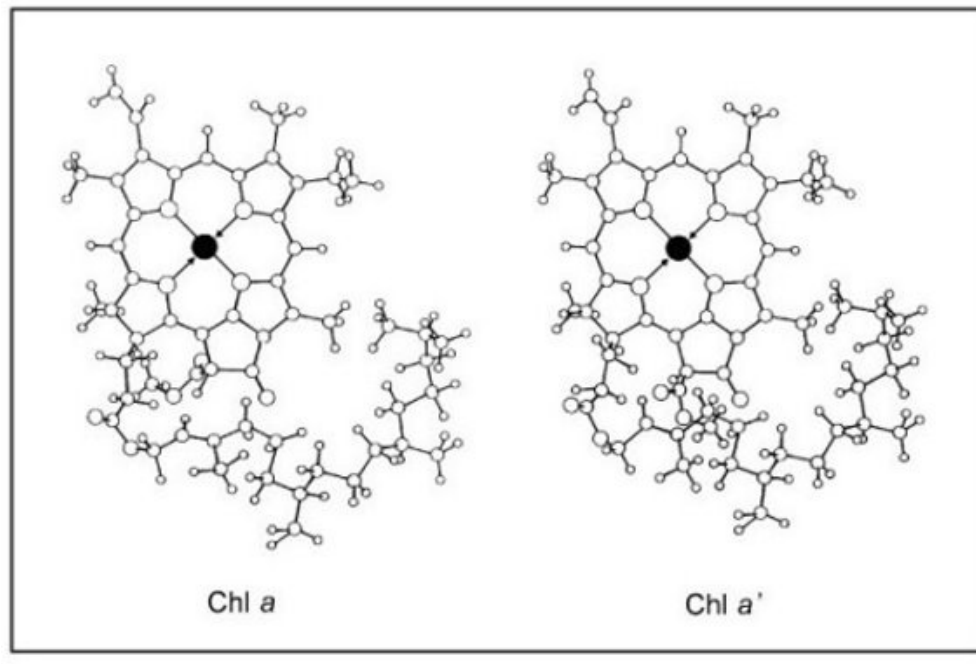
Figure 4. Structures of Chl *a* and Chl *a*' on the basis of ^{1}H NMR measurements and molecular models. Chl *a* = $13^{2}(R)$ – Chl *a* and Chl *a*' = $13^{2}(S)$–Chl *a*. Source: adapted from P.H. Hynninen and S. Lötjönen (1985). Steric interaction between the peripheral substituents of 10(*S*)-chlorophyll derivatives and its conformational consequences: a proton magnetic resonance study. *Magnetic Resonance in Chemistry* 23, 605–615.

The $13^{2}(S)$-epimeric chlorophyll can only be produced through the chlorophyll enol intermediate. Thus the occurrence of such an epimer in the photosystems would mean that, at some phase of the photosynthetic process, the $13^{2}(R)$-chlorophyll molecule would have to enolize. In this connection, it is noteworthy that every chlorophyll species that is known with certainty to function in a photosynthetic RC bears an easily enolizable β-keto ester system in its isocyclic ring (see the structures of Chl *a*, BChl *a* and BChl *b* in Figure 3). This suggests that the chlorophyll enolates might play some role in the primary events of photosynthesis.

To see in further detail how the salient structural features of chlorophylls influence the organization and functions of these molecules in the photosynthetic membranes, we will inspect in the following sections the results obtained by X-ray crystallography on various reaction-center and light-harvesting complexes.

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Biographical Sketches

Professor P.H. Hynninen received his basic training in chemistry and biochemistry at the University of Helsinki. He was guided to a researcher's career by Professor A.I. Virtanen (Nobel Prize for Chemistry in 1945). After his doctoral dissertation in 1973, he moved to the University of Kuopio, acting there as a pro tempore Professor of Biochemistry during a period of two years. In 1976–1977, he served as a research associate at Argonne National Laboratory (IL, USA), receiving his post-doctoral training under the supervision of Dr. J.J. Katz (Member of the USA Academy of Sciences). After returning to Finland in 1978, he served as an Assistant Professor of Biochemistry at the University of Kuopio. In 1986, he was appointed Associate Professor of Organic Chemistry at the Chemistry Department of the University of Helsinki, and in 1998 he became Professor in Organic Chemistry at the same department. His research interests are distributed over wide areas of chemistry such as organic and bioorganic chemistry, analytical chemistry and photochemistry. He has collaborated with many universities and research institutes both on national and international levels.

T.S. Leppäkases is a Graduate Student of Chemistry at the University of Helsinki, majoring in Organic Chemistry. He performed his M.Sc. thesis in 2001 under the supervision of Professor P.H. Hynninen. The experimental part of the thesis concerns regio- and stereoselective oxidation of chlorophylls *a* and *b*. His present research interests are directed to the synthesis methods of organic chemistry and structural analysis of complex organic molecules such as chlorophylls and their derivatives. His special interests also concern organic reaction mechanisms and separation science.