The Thermodynamics and Kinetics of Electron Transfer between Cytochrome \(b_{6f}\) and Photosystem I in the Chlorophyll \(d\)-dominated Cyanobacterium, *Acaryochloris marina* *

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We have investigated the photosynthetic properties of *Acaryochloris marina*, a cyanobacterium distinguished by having a high level of chlorophyll \(d\), which has its absorption bands shifted to the red when compared with chlorophyll \(a\). Despite this unusual pigment content, the overall rate and thermodynamics of the photosynthetic electron flow are similar to those of chlorophyll \(a\)-containing species. The midpoint potential of both cytochrome \(b_{6f}\) and the primary electron donor of photosystem I (P740) were found to be unchanged with respect to those prevailing in organisms having chlorophyll \(a\), being 345 and 425 mV, respectively. Thus, contrary to previous reports (Hu, Q., Miyashita, H., Iwasaki, I. I., Kurano, N., Miyachi, S., Iwaki, M., and Itoh, S. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 13319–13323), the midpoint potential of the electron donor P740 has not been tuned to compensate for the decrease in excitonic energy in *A. marina* and to maintain the reducing power of photosystem I. We argue that this is a weaker constraint on the engineering of the oxygenic photosynthetic electron transfer chain than preserving the driving force for plastocyanin oxidation by P740 via the cytochrome \(b_{6f}\) complex. We further show that there is no restriction in the diffusion of the soluble electron carrier between cytochrome \(b_{6f}\) and photosystem I in *A. marina*, at variance with plants. This difference probably reflects the simplified ultrastructure of the thylakoids of this organism, where no segregation into grana and stroma lamellae is observed. Nevertheless, chlorophyll fluorescence measurements suggest that there is energy transfer between adjacent photosystem II complexes but not from photosystem II to photosystem I, indicating spatial separation between the two photosystems.

*Acaryochloris marina* is an unusual cyanobacterium, because it contains mainly chlorophyll \(d\) (Chl) \(^3\). It was first isolated from a suspension of algae squeezed out of *Lissoclinum patella*, a colonial ascidian (1). It mainly grows as a biofilm on the underside of the thallus of red algae (3) and free living in a salt lake (4). The general feature of its habitat is that it lives at relatively low light intensities, which are enriched in the far red region of the spectrum. Therefore, its oxygenic photosynthesis provides a typical example of adaptation to specific light conditions (2), as evidenced by its pigment composition, where Chl \(d\) is predominant and the Chl \(a\) level is very low (5, 6). This pigment composition contrasts with the usual high level of Chl \(a\) found in other oxygenic phototrophes, as illustrated by the absorption spectrum of a suspension of *A. marina* cells, which is markedly red-shifted when compared with other Chl \(a\)-containing photosynthetic organisms. The extent of the red shift of the absorption spectrum of *A. marina* is of similar magnitude to that observed between Chl \(d\) and \(a\) in organic solvent (e.g. methanol) (7).

Not surprisingly, the unusual absorption spectrum of *A. marina*, due to the high level of Chl \(d\), influences the spectroscopic signatures of the various cofactors involved in the primary photochemistry. For example, light-induced charge separation in photosystem I (PSI) is associated with a main absorption band (in the Qy region) bleaching maximally at 740 nm, indicating that the primary donor is composed of Chl \(d\) rather than Chl \(a\). Hence, this pigment was named P740 (8). Clearly, the energy of the quantum inducing charge separation by P740 is lower than that of P700 present in Chl \(a\) containing PSI (1.68 eV instead of 1.77 eV). This decrease by about 100 meV is significant when compared with the ~800 meV required to drive electron transfer between the soluble electron donor to P740 (plastocyanin) and the first soluble electron acceptor (ferredoxin). However, some specific strategies may have been developed by this organism to cope with this peculiar situation and maintain a photosynthetic activity compatible with its...
growth requirements. A possible rationale came from the study by Hu et al. (8), who measured the midpoint potential of P740 and found that it was down-shifted by about 100 mV when compared with that of P700, the primary electron donor in PSI, where only Chl a is present. On this basis, they suggested that the lower midpoint potential of P740 would compensate for the lower energy of the absorbed quantum, thereby maintaining the reducing power of the excited state. Although such a tuning of the midpoint potential of P740 would allow a similar energetic picture for the electron acceptor side of PSI in A. marina and in Chl a-containing species, it would result in a significant thermodynamic pitfall on the donor side of PSI by decreasing the free energy gap between P740 and its electron donors. For example, the midpoint potential of cytochrome (cyt) f, in Chl a-containing organisms is in the 320–370 mV range (9–11). If cyt f in A. marina has the same midpoint potential, the driving force for electron transfer between cyt f and P740 would be slightly uphill. This would lead to the accumulation of a significant fraction of P740 under steady state illumination, which would obviously reduce the overall photochemical efficiency by closing most of the PSI photochemical traps. Alternatively, the midpoint potential of the redox-active cofactors involved in electron donation supply to P740 (the Rieske FeS center, cytochrome f, and plastocyanin or cyt c1) would have to be tuned to compensate for the loss in oxidizing power of P740 so as to provide a driving force for maintaining efficient electron flux through PSI.

These issues are addressed in the present work. We first determined the thermodynamic equilibrium constant of the electron transfer reaction between cyt f and P740 in actively growing cells. The equilibrium constant for this reaction was found to be ~15, showing that the midpoint potential of cyt f is about 70 mV lower than that of the P740/P740 couple. Consistent with this, the directly measured midpoint potentials of cyt f and of the P740/P740 couples were 345 and 425 mV, respectively, in contradiction of the previous report by Hu et al. (8) of a midpoint potential of 335 mV for the latter. The values that we have determined are similar to those found for the analogous redox cofactors in Chl a organisms for cytochrome b,f (9–11) and P700 (12, 13), suggesting that the midpoint potential of the primary electron donor in PSI is not necessarily affected by the chemical nature of the Chls that comprise it. This finding raises interesting questions concerning the overall energetics of PSI-driven charge separation in A. marina, which are discussed in this paper.

**EXPERIMENTAL PROCEDURES**

**Growth Conditions**—A. marina cells were grown under low white light illumination (5–10 microeinsteins m⁻² s⁻¹) in a modified K⁺ ESM medium (14, 15) supplemented with extra iron to a final concentration of 14 μM. The cyanobacterium was harvested in its midexponential phase, and the cells were resuspended in fresh medium at the desired density for experiments.

**Membrane Isolation**—Thylakoid membranes were prepared from freshly harvested cells, which were resuspended in a medium containing 50 mM MES, pH 6.5, 20–25% glycerol (W/v), 10 mM CaCl₂, 5 mM MgCl₂, and 1 mM benzamidine. Cells were broken by several cycles of centrifugation in the presence of 0.1-mm glass beads (10 s), followed by a short (10 s) incubation in an ice bath. Unbroken cells were removed by a short centrifugation at 2000 × g for 30 min. The final pellet was resuspended in a 50 mM MES, pH 6.5, buffer, in the presence of 20 mM CaCl₂ and 2.5 mM MgCl₂.

**RNA Isolation and Reverse Transcription-PCR**—RNA was isolated using a hot phenol acid method for Synechocystis sp. PCC 6803⁴ adapted from Ref. 16. Total RNA was treated with 1 unit/μg RNA for 1 h with DNase (Promega). 350 ng of total RNA was used in a reverse transcription reaction according to the manufacturer’s instructions (BcaBEST RNA PCR kit version 1.1; TaKaRa), using a specific reverse primer (5′-TTAGC-CCTGAACCCTAATGGG-3′) to the petE genomic DNA sequence (accession number: YP_001517679), and the entire volume was added to the subsequent PCR (94 °C 30 s, 57 °C 30 s, 72 °C 1 min for 35 cycles) using a specific forward primer (5′-TCAAGTGCGTCTTCTAAGGG-3′).

**Spectroscopy**—Absorption and fluorescence spectra were measured with a laboratory-built spectrophotometer based on a diode array (AVS-USB 200; Ocean Optics). Excitation of fluorescence was at 470 nm unless otherwise indicated. Spectroscopic analysis was performed at room temperature, using home-made pump and probe spectrophotometers. Three different set-ups were employed. The kinetics of light-induced redox changes were measured with a LED-based spectrophotometer (JTS10, Biologic, France), having a time resolution of 10 μs (Figs. 2 and 5). Actinic flashes were provided by a dye laser at 690 nm, whereas measuring flashes were provided by a white LED (Luxeon; Lumileds) fitted with appropriate interference filters (10 nm full width at half-maximum). Alternatively, a second set-up having a time resolution of 10 ns (17) was employed, in which the actinic flashes were provided by a dye laser at 690 nm, whereas probe flashes were generated by an optical parametric oscillator pumped by an Nd:Yag laser (Figs. 3 and 4). The third set-up was for electrochemical redox titrations, which were measured as described previously (18, 19) (Fig. 6). In this case, the actinic flashes were provided by a xenon flash lamp (giving saturating light pulses, 5-μs duration at half-height), passed through a Schott RG695 interference filter. Probe flashes were provided by a second xenon flash lamp (3-μs duration at half-height), filtered through a monochromator (Jobin Yvon). Titration of cyt f was performed measuring the absorption changes at 554 nm, with a base line drawn between 546 and 562 nm. Mediators were para-benzoquinone, diaminodurene, 2,5 dimethyl-benzoquinine, phenazine methosulfate, and menadione, at a concentration of 1 μM for each. Similarly, the redox potential of P740 was measured using the amplitude of the flash-induced absorption changes (at 740 nm) at various redox potentials, poised either electrochemically or chemically.

**RESULTS**

**Absorption and Fluorescence Emission Spectra**—Cells of A. marina, in midexponential phase, were used to characterize the main properties of the intersystem electron transport chain in

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⁴ G. Ailani, personal communication.
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Figure 1. A and B, absorption spectrum (A) at room temperature and fluorescence spectra (B) at room temperature (solid line) and 77 K (dotted line) of a suspension of A. marina cells. Room temperature fluorescence induction kinetics (C and D, on an expanded scale) was measured in the presence (solid line) and absence (dotted line) of the PSI inhibitor DCMU. Cells were harvested in midexponential phase and resuspended in fresh growth medium before measurements. See "Experimental Procedures" for further experimental details.

Overall Photosynthetic Activity—The overall photosynthetic activity of A. marina was evaluated from the fluorescence induction curve shown in Fig. 1, C and D. The quantum yield of PSI, \( F_{v}/F_{m} = (F_{m} - F_{o})/F_{m} \) (23–25) gave a typical value of ~0.7 for \( F_{v}/F_{m} \), which is in agreement with previous reports (20). This is higher than values for other phycobilisome-containing cyanobacteria (see Ref. 26) for a review). In these organisms, the low \( F_{v}/F_{m} \) ratio is interpreted as reflecting either the presence of sustained energy spillover from PSII to PSI (27), the loose excitonic connectivity between the phycobilisomes, or the photochemical traps (which is not the case here; see above), or the low PSII/PSI ratio (reviewed in Ref. 26). Neither of these phenomena significantly occur in A. marina, since the large \( F_{v}/F_{m} \) is incompatible with a low amount of PSII, and the shape of the fluorescence rise, measured in the presence of the PSI inhibitor, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), is clearly sigmoidal (Fig. 1C) (also see Ref. 20). This feature, which is commonly observed in photosynthetic eukaryotes but not in cyanobacteria, is interpreted as reflecting the progressive increase in the light harvesting capacity of a PSII photochemical unit as its PSII neighbors become photochemically inactive due to the reduction of the quinone acceptor QA (28, 29). Such a feature is indicative of efficient energy transfer between closed and open PSII traps, without any significant competition by energy quenching through PSI photochemistry.

Another important feature of the fluorescence data shown in Fig. 1 is the large difference between the level measured under steady state (\( F_{s} \)), noninhibited conditions (control in Fig. 1D) and the maximum level (\( F_{m} \) (+DCMU in Fig. 1D). Since \( F_{s} \) is expected to reflect the steady state amount of reduced QA, this finding suggests that the limiting step in the reoxidation of the plastoquinone pool (i.e. in the electron flow downstream of PSII) is in the same range as the rate of its reduction (i.e. the number of electrons transferred by PSII per unit of time). As discussed by Joliot (28, 30), this can be empirically estimated from the time required to reach ~66% of the \( F_{m} \) value, in the presence of DCMU. Since this value is ~10 ms in the present experiment (Fig. 1D), it can be deduced that the number of electrons transferred per photosynthetic chain is in the 100 s\(^{-1}\) range (i.e. close to the maximum rate of PQH\(_2\) oxidation in vivo) (31). In other words, there seems to be no severe bottleneck in electron transfer downstream of PSII in A. marina.

Kinetic Features of the Electron Flow Chain—Following on from above, we investigated further the properties of the electron transfer chain by directly measuring the photochemically induced turnover of the cytochrome b\(_{6}\)f complex. Fig. 2 shows the time course of the changes occurring at 554 nm upon excitation with a single turnover saturating flash. At this wavelength, the actinic flash induces a rapid bleaching (see Fig. 2). The recovery kinetics, to the initial (dark-adapted) level, was inhibited by addition of the plastoquinone analogue, 2,5-dibromo-3-methyl-6-isopropyl-p-benzoquinone (DBMIB) (32), as expected if it reflects electron flow from PSII through the luminal (Q\(_{L}\)) site of the cytochrome b\(_{6}\)f complex.
follow the redox changes of indicating that this wavelength may be used with confidence to attribution of the electrochromic band shift at this wavelength, cyt 2 and 3 can be taken as the signature of the oxidation of either oxidized redox carriers. The absorption changes shown in Figs. c6. The latter is known to act as a soluble electron carrier between the cyt b6f complex and PSI (32). As shown in Fig. 4, about 75% of the absorption changes were observed after the first flash in the series. This shows that a single charge separation at the level of PSI is sufficient to oxidize 75% of the c-type cytochrome. Thus, the pool of electron donor to P740 which gives rise to an absorption change at 554 nm is not in excess with respect to the PSI content. Since the soluble electron carriers, such as cyt c6 or plastocyanin, are usually found in an ~3:1 ratio with respect to PSI (see Ref. 36 for a review), we ascribe the 554 nm bleaching to cyt f and propose that plastocyanin, the redox changes of which are not associated with any significant absorption changes in this wavelength region, mediates electron transfer between PSI and the cyt b6f complex.

Ideally, this proposal should be backed up by the direct observation of absorption changes associated with plastocyanin redox changes. However, in the case of A. marina, the red-shifted oxidation spectrum of P740 (8, 38, 39) strongly overlaps with the oxidation spectrum of plastocyanin (the redox changes of which are commonly followed in the 810–870 nm region), making the direct observation of plastocyanin redox changes using optical spectroscopy difficult.

To circumvent this, reverse transcription-PCR was performed on total RNA samples from A. marina to test expression of the petE gene hypothetically coding for plastocyanin. We observed a band of the expected size (285 nt) when reverse transcriptase was added to the reaction and no product in the negative control (Fig. 5), suggesting that the petE gene is expressed under the present conditions and providing support for the presence of plastocyanin as a soluble carrier under these conditions. Similar experiments aimed at testing the expression of the two petf genes hypothetically coding for cytochrome c6 proved inconclusive (data not shown). Thus, cytochrome c6 cannot be strictly ruled out, but it would have to be present in largely substoichiometric amounts with respect to PSI to account for the fact that 75% of the absorption changes resulting from the complete oxidation of the cytochrome pool are obtained after the injection of a single oxidizing equivalent in the chain, as shown in Fig. 4.

**Redox Equilibration within the Electron Transfer Chain—** The fact that 75% of the oxidizing equivalents generated by PSI eventually end up on cyt f itself suggests that the equilibrium

The observation of a light-induced oxidation of one or more c-type cytochrome(s) raises the question of the identity of these oxidized redox carriers. The absorption changes shown in Figs. 2 and 3 can be taken as the signature of the oxidation of either cyt f alone or with a soluble cyt c6. The latter is known to act as a soluble electron carrier between the cyt b6f complex and PSI in some cyanobacteria (35) (see Ref. 36 for a review), and it cannot be ruled out as an electron carrier in A. marina, where two putative genes coding for cyt c6 are present in the genome sequence (37). On the other hand, a gene encoding for plastocyanin is also found in this cyanobacterium (37).

To determine the nature of the soluble electron carrier between the cyt b6f complex and PSI, we measured the progressive increase in the absorption changes associated with the c-type cytochrome oxidation, at 554 nm, during a series of 10 flashes (Fig. 4). Again, DBMIB was added to prevent the re-reduction of the cytochromes by plastoquinol (32). As shown in Fig. 4, about 75% of the absorption changes were observed after the first flash in the series. This shows that a single charge separation at the level of PSI is sufficient to oxidize 75% of the c-type cytochrome. Thus, the pool of electron donor to P740 which gives rise to an absorption change at 554 nm is not in excess with respect to the PSI content. Since the soluble electron carriers, such as cyt c6 or plastocyanin, are usually found in an ~3:1 ratio with respect to PSI (see Ref. 36 for a review), we ascribe the 554 nm bleaching to cyt f and propose that plastocyanin, the redox changes of which are not associated with any significant absorption changes in this wavelength region, mediates electron transfer between PSI and the cyt b6f complex.

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![Figure 3](image-url) Light-induced absorption changes between 500 and 570 nm. DBMIB (5 μM) was added to slow down signal recovery between each flash and allow integration of the absorption signal. Absorption was measured 100 μs and 10 ms after the last flash. The rather featureless difference between those two spectra (triangles) is attributed to electrochromic band shift.

![Figure 4](image-url) Relative amplitude of the signal at 554 nm, measured 10 ms after a saturating flash as a function of the number of actinic flashes during a series of 10 closely spaced (150 ms) saturating pulses. Conditions were the same as in Fig. 3. DBMIB was present at a concentration of 5 μM.
constant of the electron transfer reaction between these two redox cofactors is larger than one. This is at odds with the expectation that could be drawn from the difference in midpoint potential between the \( P_{740}^+ \) and \( P_{740} \) and \( \text{cyt f}^\circ \)/cyt f couples based on the results of Hu et al. (8).

By definition, the equilibrium constant \( K_{fp} \) between the \( P_{740}^+ / P_{740} \) and \( \text{cyt f}^\circ / \text{cyt f} \) couples is as follows,

\[
K_{fp} = \frac{[\text{cyt f}^\circ][P_{740}]}{[\text{cyt f}][P_{740}^\circ]} \tag{1}
\]

where \([\text{cyt f}]\), \([\text{cyt f}^\circ]\), \([P_{740}]\), and \([P_{740}^\circ]\) represent the concentration, at equilibrium, of the oxidized and reduced form of \( \text{cyt f} \) and \( P_{740} \). Thus, the equilibrium constant may be experimentally determined by assessing the relative amount of \( P_{740}^\circ \) for a given value of the relative amount of \( \text{cyt f}^\circ \). To do so, we measured the slow rereduction in the dark of both \( P_{740}^\circ \) and \( \text{cyt f}^\circ \) (generated by continuous illumination beforehand). As shown in Fig. 6, this illumination regime, in the presence of DBMIB, leads to the full oxidation of both cofactors. When the actinic light is switched off, they are slowly rereduced and return to their dark-adapted redox state within the second time range. Since this is more than 4 orders of magnitude slower than the overall electron transfer between \( P_{740}^\circ \) and \( \text{cyt f} \) (the latter is oxidized in the hundreds of microseconds time range; see Fig. 3), it can be reasonably assumed that the slow rereduction of both components occurs at equilibrium. Consequently, the relative amounts of oxidized \( \text{cyt f} \) and \( P_{740} \) at a given time can be derived from the kinetics shown in Fig. 6A and plotted one against another (Fig. 6B). Qualitatively, it can be seen that the reduction of \( \text{cyt f} \) is significantly slower than that of \( P_{740} \), leading to a difference of \( P_{740}^\circ \) indicating that when an electron is reinjected into the PSI donors chain, the probability that it reduces \( P_{740} \), the expected equilibrium constant would be \( K_{fp} \) rather than \( \text{cyt f}^\circ \) is \( \gg 1 \), consistent with the above conclusion that \( K_{fp} \) is larger than 1.

From Equation 1, the following relationship between the relative amount of oxidized \( P_{740} \) and of \( \text{cyt f} \) can be derived.

\[
y = \frac{K_{fp} \cdot x}{1 + x \cdot (K_{fp} - 1)} \tag{2}
\]

with

\[
y = \frac{\text{cyt f}^\circ}{\text{cyt f} + \text{cyt f}^\circ} \tag{3}
\]

and

\[
x = \frac{P_{740}^\circ}{P_{740}^\circ + P_{740}} \tag{4}
\]

As shown in Fig. 6, the data could be satisfactorily fitted with this function, and the fit yielded \( K_{fp} = 15 \pm 1 \), leading to a difference between the midpoint potentials of the \( P_{740}^+ / P_{740} \) and \( \text{cyt f}^\circ / \text{cyt f} \) couples of ~70 mV. Taking a value of 345 mV for the latter (9–11) and 335 mV for the former (8), the expected equilibrium constant would be ~0.8 (see dashed line in Fig. 6). Conversely, the value estimated by Hu et al. (8) for the \( P_{740}^+ / P_{740} \) midpoint potential combined with \( K_{fp} = 15 \) would lead to a midpoint potential for the \( \text{cyt f}^\circ / \text{cyt f} \) couple of ~265 mV (i.e. downshifted by about 100 mV with respect to Chl a-containing organisms). Although such a variation in the midpoint potential of a given cofactor would not be unprecedented, as illustrated by the down-shifted midpoint potential of the cytochrome \( b_{14} \).
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Energetics of PSI in A. marina—Our analyses of the kinetics and thermodynamic properties of the electron flow chain in A. marina reveals a scenario that is very similar to that found in Chl a-containing organisms. Indeed, despite the large displacement in the overall absorption spectrum, resulting in an overall decrease in the energy content of the photons absorbed by both PSI and PSII, photosynthetic electron flow takes place very efficiently in this cyanobacterium, where the rate of the intersystem electron transfer is very similar in both Chl a- and Chl d-based organisms, as required for an efficient oxidation of the plastoquinone pool. At first sight this seems to be a surprising finding given that there is a significant difference between the ground and excited states of the absorbing pigments. Yet, this does not necessarily translate into a decreased driving force for electron transfer, since the primary electron donor in A. marina could be a Chl a rather than a Chl d. Such a hypothesis would be in line with the combined proposals that (i) the primary electron donor in Chl a containing PSI is not the Chl a heterodimer of P700 but an accessory Chl a (ec2) monomer (43, 44) and (ii) in A. marina PSI, at least one of the two accessory Chls involved in charge separation has an absorption maximum at 680 nm, suggesting that it is a Chl a (45). It is of note, however, that Kumazaki et al. (45) assigned the bleaching they observed at 680 nm, which is formed and decays with time constants of ~6 and 50 ps, respectively, to the primary electron acceptor rather than to the primary electron donor as we consider here.

Clearly, the hypothesis that Chl a is involved in primary charge separation in PSI of A. marina would require a thermally activated transfer of the exciton between the light-harvesting Chl d and the photochemical trap. Although a hint pointing to such an activated excitonic transfer can be found in the ultrafast studies by Kumazaki et al. (45), who reported a rather slow exciton trapping time (~50 ps), the mere observation that PSI from A. marina is still photochemically active at 77 K (39) is at odds with a significant difference in energy level between the excited states of the primary electron donor and of the antenna Chls.

Thus, an alternative hypothesis would be that either the reducing power (i.e. the midpoint potential of the P+/P0 couple, where P denotes the primary electron donor without any assumption as to whether it is either one of the two P740 (ec1) or accessory (ec2) Chls) or the oxidizing power (i.e. the midpoint potential of the P/P+ couple) is decreased.

In a survey of the available data on type I reaction centers from a variety of organisms, including A. marina, Itoh et al. (46) proposed that the reducing power of the acceptor side of type I reaction centers is conserved in all of the photosynthetic organisms (green sulfur bacteria, heliobacteria, and oxygenic organisms in general). According to these authors, the requirement for preserving the reducing power of the P+ state, despite different transition energies between the ground and excited states of their primary Chl electron donors, stems from the necessity to optimize the primary and secondary electron transfer reactions. Thus, any change in the energy of the exciton that drives the primary photochemistry would require a compensatory change of the Em of the primary electron donors to the reaction center in order to preserve this optimization.

This proposal is supported, at first sight, by both green sulfur bacteria and heliobacteria, in which the decreased Em of the primary donor (the midpoint potential of the Chl dimer corresponding to P700 or P740) is down-shifted by ~200 mV (for reviews, see Refs. 47 and 48), which matches the decrease in energy supply to PSI photochemistry (in the case of green sulfur bacteria, the absorbed photon is at ~800 nm; i.e. ~250 mV lower than for a 700 nm photon). However, the overall photosynthetic electron transfer chain from either of these strictly anaerobic photosynthetic bacteria differs from those from Chl...
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a and probably also from Chl d containing oxygenic phototrophs in several respects beyond the nature of the Chls involved in electron transfer. Indeed, the quinone serving as the electron donor to the cytochrome b6/f complex in these anaerobes is a menaquinone-9 and not a plastoquinone. The midpoint potential of the MKH2/MK couple is more negative than that of the PQH2/PQ couple, which acts as the electron carrier between PSII and the cyt b6/f complex in oxygenic photosynthetic chain. Although the available reducing power for electron transfer from the quinol is thus larger, it is remarkable that not only the midpoint potential of the P+/P couple but also those of the cyt c, b6/f, and b6/f complex, as discussed in H. mobilis, are larger than the free energy change of 500 meV associated with the formation of the P+P complex to drive the Q-cycle. Accordingly, the lack of changes in the E_m of P740 and cyt f observed in A. marina on the one side and the cyt b6/f complex to drive the Q-cycle. Accordingly, the lack of changes in the E_m of P740 and cyt f observed in A. marina on the one side and the down-shifted midpoint potential of the Chl dimer in type I reaction centers from heliobacteria and green sulfur bacteria can both be explained in terms of the stronger reducing power of menaquinol, with respect to plastoquinol, rather than by the lower excitonic load that drives the photosynthetic chain in these organisms.

Electron Flow Is Not Restrained by Diffusion-limiting Domains in A. marina—A corollary to our characterization of electron flow in A. marina is the finding that the experimentally determined equilibrium constant, K_fP—theor, expected from the midpoint potentials of these two cofactors. Although one would expect such an agreement to prevail, this is, to our knowledge, the first reported case where it is met and, as we will now briefly discuss, it may be a reflection of significant ultrastructural differences between the supramolecular organization of the photosynthetic chain in thylakoids from vascular plants and cyanobacteria. The difference between K_fP—theor and K_fP—theor has been interpreted as reflecting the existence of plastocyanin (PC) diffusion domains, with different stoichiometries of PSI, PC, and cyt b6/f complexes. These domains have been tentatively suggested to be the different chloroplast compartments previously identified by Albertsson (52, 53), namely the grana stacks, the end and margin membranes, and the stroma lamellae. Within this structurally based model, the rationale for the discrepancy between K_fP—theor and K_fP—theor is the following. Due to even stoichiometry of the different membrane complexes, P700 can be photo-oxidized in the domains with a high P700/(cyt f + PC) ratio, well before cyt f oxidation is fully completed in the other domains with a low P700/(cyt f + PC) ratio. In this case, coexistence of cyt f and P700 is observed when the redox state of the two cofactors is probed simultaneously in all of the domains, resulting in the estimation of a low K_fP—theor (e.g., see Refs. 54–56 and also Ref. 57).

In the case of the A. marina cells used in the present study, the thylakoids do not show the presence of any grana and lamella domains (58). Thus, the good agreement between K_fP—theor and
exp and $K_{f}\theta$ observed in this organism supports the hypothesis of a correlation between the presence of confined diffusion domains and the macroscopic differentiation of the thylakoid membranes in plants and green algae.

In such a homogeneous system, one could expect PSI and PSII to coexist in the same domains. However, the fluorescence induction curves in the presence of DCMU show both a high $F/F_m$ and a sigmoidal shape, indicating that, on the one hand the probability for energy transfer between PSII units is significant and that on the other hand there is little excitonic transfer between PSI and PSII. Altogether, these data suggest that PSII and PSI are present in the same membrane domains but do not share their antenna, raising the issue of the segregation between the two PS types. Previous analysis of the cyanobacterium Synechocystis 6714 provides a structural answer to this question. Freeze-fracture pictures of thylakoid membranes frequently show rows of EF particles (e.g. see Ref. 59), which are attributed to an organized arrangement of PSI complexes in the membranes. Clearly, a similar situation may take place in A. marina, as suggested by the ultrastructural data, which show this photosystem to be arranged as a dimer within a giant complex surrounded by light-harvesting Pcb proteins (60), a feature that may extend to even larger aggregates of PSII within the membrane (5), thereby accounting for the energetic segregation between PSI and PSII in an otherwise freely diffusing system.

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REFERENCES

26.以前に豆科植物のサツニレも、Asparagalesで見られることが示唆されている。したがって、このような系においても同様の状況が起こることが考えられる。
The Photosynthetic Chain of A. marina