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REVIEW

A viewpoint: Why chlorophyll *a*?

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Abstract Chlorophyll *a* (Chl *a*) serves a dual role in oxygenic photosynthesis: in light harvesting as well as in converting energy of absorbed photons to chemical energy. No other Chl is as omnipresent in oxygenic photosynthesis as is Chl *a*, and this is particularly true if we include Chl a_2, (=[8-vinyl]-Chl *a*), which occurs in *Prochlorococcus*, as a type of Chl *a*. One exception to this near universal pattern is Chl *d*, which is found in some cyanobacteria that live in filtered light that is enriched in wavelengths >700 nm. They trap the long wavelength electronic excitation, and convert it into chemical energy. In this *Viewpoint*, we have traced the possible reasons for the near ubiquity of Chl *a* for its use in the primary photochemistry of Photosystem II (PS II) that leads to water oxidation and of Photosystem I (PS I) that leads to ferredoxin reduction. Chl *a* appears to be unique and irreplaceable, particularly if global scale oxygenic photosynthesis is considered. Its uniqueness is determined by its physicochemical properties, but there is more. Other contributing factors include specially tailored protein environments, and *functional compatibility* with neighboring electron transporting cofactors. Thus, the same molecule, Chl *a* in vivo, is capable of generating a radical cation at +1 V or higher (in PS II), a radical anion at −1 V or lower (in PS I), or of being completely redox silent (in antenna holochromes).

Keywords Chemistry of chlorophylls · Chlorophyll *a* · Chlorophyll *d* · Chlorophylls in proteins · Color of plants · Cyanobacteria · Evolution of photosystems · Oxygenic photosynthesis · Photosystem I · Photosystem II · Reaction centers · Spectra of chlorophylls

Abbreviations

Chl	Chlorophyll
Pheo	Pheophytin
PS	Photosystem
RC	Reaction center
TMH	Transmembrane helix

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> "*Man cannot give a true reason for the green under his feet Why it should be green rather than red or any other colour*."
>
> Sir Walter Raleigh

Introduction

Chlorophylls (Chls) are ubiquitous participants in photosynthesis and this prompted Mauzerall (1973) to ask "Why Chl?" While various Chls function as light-harvesting

pigments, only one of them, Chl *a*, depending on its protein environment, functions either as a light harvester or as a redox participant in electronic excitation trapping (primary charge separation) and electron transporting events in the reaction centers of Photosystems II and I (PS II, PS I) of oxygenic organisms. Only Chl *a* (see Section "Is chlorophyll *d* a match for chlorophyll *a* everywhere?" below for a discussion on Chl *d*) is indispensible for oxygenic photosynthesis; it is the only member of the Chl family that is present in all organisms that carry out oxygenic photosynthesis, from primitive cyanobacterial cells to sequoia trees. Depending on their evolutionary ancestry, various taxa of photosynthetic organisms contain different sets of light harvesting Chls. Thus, Chl *a* occurs in red algae and glaucophytes, Chls *a*, *b*, *d* and [8-vinyl]-Chls *a* and *b* in cyanobacteria, Chls *a* and *b* in green algae and higher plants, and Chl *a* and *c* in chromophytic algae (Govindjee and Satoh 1983; Green and Parson 2003; Larkum et al. 2003; Zapata et al. 2003; Murakami et al. 2004; Papageorgiou 2004; Grimm et al. 2006; Scheer 2006). Here, we do not consider the bacteriochlorophylls that are found in anoxygenic photosynthetic bacteria.

That a single type of molecule has become so dominating in oxygenic photosynthesis is surprising, considering the enormous variation in the living world, and the long time that evolution of photosynthesis has been going on (Olson and Blankenship 2004). Why Chl then, and particularly, why Chl *a*?

Trying to answer this question is important for attempts to look for life and photosynthesis on far away planets. Earth-like photosynthesis appears to be unique in our Solar System, but is it so elsewhere in the space? What we must be looking for and what can we expect to find there (Kiang et al. 2007a, b)? We consider here different answers to the question, and we classify them into three categories (not mutually exclusive): Historical ("accidental," due to how evolution happened to proceed—Section "Historical"); Spectral (Section "Spectral"); and Chemical (Section "Chemical").

Historical

Before photosynthesis arose, there was already electron transport mediated by metal porphyrins; thus, much of the biosynthetic pathway for Chl was already in place. Under certain circumstances light can drive electron transport between such molecules (Widell and Björn 1976; Qin and Kostic' 1994). Once the evolution via porphyrins to chlorins had gone on for a while, new alternatives would be at a disadvantage in the competition. To be successful, however, this evolutionary path had to end in Chl *a* because it is the only redox-active Chl form in vivo found in oxygenic organisms so far. And this was achieved quite early in the course of evolution (Björn and Govindjee 2008). Further, the molecular machinery for assimilation of carbon dioxide was in place before the advent of photosynthesis (Björn and Govindjee 2008).

Spectral

A light-harvesting pigment must absorb in a spectral region where radiant energy is available, and where quanta are energetic enough to move electrons uphill from a high potential electron donor to a low potential electron acceptor molecule. On the present Earth, and probably when the Earth was young as well, this means light of 300 to about 1200 nm wavelength. In considering the combination of photon availability in unfiltered sunlight and the photon energy content, Björn (1976) suggested[1] an optimal location for the absorption peak of a light-harvesting pigment of about 700 nm. Absorption of light at the low frequency end of the visible spectrum requires a fairly extensive system of conjugated bonds and this means a fairly large molecule (see below). Several absorption transitions will be the consequence.

This is true, indeed, for Chls. In particular, the absorption spectrum of Chl *a* in diethyl ether shows four bands on the red side of the spectrum (Q-region, at 660, 612, 572, and 519 nm) and another four on the blue side (Soret or B-region, at 428, 409, 379, and 326 nm; Fig. 1). According to the four-orbital model of Gouterman (1961), these bands originate from singlet π–π^* transitions between the two highest occupied molecular orbitals (HOMO) and the two lowest unoccupied molecular orbitals (LUMO; Weiss et al. 1965; reviewed by Shipman 1982). These transitions are strong and polarized along the X and Y axes of the asymmetric porphyrin ring and are assigned as follows: 660 nm Qy(0,0), 612 nm Qy(1,0), 572 nm Qx(0,0), 510 nm Qx(1,0), and 428 nm (Bx(0,0) plus By(0,0). The absorption bands below 428 nm are attributed to mixed transitions (Houssier and Sauer 1970; Weiss 1972; reviewed by Papageorgiou 2004). See also Gouterman (1978)

[1] The calculation of 700 nm was based on, among others, the following assumptions and approximations: (1) The sunlight spectrum was approximated as a Planck 6000 K blackbody spectrum, modified by a factor depending on the solar system geometry. (2) The shape of the long-wavelength band of the photosynthetic pigment was approximated by a Gaussian function. (3) The photosynthetic system was considered to be at 300 K. (4) The maximum chemical potential that can be extracted from the photons was accepted as described by Ross and Calvin (1967). (5) A limiting value for the oscillator strength can be accommodated within a certain volume. (6) The maximum extractable power (energy per time) is the product of chemical potential achieved by the photon absorption and the rate of photon absorption. For details, see Björn (1976); and for basics, see Knox (1969).

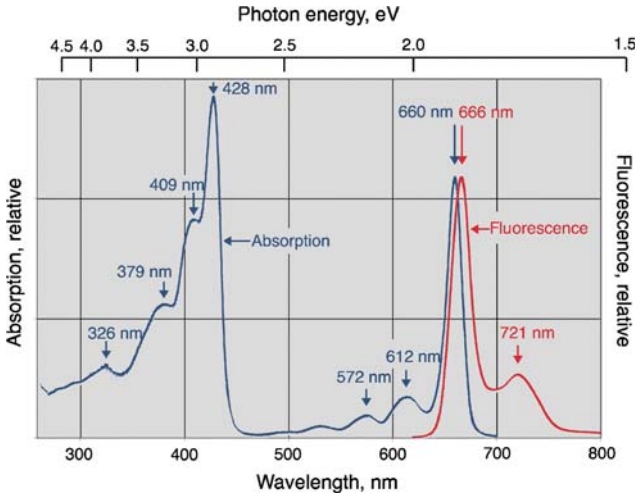

Fig. 1 Relative absorption (*blue line*) and fluorescence spectra (*red line*) of Chl *a* in diethyl ether. Spectral band maxima are indicated in nm and the two spectra are displayed after normalization of the 660 nm absorption and 666 nm fluorescence bands to equal heights. The millimolar absorptivity (extinction coefficient) of the 428 nm band is 111.7 mM^{-1} cm^{-1}. An energy scale in eV, corresponding to the wavelength scale, is shown at the top. Data obtained from http://ww.photochemcad.com; figure modified from Papageorgiou (2004)

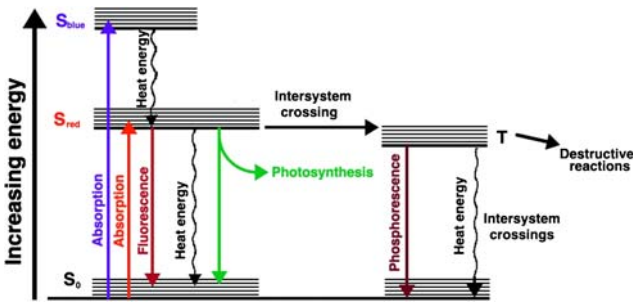

Fig. 2 A "Jablonski diagram" of the energy levels in a Chl molecule and the transitions between them. Independently of what kind of light that is absorbed, the molecule will reach the first excited singlet state before part of its energy will be used in photosynthesis. Photosynthesis competes with radiationless energy dissipation as heat, fluorescence emission, and intersystem crossing to the first excited triplet state. The vertical scale is uncalibrated, since it is different for different Chl molecules (*a*, *b* etc.) and depends on the protein environment. Energy dissipation for photosynthesis includes energy transfer to other Chl molecules, which is how Chl in the antenna proteins contributes to photosynthesis

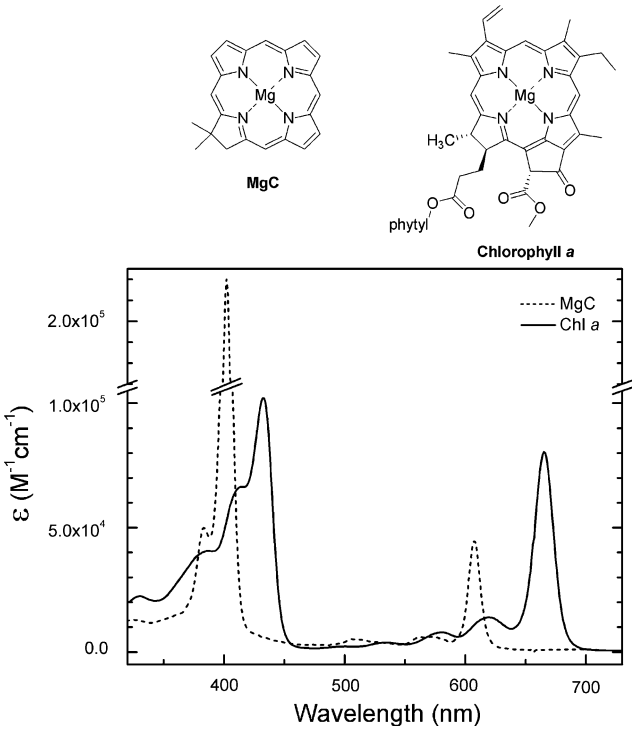

Fig. 3 A comparison between molecular structures and absorption spectra (in toluene) of magnesium chlorin (MgC) and Chl *a* (Chl *a*). Note how the addition of the E ring and the side chains increase the red/blue absorbance ratio (*Source*: Kee et al. 2007)

for details on the relationship between optical spectra and electronic structure of all porphyrins (including chlorins and bacteriochlorins).

Blue photons contain more energy than red photons due to the Planck relation, $E = hc/\lambda$, where E is the energy of the photon, h is Planck's constant, c is the speed of light in vacuum, and λ is the wavelength of light. Excited states resulting from absorption of blue photons are degraded, within subpicoseconds, to the level of the red ones before they are used (Fig. 2). In this process, energy is degraded intramolecularly, by *internal conversion*, as heat. In addition, the ability to absorb only blue light could not have worked as a selective advantage in the evolutionary development of the Chl *a* because all its biosynthetic precursors absorb blue light strongly and red light weakly (Larkum 2006). We may ask the question: Why did nature not choose blue-light absorbing pigments to do photosynthesis? In the 4.5 Ga lifetime of Earth, no other blue, or green, or red light absorber has challenged the dominance of Chl *a*. Chls use only red photons/excitons to drive water-splitting and ferredoxin-reducing photochemistry, no matter what other wavelengths of light they absorb. Clearly, only Chl *a* absorbs strongly in the red (Mauzerall 1976; for Chl *d*, see below). There are two reasons for this strong absorption (Kee et al. 2007; Fig. 3):

(1) The system of conjugated bonds, representing the "π-electron box" determining the wavelength of absorption bands, is extended in the Y direction by the CH_2=CH– substituent in the 3 position and the carbonyl in the 13 position of the closed tetrapyrrole ring (cf. Björn and Ghiradella 2008).

(2) The asymmetry of the π-electron system in the X and Y directions makes possible multiple absorption transitions. The porphin nucleus, present in Chls *c* (c_1, c_2, c_3), with double bonds in "the backs" of all four pyrrole rings, has fourfold symmetry. This results in

very weak red absorption bands. If the biosynthetic pathway imitates, in a way, the course of evolution, then the importance of Chl *a* must lie in its ability to absorb red light efficiently (see Granick 1965).

Larkum (2006), on the other hand, points out that strong absorption in the blue region would be advantageous for organisms living under the filtering action of water. Stomp et al. (2007) have eloquently summarized how the filtering properties of water have modified light, resulting in different optima in different environments. All these environments have been exploited by various organisms, with suitable modifications of the light-harvesting Chls but not of the Chl *a* molecules of the reaction centers of PS I and of PS II.

At first glance, the weak absorption of Chl in the green region could be regarded as a disadvantage. One might think that an ideal pigment should be black to absorb all available energy. Some cyanobacteria (e.g., *Oscillatoria* sp.) do, in fact, appear almost black, partly because of the phycobiliproteins they contain, and they do absorb almost all *visible* light (Fig. 4). Furthermore, many higher plant leaves are dark green (almost black) because the absorption of green light is increased by having many layers of thylakoid membranes containing both Chl *a* and Chl *b*, leading to 95% absorption even in the green (Fig. 5). So indeed, plants are effectively black. Thus, the perception of green color is not an accurate measure of the true optical properties of leaves. The green "trough" in the absorption spectrum of chlorophyll thus does not prevent plants from utilizing green light,

Fig. 4 The cyanobacterium *Oscillatoria princeps* is almost black, due to the presence of both Chls and phycobiliproteins. The sample, shown here, was collected by David Krogmann (Purdue University) and Mark Schneegurt (Wichita State University) from a road side pond in Auburn, South Carolina; the hand is that of Krogmann (From Cyanosite : http://www-cyanosite.bio.purdue.edu; available at http://www.biologie.uni-hamburg.de/b-online/library/webb/BOT311/Cyanobacteria/Cyanobacteria.htm, accessed July 16, 2008)

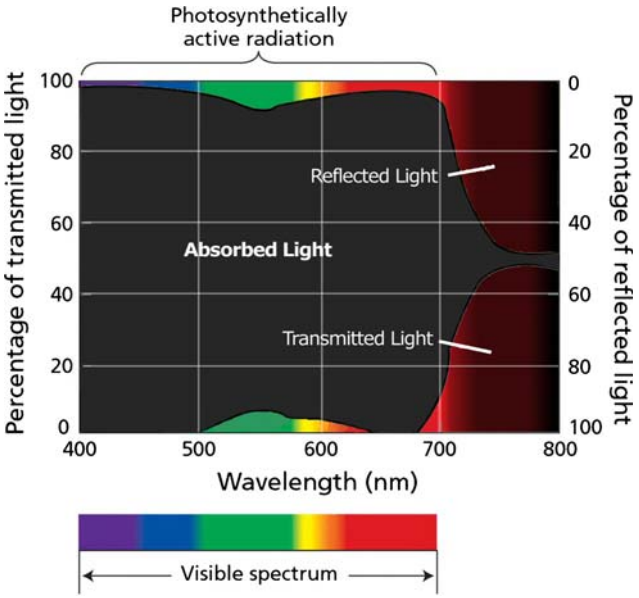

Fig. 5 Spectral distribution of absorbed, transmitted, and reflected light from a maize leaf. Redrawn and modified by Hyunshim Yoo and Govindjee from Chapter 9 in Taiz and Zeiger (2006)

but rather helps to distribute the energy more evenly throughout the leaf. Various methods have been developed for measuring the distribution of light within plant leaves (Vogelmann and Björn 1984; Vogelmann 1993; Vogelmann and Evans 2002; Seyfried and Fukshansky 1983).

We may raise the question as to why higher plants did not continue to use phycobilisomes (present in cyanobacteria and red algae) to capture green light. The answer may lie in the suggestion that on land there was plenty of light available and in the interest of conservation of energy, there was no need to use phycobilisomes. Because of their high nitrogen content and the high energy cost of nitrogen fixation, phycobilisomes are expensive to produce whenever nitrogen is limiting. However, plants evolved to have multiple layers of thylakoids to capture quite a bit of green light (Nishio 2000). The simultaneous presence of highly stacked thylakoids and phycobilisomes would also appear to be mutually exclusive, since the bulky phycobilisomes prevent the close appression of multiple stacked membranes.

We must recognize the quantum nature of photosynthesis, i.e., that it uses energy that comes in packets (quanta) of finite size. The absorption spectrum of Chl does, in fact, drop very rapidly for photon energies below what is required for driving photosynthesis. This is the declining 'red edge' of the absorption spectrum; the inverse of absorption spectrum is transmission spectrum, and, thus, equivalent to the rising red edge in it. This prominent "red edge" could be one of the biosignatures people will look for in exoplanet spectra.

In fact, Kiang et al. (2007b) have discussed the co-evolution (and/or retention) of Chl *a*, absorbing in the red: it relates to the absorption edge of the oxygen molecule in the

atmosphere, which matches the transmission red edge of Chl *a* in vivo. However, this would be important only after accumulation of substantial amounts of oxygen, and Chl *a* must have been selected before that occurred. Furthermore, the P680 referred to by Kiang et al. (2007a, b) is the special cluster of 4 Chl *a* molecules in PS II (see Dekker and van Grondelle 2000, and cited literature) while antenna pigments (which have evolved already at the anoxygenic stage) have, in general, absorption bands at shorter wavelengths. Finally, even in the present high-oxygen terrestrial atmosphere, oxygen absorbs only a small fraction of the incident light at 687.5 nm. Thus, light absorption by oxygen may not have contributed significantly to evolution's choice of Chl *a*. The absorption band of water at 725 nm may have contributed to making absorption by a photosynthetic pigment above 700 nm less useful.

Accessory antenna pigments can extend the spectral range. Such pigments have evolved many times, not only in photosynthetic systems, but also in others. Among others, they occur in photolyases (Fujihashi et al. 2007), in vision (Gemperlein et al. 1980; Vogt and Kirschfeld 1983, Douglas et al. 1999), and in light-driven proton pumping (Lanyi and Balashov 2008); further, energy transfer between pigments also takes place in some cases of bioluminescence (Ruby and Nealson 1977; Ward and Cormier 1976, 1978; Ward et al. 1980). The possibility of extending the spectral range in this way may have made the spectral properties of chlorophyll less critical than they would otherwise have been.

Chemical

Chlorophylls in solution

What should a molecule have if it were to act the role of Chl in photosynthesis? According to Mauzerall (1976):

(i) It should be fairly large (to allow for a large "π-electron box," allowing absorption of long-wavelength light).
(ii) It's π-system should preferably be asymmetric so it will have a strong absorption band in the red (in addition to that in the blue, cf. above, and see Figs. 1 and 3).
(iii) Its lowest excited singlet state should be sufficiently long-lived ($\sim$1 ns) to allow its direct involvement in redox reactions.
(iv) Its lowest excited state should be separated by a sufficiently large energy gap from the ground state in order to make radiationless de-excitation (or non-photochemical quenching) less probable, and also in order to be able to deliver enough energy for photosynthesis.
(v) It should be capable of losing or gaining electrons photochemically, and thereby providing "a rich supply of redox potentials."
(vi) In spite of its complexity, it should be a fairly stable molecule, at least under the influence of a suitable scaffold, such as protein.

Mauzerall (1976) discussed Chls in solution in general, and indeed all of them do fulfill these prerequisites but not exactly to the same extent. For example, according to Table 1 and compared to the Soret absorption band, the red band of Chl *c* molecules is insignificant. On the other hand, Chl *d* has the strongest red absorption, but its photonic energy is significantly lower than that of the next best red light absorber, Chl *a*. In the particular case of Chl *a* (see i, ii, above), it has strong absorption both in the blue and in the red, plus several weaker absorption bands in-between (Fig. 1); (iii) the natural singlet excitation lifetime of Chl *a*, that is calculated from its absorption spectrum, is approximately 15 ns, but its mean measured excitation lifetime in vivo ranges between 0.3 and 0.4 ns (Brody and

Table 1 Main absorption maxima and corresponding photonic energies of chlorophylls in solution

Chlorophyll	Solvent	Absorption maxima, nm [photonic energy, eV]		Band ratio (red/blue)
		Blue band	Red band	
Chl *a* (i)	Diethyl ether	430 [2.888]	662 [1.876]	0.79
[8-Vinyl]-Chl *a* (ii, iii)	Acetone	438 [2.836]	664 [1.870]	0.73
Chl *b* (i)	Acetone	457 [2.718]	646 [1.923]	0.35
[8-Vinyl]-Chl *b* (ii, iii)	Diethyl ether	468 [2.654]	651 [1.909]	0.41
Chl *c1* (iv, v)	Acetone	446 [2.785]	628 [1.978]	0.01
Chl *c2* (iv, v)	Acetone	449 [2.766]	629 [1.975]	0.07
Chl *c3* (iv, v)	Acetone	451 [2.754]	626 [1.984]	0.03
Chl *d* (vi)	Diethyl ether	447 [2.779]	688 [1.805]	1.17

(i) Scheer (2006); (ii) Bazzaz (1981); (iii) Shedbalkar and Rebeiz (1992); (iv) Govindjee and Satoh (1983); (v) Zapata et al. (2003); (vi) Kobayashi et al. (2007)

Rabinowitch 1957; Schmuck and Moya 1994; Schilstra et al. 1999; Morales et al. 2001; Brody 2002). We also note that this excitation lifetime exceeds, by one order of magnitude, the time required for charge separation in the PS II-RC (5–7 ps; Greenfield et al. 1997; Miloslavina et al. 2006; Broess et al. 2008) and in PS I-RC (1–2 ps; Savikhin et al. 2001; Holzwarth et al. 2005); and (iv) its lowest excited singlet state (cf. Fig. 2) is $\sim$1.8 eV above the ground state (about the same for all Chls; see Table 1). Furthermore, (v) all porphyrins are capable of photochemical oxidoreduction in solution if there is an electron acceptor or donor nearby. However, this is not a desirable property in vivo, and it should be kept under strict regulation, if photo-oxidative damage is to be avoided. Highly elaborate systems have evolved to protect antenna Chls (Adams and Demmig-Adams 2004; Gilmore 2004; Golan et al. 2004) and reaction center Chls (Amarie et al. 2007; Kirilovsky 2007; Vass et al. 2007) from the destructive effect of excess excitation. On the other hand, only few Chls *a*-type pigments—and none of the other Chls—take part in excited state and ground state electron transfers. And (vi) according to Mauzerall (1976) "the stability of porphyrins is legendary," but this of course is not the case for Chls in solution. In vivo, Chls are more stable because they are coordinated and hydrogen-bonded to proteins. Indeed, there are reports of how Chls in vivo, or derivatives thereof, have persisted over tens of millions of years in plant fossils (Treibs 1934; Niklas and Giannasi 1977; Keely 2006). This stability must have provided selective pressure in the process of evolution and must have helped in the survival of the system during extreme and fluctuating environments.

Objections can be raised to some of the above prerequisites. With regard to (iii), see above, we know now that the only redox reactions in which Chls are involved are those occurring in the RCs. Although according to Mauzerall (1976) triplets are "the source of most solution photochemistry," they are unnecessary and potentially dangerous in the RCs where redox reactions are not diffusion-limited.

As noted in this Viewpoint, the great majority of Chls *a* in vivo are neither photoactive nor redox active. All Chls (be it *a, b, d* or 8-vinyl ones) attach to antenna and reaction center proteins with phytyl chains, while Chl *c* uses other groups. Certainly, it is not phytyl that makes a few Chls photoactive or redox active in vivo. However, in addition to serving as a lipophilic anchor in apoproteins, the phytyl probably helps in maintaining the central Mg^{2+} in the 5-coordinate state, and thus stereochemically affecting H-bonding interactions. Although most of the properties are due to the conjugated ring system, phytyl may ultimately influence excited state properties of Chls (Fiedor et al. 2008).

Obviously, the above-described prerequisites are not enough. The fact is that there has never been a photosynthetic organism found that contained only Chl *b*, or *c*, or *d*. In contrast, organisms having only Chl *a* have existed and still exist (most cyanobacteria, red algae, and glaucophytes). Therefore, the question what does Chl *a* have that other Chls do not, still remains unanswered.

Chlorophylls in the environment of thylakoid membrane proteins

To advance our understanding of the answer to our question "Why Chl *a*?" we must recognize that Chls associate with particular proteins specifically and stoichiometrically and that these associations and the immediate protein environment critically modify their properties. The great majority of Chls *a*, and all Chls *b* and *c* exist in the light harvesting Chl-protein complexes of the peripheral antennae (Lhc, Pcb) and of the core antennae (CP43, CP47; CP43') (Bibby et al. 2003; Green and Parson 2003; Liu et al. 2004). These antenna Chls do not eject an electron when they are electronically excited, neither does Chl *a* in solution. Only 4 Chls *a* (or, including the pheophytins *a*, Pheo *a*, 6 Chl *a*-type pigments) in PS II RC (Dekker and van Grondelle 2000; Ferreira et al. 2004; Loll et al. 2005; Barber 2008; see Fig. 6, left) and 5 Chls *a* and 1 13-C^2 epimer of Chl *a* (Chl *a*′) in PS I RC (P700; Jordan et al. 2001; Fromme et al. 2001; Grotjohann and Fromme 2005; Amunts et al. 2007; see Fig. 6, right) do somehow cooperate in moving electrons from electronically excited states to an electron acceptor strategically placed near it; thus, electrons are transported to the ground state of the acceptor molecule and, in this way, stabilized ground state cation–anion radical pairs are produced. We may add the possibility that one of the major tasks of a protein may be to exclude or position functionally compatible electron donors or acceptors, depending upon whether the complex will act as an antenna or a reaction center. If one decides to count the electron acceptor Pheo *a*, the number of redox-active chromophores in PS II RC would simply increase by one, but not the number of Chls *a*.

In both RCs, the primary electron donor is thought not to be a special pair Chl *a* (i.e., P_{D1}/P_{D2} in PS II and P_A/P_B in PS I) but an accessory Chls *a* (Chl_{D1}, Chl_{D2} in PS II RC and Chl A_A, Chl A_B in PS I RC) that are located closer to the respective primary electron acceptor (Pheo *a*, and A_{0A}, A_{0B}; Holzwarth et al. 2006a, b). However, the stabilized Chl *a* cation radicals are located on the special pairs of PS II (P_{D1}/P_{D2} in P680) and PS I (P_A/P_B in P700). Actually, in the PS II RC only 2 Chls *a* (Chl_{D1} and P_{D1}) and 1 $Pheo_{D1}$ *a*, all attached to protein, D1 can reduce the plastoquinone acceptor Q_A(D2) to Q_A^- (D2) (active branch). The 6 Chls *a* of the PS I RC are also arranged in two electron

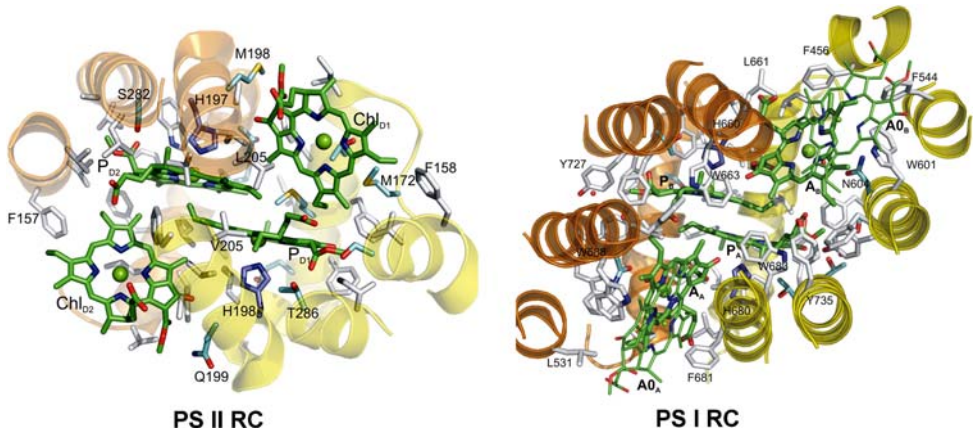

Fig. 6 Structures of the photosynthetic reaction centers, as viewed from the stromal side of the thylakoid membrane and parallel to the membrane normal. Carbon-to-carbon bonds are colored green in Chls *a*, gray in non-polar amino acids, cyan in polar amino acids, and blue in positively charged amino acids. O, N, and S atoms are shown in red, blue, and yellow. *Left*, *detail of PS II RC*. The polypeptide backbones of proteins D1 and D2 are represented as yellow and orange ribbons, the two special pair Chls *a* are identified as P_{D1} and P_{D2}, and the two accessory Chls *a* as Chl_{D1} and Chl_{D2}. The central Mg atom of P_{D1} forms a covalent coordinate bond with His-198(D1), and of P_{D2} with His-197(D2). In addition, P_{D2} has a possible interaction with D1-Ser 282. Further, Chl_{D1} (the primary electron donor) has an environment very different from that of P_{D1} and P_{D2}, and Chl_{D2} (see Table 2). *Right*, *detail of the PS I RC*. The polypeptide backbones of proteins A and B are represented as yellow and orange ribbons, the special pair P_A and P_B are Chl *a*′ ($13C^2$-epimer) and Chl *a*, respectively, the two accessory Chls *a* are identified as A_A and A_B and the two stable Chl *a* anions as $A0_A$ and $A0_B$. The central Mg atoms of P_A and P_B form coordinate covalent bonds with His residues, A-His 680 and B-His660, respectively. In addition, there is the possibility of H-bond interaction of P_A with A-Tyr735, and of P_B with B-Tyr 727. $ChlA_A$ and $ChlA_B$ coordinate with water molecules, which are further hydrogen-bonded to A-Asn-591and B-Asn604, and of $A0_A$ and $A0_B$ Chls *a* with A-Met-668 and B-Met-668; after Grotjohann and Fromme (2005). Structural water molecules are shown as red spheres. In all cases, RC Chls have, within van der Waals distances, different sets of hydrophobic amino acids, with different polarity (see Table 2). The figure shown here is courtesy of Jan Kern; it was made by using PyMol and is based on pdb coordinates of 2 AXT file (Loll et al. 2005) for PS II, and of 1JBO file (Jordan et al. 2001) for PS I

transporting branches on the core proteins A and B but in this case both branches are active although unequally (for literature, see reviews in the books edited by Wydrzynski and Satoh (2005), and by Golbeck (2006) for PS II, and PS I, respectively).

Of the several hundreds of chlorins *a* (i.e., Chls and Pheos) that are present in the photosynthetic units of oxygenic organisms, only 4 Chls *a* and 2 Pheos *a* per PS II RC monomer and 6 Chls *a* per PS I RC monomer are actually redox active. Recent evidence indicates that even this six-plus-six chlorin *a* minimum can be further pared down by replacing Chls *a* with Chls *d*, though perhaps the P_{D1} Chl *a* of the PS II RC is irreplaceable (vide infra). There is also one Chl *a* in the Cyt b_6f complex, which is involved neither in light harvesting nor in transporting electrons (reviewed by Cramer et al. 2005).

If only 10, or fewer, redox-active Chls *a* in PS II and in PS I do really make the difference, then it is quite intriguing to speculate how this came about, or how different protein environments tune their properties differently from, let us say, those of the antenna Chls *a*. Chls bind to proteins in at least three ways: (1) by forming coordinate covalent (or semi-polar) bonds with their central Mg atom (the electron acceptor) and with unshared electron pairs donated by N, O, and S atoms (the donor) of amino acid side chains, peptide backbones and water molecules; (2) by forming H-bonds with their keto and ester carbonyl oxygens as acceptors and –OH, –SH, and –NH groups of amino-acid side chains as donors; and (3) by binding to the phytyl chain. Probably there is at least one more component interacting with the π-electrons of the macrocycle. These various kinds of ligands plus the differences in the electron shielding of the central Mg, due to the presence of different electron donating (alkyls) and electron withdrawing (formyl) peripheral substituents, afford a wide range of possibilities for selective Chl binding. Our knowledge is only fragmentary, although elegant laboratory demonstrations do exist in the literature (Rau et al. 2001; Chen et al. 2005; Chen and Cai 2007; see also Hoober and Argyroudi-Akoyunoglou 2004, and Hoober et al. 2007 for reviews). There is no easy way to know why the different RC Chls have totally different properties. Although the dominant force is H-bonding, the overall electronic milieu must have the greatest contribution (see Table 2 for differences).

In the protein environment of the PS II reaction center (Fig. 6, left), the redox-active Chls *a* P_{D1}, P_{D2}, Chl_{D1}, and Chl_{D2} are all excitonically coupled and share, at room temperature, the energy of a 680 nm photon (see review by Dekker and van Grondelle 2000) with which they generate stable oxidant $P_{D1}^{+\bullet}$ ($E_m(P_{D1}^{+\bullet}/P_{D1})$ ~1.1–1.3 V; see Rappaport et al. 2002; Grabolle and Dau 2005; Ishikita

Table 2 Amino acid environment of reaction center chlorophylls of PS II (P_{D1}; P_{D2}; Chl_{D1};Chl_{D2}) and of PS I (P_A; P_B; Ao (A): Chl_A (A branch); Ao (B): Chl_A (B branch))[a]

RC Chls	Amino acids within H-bonding distance, ligands	Amino acids within van der Waals distance
PS II P_{D1}	D1-His198	D1-Met183; D1-Phe186; D1-Gln187; D1-Leu193; D1-Gly201; D1-Val205; D2-Leu205, and some more
PS II P_{D2}	D1-His197	D2-Val156; D2-Gln186; D2-Trp191;
	D1-Ser282	D2-Thr192; D2-Gly200; D1-Phe206, and some more
PS II Chl_{D1}	–	D1-Val157; D1-Phe158; D1-Met172;
		D1-Thr179; D1-Phe182; D1-Met183;D2-Met 198, and some more
PS II Chl_{D2}	–	D1-Gln199; D1-Val202; D2-Phe157;D2-Phe181; D2-Leu182, and some more
PS I P_A	A-His680	A-Phe611; A-Trp683; A-Thr742; A-Thr743; A-Phe746, and some more
	A-Tyr603(?)	
	A-Tyr735 (?)	
PS I P_B	B-His660	A-Leu654; B-Phe656; B-Trp663; B-Tyr723
	B-Tyr727	
PS I Chl A_A (Chl_A on A branch;Nr 1012)	–	A-Leu 677; A-Ala684; B-Leu531;
		B-Trp588; B-Asn591; B-Trp663
PS I Chl A_B (Chl_A on B branch;Nr. 1022)	–	A-Phe456; A-Phe544; A-Trp601; A-Trp683; B-His660; B-Ala664
PS I $A0_A$ (Ao on A branch; Nr. 1013)	A-Met 688; A-Tyr 696;	A-Phe681; A-Ala 684; A-Trp697;
	B-Ser429; B-Trp 588	B-Ser426; B-Phe587
PS I $A0_B$ (Ao on B branch; Nr 1023)	B-Ala664; B-Threo665;	B-Trp677;
	B-Met668	A-Asp446; A-Val548; A-Ile 552; A-Phe600

[a] Data provided by Jan Kern, using pdb file 2AXT (Loll et al. 2005) and 1 JBO (Jordan et al. 2001)

et al. 2005) and reductant Pheo a^- (E_m (Pheo a^-/Pheo a) −0.60 to −0.65 V; Klimov et al. 1979; Rutherford et al. 1981). Furthermore, these redox-active chlorins *a* exist at short distances from other cofactors, such as to Tyr_Z and Tyr_D (~13.5 Å, center-to-center) and to Q_A(D2) (~13.1 Å center-to-center; Ferreira et al. 2004; Loll et al. 2005). Edge-to-edge distances, which are more relevant for intermolecular electron exchanges, are of course shorter. It should be noted that the widest difference of the above redox potentials (~1.3 − (−0.65) = 1.95 V) exceeds the energy of the 680 nm photon (1.826 eV); therefore, the narrower difference (~1.1 − (−0.60) = ~1.70 V) seems more reasonable. For uncertainties in the estimation of redox potentials, see Ishikita et al. (2005) and Rappaport and Diner (2007).

In the protein environment of the PS I reaction center (Fig. 6, right), the 6 redox-active Chls *a* (and other electron transporting cofactors) are linked to the the 5 central transmembrane helices (TMH; RC domain) of the core proteins A and B, while antenna Chls *a* are linked to the remaining 6 TMH (antenna domain) of these 11-TMH proteins. The similarities of the A, B proteins of PS I to the 5 TMH (D1, D2) and the 6 TMH (CP43, CP47) proteins of the PS II core prompted hypotheses of a linear evolutionary relation (branched from a common precursor) between the two photosystems, either by gene fission (PS I first) or by gene fusion (PS II first; see Grotjohann and Fromme (2005) and cited literature). Details of the cofactor arrangement in PS I, however, is remarkably dissimilar to that in PS II. Thus, in contrast to PS II in which P680 comprises a special pair of 2 identical Chl *a* molecules and only 1 of the 2 electron transporting branches that originate from it is active (that on D1), the special pair of PS I (P700) is heterodimeric (Chl *a*′/Chl *a*) and both electron transporting branches originating from it are active, although to different degrees. The asymmetry of P700 and the presence of the epimeric Chl *a*′ are considered to be essential for the function of PS I, which uses the energy of a 700 nm photon (1.774 eV) to produce a weak oxidant ($P700^+$; E_m = 0.423 V in *Thermosynechococcus elongatus*, 0.468 V in spinach; Nakamura et al. 2005) and a very strongly reducing Chl *a* anions (A_{0A}^-, A_{0B}^-; E_m = −1.05 V; Kobayashi et al. 2007). Here the energy fraction stored as redox potential difference after the primary charge separation is 1.43–1.47 eV, about 83–86% of the photonic energy.

For the chlorophylls and bacteriochlorophylls, Haehnel et al. (2009) point out that it is the coordinatively unsaturated central Mg that has the best established interaction with the proteins. Mg has an extra ligand, and in 50% of natural binding sites, the amino acid is histidine; in other

cases, the binding may be to glutamine, asparagine, rarely cysteine, as well as the backbone C=O groups, N-terminal formyl groups, and also water. This information becomes important when we begin to build artificial pigment-protein complexes in order to further understand the role played by the protein environment in determining the chemistry of the native chlorophylls.

Is chlorophyll *d* a match for chlorophyll *a* everywhere?

Judging only from the energy of the photons absorbed (approx. 1.8–2.0 eV; see Table 1) and from the energy stored as redox potential difference after the primary charge separation (roughly 1.2 eV), there is no reason why another Chl could not replace Chl *a* in the RC environments. However, we do not know if this alternate Chl could be tuned by the protein environment to produce an oxidant above ~ 1 V in one case (PS II RC), a reductant below ~ -1 V in another (PS I RC), and be redox silent in a third (antenna holochromes). In the case of *Acaryochloris*-like cyanobacteria Chls *d* are known to replace Chls *a* both in the antenna and the reaction center proteins. These cyanobacteria occur in habitats in which far red light ($\lambda > 700$ nm) predominates and they are either free-living organisms (Miller et al. 2005) or they are attached on red, green, or brown macroalgae (Murakami et al. 2004; Ohkubo et al. 2006). Recently, Chl *d* has been detected chemically, always together with Chl *a*, in the sediments of various oceanic and lake environments that span a range of salinities and temperatures. Thus, the habitats of Chl *d*- containing organisms may be more extensive and widespread than it was supposed hitherto (Kashiyama et al. 2008).

The most extensively and intensively studied case of a Chl *d*-containing cyanobacterium is that of *Acaryochloris marina* (Swingley et al. 2008). Its PS I complex contains per each PS I RC monomer 97 Chls *d*, 1 Chl *d*′, and 2 Chls *a* (Tomo et al. 2008a). Two Chls *a* are assigned to the primary electron acceptors A_{0A} and A_{0B}; Itoh et al. 2007), the special pair is a Chl *d*/Chl *d*′ heterodimer (named P740 by Hu et al. 1998) and the 2 accessory Chls A_A and A_B are again Chls *d* (Itoh et al. 2007). Primary photochemical charge separation occurs between A_A and A_{0A} in one branch and A_B and A_{0B} in the other and yields stable low potential anions A_0^- ($E_m \sim -1.02$ V; Brettel 1997). On the other hand, the complex that consists of PS II RC plus the core antenna proteins CP43′, CP47 in *A. marina* contains 55 Chls *d*, 3 Chls *a*, and 2 Pheos *a* (Tomo et al. 2008b).

There is consensus that in the *Acaryochloris* PS II RC, the primary charge separation occurs between accessory Chl *d* (D1) as donor and Pheo *a* (D1) as acceptor (Itoh et al. 2007; Schlodder et al. 2007; Tomo et al. 2008b), but there is disagreement whether the special pair P_{D1}/P_{D2} is a Chl *d*(D1)/Chl *d*(D2) homodimer (Itoh et al. 2007; Tomo et al. 2008b) or a Chl *a*(D1)/Chl *d*(D2) heterodimer (Schlodder et al. 2007; Renger and Schlodder 2008). Interpretations are based on flash induced difference absorption spectra, light–dark difference absorption spectra, FTIR difference spectra, and pigment stoichiometries. The resolution of this dilemma is crucial for the absolute exclusiveness concept of Chl *a*. In any case, it appears that in *A. marina*, Chl *d* does not totally substitute for all reaction center chlorins *a*. Exempted are seven chlorins *a*: The two A_0, when reduced by PS I, serve as strong reductants in electron transporting branches of PS I; a third that possibly serves as strong oxidant in PS II RC; two Chls *a*, which have not yet been assigned, although they have been recognized as redox-active species in the PS II RC-core antenna complex (Tomo et al. 2008b); and two Pheos *a*.

In general, no replacement of Chls *a* in RCs by a different Chl, either in nature or in the laboratory, has been reported up to now. In one remarkable experiment, Sato et al. (2001) transformed cyanobacterium *Synechocystis* that synthesizes only Chl *a* with the chlorophyllide *a* oxygenase gene of *Arabidopsis thaliana*, a higher plant that synthesizes both Chl *a* and Chl *b*. The transformant did synthesize Chl *b* which, however, replaced Chls *a* only in the core antenna complexes of PS II and PS I. In contrast, Vavilin et al. (2003) succeeded in replacing Chl *a* with Chl *b* in the PS II RC of a *Synechocystis* mutant that was first deprived of the PS I complex, then transformed with the *lhcb* gene that codes for the peripheral light harvesting complexes (LHC) of eukaryotic plants, and lastly with the chlorophyllide *a* oxygenase gene. Although the Chl *b* of PS II RC Chl *b* was reported to be redox active, there was no evidence that it could photogenerate a high-potential cation, analogous to $P_{D1}^{+\bullet}$ of Chl *a*. As far as we know, this remarkable report is the first proof that Chl *b* can be made redox active in vivo.

The answer, therefore, to the question in the subsection heading is that Chl *d* is indeed a good match for Chl *a,* but not everywhere. Chl *a*, in general, appears unique and irreplaceable (at least on our planet), particularly if global scale photosynthesis is considered. In all likelihood, this is due to its physicochemical properties as Mauzerall (1973, 1976) had suggested, which however are greatly modified by its various ligations to protein (*via* axial ligands and H-bonding) and by the immediate proximity of functionally compatible redox cofactors and π-electron systems (quinones, aromatic amino acids, etc.). Upon extraction with diethyl ether, or with another solvent, all Chl *a* molecules become indistinguishable, regardless of their origin (but Chl *a*′ and other Chls of course maintain their chemical characteristics). In antenna holochromes, excited Chls *a* do not eject electrons, probably because of the lack of a compatible nearby redox cofactor. In contrast, the proximity of high potential redox cofactors (Tyr_Z, $CaMn_4$) must

be conducive to the formation of the high redox potential Chl a^+ or P(D1)$^+$ cation in PS II RC and the proximity of a low potential redox cofactors (A1, F_X) conducive to the formation of the two low potential Chl *a* anions Chl a^- (A_0 (A)$^-$ and A_0 (B)$^-$) in PS I RC.

Evolution of PS II RC and of PS I RC

Protein structure similarities between type II (BII-R) and type I (BI-R) bacterial reaction centers and the plant reaction centers PS II and PS I suggest that all have descended from a common ancestor (Rutherford and Faller 2002; Ben Shem et al. 2004; Olson and Blankenship 2004; Raymond and Blankenship 2004; Sadekar et al. 2006). This ancestor was more bacteria-like because its light transducer chromophores formed homodimers and not heterodimers like those of Chls *a* and *d* in the PS I-RCs (and perhaps in the PS II-RCs) of plants and of the cyanobacterium *A. marina*. According to Rutherford and Faller (2002) heterodimeric transducers handle incoming photons more efficiently and more safely and so they afford an evolutionary advantage. In addition, the change from BChls to Chls *a* would enable (a) the Type II-RCs to achieve oxidation potentials of $\sim$1 V, or higher, in excess of what is needed to oxidize water at slightly acidic pH (0.89 V/e$^-$; Pecoraro and Hsieh 2008); and (b) the Type I-RCs to achieve reduction potentials of ~ -1 V, or lower, in excess of what is needed to reduce low potential nonheme iron sulfur centers (e.g., F_X at -0.73 V; Demeter and Ke 1977).

We can imagine (after Allen and Martin 2007) an ancestral symbiont of such Type II and Type I bacteria (a *protocyanobacterium*) capable of switching between the one or the other one-step photoreaction, and in which the loss of the switching ability would be lethal (because of accumulated oxidants) unless it occured simultaneously with the incorporation of the charge accumulator, the Mn_4Ca cluster, in its PS II-RC. This would enable the biphotonically powered electron transport to be coupled on one side to water as electron donor and on the other to slowly autoxidizable low potential electron acceptors, such as pyridine nucleotides (Asada 2000).

Concluding remarks

Why is, then, Chl *a* so unique, at least on the Earth? Here, we summarize our views.

First, its omnipresence must derive from the fact that it appeared ahead of Chl *b* on the Earth. Also, the absorption spectrum of Chl *b* is less suitable when not combined with that of Chl *a*. Chl *c* may have preceded Chl *a*, but it was less suitable for oxygenic photosynthesis due to its weak absorption in the red region of the spectrum (Table 1; see Larkum 2006 for discussion of the evolution of Chls).

Second, neither in vivo nor in vitro the 6 Chls *a* of the PS I RC and the 6 Chls *a* of PS II RC were ever replaced completely by another Chl, in spite of the long ancestries and the great varieties of photosynthetic organisms. Even in the exceptional case of the *Acaryochloris*-like cyanobacteria, the primary stable low potential anion in RC I is formed either on acceptor A_{0A} or acceptor A_{0B}, both Chls *a*, while most probably (but not yet certainly) the high potential cation in RC II PC is formed on the P_{D1} Chl *a* (see Section "Is chlorophyll d a match for chlorophyll a everywhere?"). Indeed, RC Chls *a* can be replaced partly by redox-active Chls *d*, but the Chl *d*-containing organisms were probably never a challenge to the dominance of the Chl *a*-containing organisms.

Third, excited Chls *a* become redox active as long as compatible redox-active cofactors exist at short distances from them. This is the case in the protein environments of PS I RC and PS II RC, but not in the protein environments of the core and peripheral antenna complexes.

We conclude that the uniqueness of Chl *a* stands unchallenged in our Earth. In the wider space, which encompasses the recently discovered exoplanets, and as far as carbon-based life is concerned, still Chl *a* can be either unique, or one of the very rare solutions that are chemically and energetically feasible.

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