The cytochrome $b_{6f}$ complex of oxygenic photosynthesis carries out "dark reactions" of electron transfer that link the light-driven reactions of the reaction centers, and coupled proton transfer that generates part of the electron potential utilized for ATP synthesis. In contrast to the $bc_1$ complex of the respiratory chain, with which there are many structural and functional homologies, the $b_{6f}$ complex contains bound pigment molecules. Along with the specifically bound chlorophyll $a$ previously found to be bound stoichiometrically in the dimeric $b_{6f}$ complex, it was found in the present study that $b$-carotene is also present in the $b_{6f}$ complex at stoichiometric levels or nearly so. Chlorophyll and carotenoid pigments were quantitatively extracted from $b_{6f}$ complex purified from (i) the thermophilic cyanobacterium, *Mastigocladus laminosus*, (ii) spinach chloroplasts, and (iii) the green alga, *Chlamydomonas reinhardtii*. Visible and mass spectra showed the carotenoid to be a $b$-carotene of molecular weight = 536, with a stoichiometry of 1:0:1 relative to cytochrome $f$ in the highly active *M. laminosus* complex but somewhat lower stoichiometries, 0.77 and 0.55, in the $b_{6f}$ complex obtained from spinach chloroplasts and *C. reinhardtii*. A photoprotective function for the $b$-carotene was inferred from the findings that the rate of photobleaching of the chlorophyll $a$ bound in the complex was found to vary inversely with $b$-carotene content and to decrease markedly in the presence of ambient $N_2$ instead of air. The presence of $b$-carotene in the $b_{6f}$ complex, and not in the related $bc_1$ complexes of the mitochondrial respiratory chain and photosynthetic bacteria, suggests that an additional function is to protect the protein complexes in oxygenic photosynthetic membranes against toxic effects of intramembrane singlet $O_2$.

Elucidation of the structural basis of energy transduction in the cytochrome $bc$ complexes is proceeding rapidly. High resolution structure data from x-ray diffraction have been obtained for the 11 subunits of the intact cytochrome $bc_1$ complex from the bovine respiratory chain (1–3). For the cytochrome $b_{6f}$ complex, atomic structures (<2.0-Å resolution) have been obtained for the luminal side domains of cytochrome $f$(4–5) and the Rieske iron-sulfur protein (6). It is clear that along with many similarities between the $b_{6f}$ and $bc_1$ complexes (7), there are significant differences in structure, now most obvious in the comparison of the structures of cytochromes $f$ and $c_1$ (Refs. 2 and 3 versus 4). The presence of stoichiometrically bound pigment molecules in the cytochrome $b_{6f}$ complex, discussed in the present work, is another contrasting aspect.

The electronic connection between the reducing and oxidizing sides of photosystems II and I, provided by the cytochrome $b_{6f}$ complex, through which the proton electrochemical potential is generated, is traditionally considered part of the "dark" reactions of oxygenic photosynthesis (8). Thus, the finding of a molecule of the light-absorbing pigment, chlorophyll $a$, to be present at an approximate 1:1 stoichiometry in active cytochrome $b_{6f}$ complex after exhaustive chromatography, was unexpected (9). In addition to the unit stoichiometry, a specific binding site for the chlorophyll molecule in the complex was implied by the dichroism of the bound chlorophyll $a$ in oriented monomeric $b_{6f}$ complex isolated without the Rieske protein from the cyanobacterium, *Synechocystis* sp. PCC 6803 (10). A specific binding site of a chlorophyll $a$ in the complex from the green alga, *Chlamydomonas reinhardtii*, was implied by spectroscopic identification of a specific hydrogen-bonding mode of the chlorophyll and slow rates of exchange of the $b_{6f}$-bound Chl$^+$ with $^3$[H]Chl in detergent micelles (11).

The conclusion that the chlorophyll $a$ molecule is bound at a specific site in the $b_{6f}$ complex raises the questions of where the two chlorophyll molecules per dimer are located and the functional role of the pigment molecules in the complex. It has been proposed that the function of the chlorophyll $a$ may be to stabilize the dimeric form of the $b_{6f}$ complex (12). The presence of a chlorophyll molecule also implies that there should be a neighboring carotenoid to quench the chlorophyll triplet state that, otherwise, will inevitably lead to photodamage through formation of singlet oxygen (13). It is reported in the present study that $b$-carotene is present in the cytochrome $b_{6f}$ complex of the cyanobacterium, *Mastigocladus laminosus*, at a unit stoichiometry relative to cytochrome $f$, approximately equivalent to that of the bound chlorophyll $a$. The Chl $a$ in the *M. laminosus* $b_{6f}$ complex, compared with the complex isolated from *C. reinhardtii* with half the $b$-carotene content, is twice as resistant to bleaching by actinic light, which was found to be $O_2$-dependent.

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1 The abbreviations used are: Chl, chlorophyll; Car, carotenoid; $^1$Car$^*$ or $^3$Car$^*$, first excited singlet or triplet state of carotenoid; $^1$Chl$^*$, first excited triplet state of chlorophyll; cyt, cytochrome; c, dielectric constant; $e_{	ext{max}}$, millimolar extinction coefficient; HPLC, high performance liquid chromatography; LHC, light-harvesting chlorophyll pigment protein; PAGE, polyacrylamide gel electrophoresis; PC, plastocyanin; PS, photosystem; μE, microeinsteins; MOPS, 4-morpholinepropanesulfonic acid; $^3$O$_2$, singlet $O_2$.
\(\text{\beta-Carotene in Cytochrome b}_6^f\) Complex

**Materials and Methods**

Isolation and Purification of Cytochrome b\(_6^f\) Complex

The b\(_6^f\) complex was purified from spinach chloroplasts and \(C.\) reinhardtii according to the procedures described previously (9) and from cyanobacteria as described (14). The differences in the latter procedure are the use of a propyl-agarose column before the sucrose gradient and of dodecylmaltoside in the sucrose gradient step.

Determination of Chlorophyll and Carotenoid Concentration

**Pigment Extraction**—Pigments were extracted with 80% acetone, 20% methanol at 4 °C under dim light (15), vortexed (1 min), sonicated (2 min), and centrifuged at 5,000 × g (10 min). The supernatant was decanted and filtered through a Millipore SLCR L04 NS (Millipore) 0.5-μm filter. A second extraction was carried out with 80% acetone, 20% methanol to ensure complete extraction. Because of the prominence of the spectrum of the bound chlorophyll \(a\), \(\varepsilon_{400} = 79\) for the absorbance peak at 669 nm (16), the efficiency of extraction based on the absorbance of first and second extracts could be determined to be >98%. The second extract was colorless by visual inspection.

**HPLC Separation**—The pigments were resolved on HPLC following procedures in (17), using a Microsorb-MV C-18 column (Raimin Instruments ODS (4.6 mm, inner diameter × 25 cm)), and eluted isocratically (0–4 min) with acetonitrile/Tris-HCl (0.1 M, pH 8.0, 80:3 (v/v), and then by a 4.5-min linear gradient to methanol:hexane, 4:1 (v/v). All solvents were filtered through GH-Polyprop 0.2-μm filters (Gelman Sciences). Sample injections were 20 μl, the flow-rate for all separations was 2 ml/min, and the eluent was monitored at 440 nm.

**Pigment Identification and Calibration**—Pigments were identified by UV-visible spectrophotometry and mass spectrometry. For visible excitation and emission wavelengths, for measurements of emission spectra, respectively, were 440 and 676 nm, with bandwidths of 4 and 8 nm, respectively.

**Photobleaching of Chl a in the cyt b\(_6^f\) Complex**

A stirred 1-ml cyt b\(_6^f\) sample, ~1 μM cyt f in 25 mM Tris-HCl, pH 7.5, 1 mM MgCl\(_2\), 0.02% \(\beta\)-dodecyl maltoside, was illuminated with 1.6 × 10\(^6\) E/m\(^2\) of white light filtered through a 12.5-cm 1% CuSO\(_4\) solution as a heat filter. 0.2 ml of sample were taken at different time intervals and diluted 5-fold into the same buffer. The chlorophyll a visible spectrum was measured with a Cary 3 UV-visible spectrophotometer. The photobleaching was measured in the presence and relative absence of air, in the latter case by de-aerating the stirred sample on ice for 2 h with \(N_2\) gas, adding the b\(_6^f\) complex to the degassed buffer with a syringe, and then flushing the air space above the sample with \(N_2\) gas before and during illumination.

**Circular Dichroism Spectra**

Circular dichroism (CD) spectra were measured on a JASCO J600 spectropolarimeter. Spectral parameters were as follows: sampling, 0.2-nm wavelength increments; time constant, 2 s; spectral half-bandwidth, 2 nm; optical path length, 1 cm; spectra, average of 4 scans after subtraction of the buffer blank. Cyt b\(_6^f\) (12.5 μM from spinach thylakoid membranes) was suspended in 20 mM MOPS, pH 7.2, 50 mM NaCl, and 20 mM octyl-\(\beta\)-D-glucoside.

**Characterization of the Cytochrome b\(_6^f\) Complex**—The cytochrome b\(_6^f\) complex contains the four “large” petA–D gene products, cytochrome f, cyt b\(_6^f\), the Rieske protein, and subunit IV, seen in the first dimension SDS-PAGE (9, 20) and three “small” polypeptides, the petG, M, and L hydrophobic polypeptides (21–24), separated on SDS-PAGE in the second dimension after blue native gel electrophoresis (8). The turnover of the chloroplast b\(_6^f\) complex used in the present work was approximately 100, 150, and 450 electrons transferred from decylplastoquinol to plastocyanin-ferricyanide per cyt f per second at 25 and 28 °C, respectively, for the complex from spinach chloroplasts (9), \(C.\) reinhardtii and the thermophilic cyanobacterium, \(M.\) laminosus.

The ratio of f to b heme in the complex from spinach chloroplasts (Fig. 1A) \(C.\) reinhardtii or \(M.\) laminosus (not shown) was determined from the amplitudes in the cytochrome a-band region of the chemical difference spectra, ascorbate minus ferri-cyanide for cyt f (Fig. 1A, a) and dithionite minus ascorbate for cytochrome b\(_6^f\) (Fig. 1A, b), whose ratio was 1:2. The concentration of cytochrome f and the complex were determined using the extinction coefficient, \(\varepsilon_{400} = 26\) (19). Although it has not been separately determined, we infer from the ratio of amplitudes and from the known structural requirement for two b hemes in bc complexes (1–3) that their average extinction coefficient is also approximately 24 mm\(^2\) cm\(^{-1}\) (19).

**Pigment Content of the Cytochrome b\(_6^f\) Complex**—The presence of carotenoid, as well as the chlorophyll a already noted and characterized (9–11, 25), can be seen in the absolute absorbance spectrum of the complex from spinach chloroplasts (Fig. 1B). An essentially identical spectrum was obtained for the complex isolated from \(M.\) laminosus (not shown). Extraction of all pigments from the b\(_6^f\) complex was carried out using 80% acetone, 20% methanol at 4 °C under dim light. Total extraction (~98%) was determined as described under “Materials and Methods.” The pigments from the chloroplast (Fig. 2A), \(M.\) laminosus (Fig. 2B), and \(C.\) reinhardtii (not shown) b\(_6^f\) complex were separated into two major components by reverse phase HPLC in hexane: methanol, 4:1, detected by their absorbance at 440 nm, whose visible and mass spectra are diagnostic of chlorophyll a and \(\beta\)-carotene. The visible absorbance spectra of the two HPLC peaks from the chloroplast b\(_6^f\) complex (Fig. 3) are characteristic, respectively, of \(\beta\)-carotene (Fig. 3A; Ref. 26) and chlorophyll a (Fig. 3B). Essentially identical spectra were obtained for the HPLC-separated (Fig. 2B) pigment compo-
The spectral bands associated with the presence of carotenoid and chlorophyll (not shown). Through the features of the major and minor peak positions at 434 and 473 nm, the spectrum for β-carotene (Fig. 2A) most closely resembles a 9-cis or 15-cis β-carotene (26). The mass of the isolated pigments was determined by fast atom bombardment ionization mass spectrometry to be 892.5421 and 536.4371, respectively (19) yields a value of 9-cis or 15-cis-β-carotene (26).

The concentration of the pigment components separated by HPLC was determined using the areas under the peaks, from addition of known concentrations of pigment standards, and by collecting and drying each of the two components, redissolving them in methanol (Chl) and hexane (β-carotene), and determining their spectra, checked through the extinction coefficients (ε_{max} (A_{665-750})) of 71.4 mmol^{-1} cm^{-1} for chlorophyll a in methanol and 134 mmol^{-1} cm^{-1} at 445 nm for β-carotene in 100% hexane. Using the differential extinction coefficient, Δε_{max} = 26 mmol^{-1} cm^{-1}, for cytochrome f, the stoichiometry of the chlorophyll a and β-carotene relative to cytochrome f is 0.97 ± 0.13 and 0.77 ± 0.07, respectively, in the chloroplast complex (Table 1, spinach chloroplasts), 1.65 ± 0.3 and 1.02 ± 0.1 in that from M. laminosus (Table 1), and 1.37 ± 0.3 and 0.55 ± 0.08 in C. reinhardtii. Recalculation of the data of Huang et al. (9) and Pierre et al. (11), using the recently redetermined Δε_{max} = 26 mmol^{-1} cm^{-1} for cyto f yields values of the Chl a/cyt f ratio of 1–1.2 and 1.1–1.6, respectively. This eliminates any problem of the significance of a stoichiometry smaller than 1:1 (25), but raises the possibility of either a second bound chlorophyll a or a small amount of nonspecifically bound chlorophyll. The latter is a crucial issue in studies of the fluorescence properties of the specifically bound chlorophyll a. Recalculation of the β-carotene/cyt f stoichiometry previously reported (11) using the cyt f extinction coefficient determined previously (19) yields a value of 0.38–0.55, close to the value found in the present study.

**Possible Inter-pigment Interactions**—Because the fluo...
cence yield and lifetime of β-carotene are small (27), these two pigments have to be located within a small distance of each other for energy transfer from β-carotene to the chlorophyll a to occur. The excitation spectrum for chlorophyll a fluorescence at 676 nm from the b_{6f} complex shows a broad band of small amplitude centered near 490 nm (Fig. 1B, inset, spectrum (b)). This band is absent in a solution mixture of chlorophyll a and β-carotene (not shown). Such a band was absent in the excitation spectrum for chlorophyll a fluorescence at low temperature (77 K) for the complex from the green alga C. reinhardtii (11).

In the latter work, the issue of a carotenoid excitation band was not specifically addressed because the carotenoid content was found to be less than stoichiometric.

Photobleaching of the Chlorophyll a, Dependence on β-Carotene Content—The existence of three preparations from different sources that have different contents and stoichiometries of β-carotene to cytochrome f creates the possibility of testing the hypothesis that one of the functions of the β-carotene, as in the photosynthetic antenna proteins, is to protect the chlorophyll a from photodamage. Such damage can arise from production of chemically reactive singlet oxygen because of the interaction of ground state triplet oxygen with the excited state (*) chlorophyll triplet (13) shown.

\[
{^3}\text{Chl}^* + {^3}\text{O}_2 \rightarrow {^3}\text{Chl} + {^3}\text{O}_2^*
\]

**REACTION 1**

β-carotene (Car) can serve as an alternative acceptor of the excited state chlorophyll triplet, preventing the production of excited singlet oxygen, \( {^1}\text{O}_2^* \).

\[
{^3}\text{Chl}^* + \text{Car} \rightarrow {^3}\text{Chl} + {^3}\text{Car}^*
\]

**REACTION 2**

Significant photobleaching of the Chl a in the C. reinhardtii b_{6f} complex could be observed after a 10-min exposure to heat-filtered white light with an intensity of \( 2.7 \times 10^5 \mu\text{E/m}^2\text{s} \) (Fig. 4A, b, dashed spectrum, compared with the spectrum of unilluminated sample, Fig. 4A, a, solid curve). The residual spectrum in the 400–450 nm region (Fig. 4A, b, dashed spectrum) arises mostly from the Soret bands of cytochrome f and the two hemes of cytochrome b_{6f} and explains why the amplitude of the β-carotene near 500 nm appears relatively small compared with that in the Soret region arising from one-two chlorophylls and three hemes from cytochromes b_{6f} and f (Fig. 1B).

The time course of the bleaching of the Chl a in C. reinhardtii is shown (Fig. 4B, curve c). Also shown in Fig. 4B is the time course of the bleaching of the Chl a in the b_{6f} complex of M. laminosus (curve a) and spinach chloroplasts (curve b). It can be seen that the bleaching of Chl a in the M. laminosus is very small. The residual absorbance of the 676 nm Chl a band, after the 10-min illumination (\( 1.7 \times 10^5 \mu\text{E/m}^2\text{s} \)) was 92 ± 2%, 70 ± 5%, and 46 ± 10% (n = 3), respectively, for the complex from M. laminosus, spinach chloroplasts, and C. reinhardtii (Fig. 4B). The relative magnitude of this residual absorbance for the Chl a from the b_{6f} complex from these three sources was 1.0, 0.76, 0.50, almost proportional to the respective stoichiometries of β-carotene to cytochrome f (Table I). The bleaching of the Chl a band in the C. reinhardtii complex was reduced substantially, the residual absorbance increasing from 47 ± 8 to 74 ± 4% (n = 3) when the sample was equilibrated with N\(_2\) instead of air before exposure to the actinic light (Fig. 4B, ■, linked by arrow, showing the change in photobleaching of the b_{6f} complex from C. reinhardtii).

Circular Dichroism Spectra Show β-Carotene Is Bound in an Asymmetric Environment—Carotenoids in a symmetric environment in organic solvent show no optical activity (26). CD spectra of β-carotene in the spinach chloroplast b_{6f} complex have minima at 430, 457, and 485 nm (Fig. 5), reasonably close to the absorbance peaks at 422, 451, and 478 nm for β-carotene in 100% hexane (Fig. 3A). In a somewhat more polar solvent, 80% acetone, these peaks are red-shifted to 429, 456, and 483 nm, very close to the extrema in the CD spectrum. The CD spectra are indicative of an asymmetric binding environment of the β-carotene in the complex. The ratio of the ellipticity peak of the β-carotene at 457 nm to that of the Chl a at 670 nm is 4.6:1. This ratio in the CP43 light-harvesting complex, which contains chlorophyll a and β-carotene in a ratio of 4:1, is 1:1.

![Image](310x358 to 552x729)
in the present work. The extent of the residual absorbance of the chlorophyll a molecule in the cytochrome \( b_{6f} \) complex after photobleaching (Figs. 4, A and B) correlates well with the stoichiometry of \( \beta \)-carotene relative to cytochrome \( f \) (Fig. 4B, a–c, \( \beta \)-carotene:cyt \( f \) = 1.0, 0.77, 0.55), and is markedly dependent upon the ambient \( O_2 \) concentration (Fig. 4B). One \( \beta \)-carotene per monomer of \( b_{6f} \) complex confers virtually complete protection against photodamage to the 1–2 Chl \( a \) molecules bound per monomer. It is inferred that the lower content of \( \beta \)-carotene in the complexes isolated from spinach chloroplast and \( C. \) reinhardtii is an artifact of preparation.

The mechanism of protection by \( \beta \)-carotene is inferred to be triplet transfer from the excited state of the chlorophyll to the \( \beta \)-carotene. However, triplet transfer to carotenoid was not found in the \( b_{6f} \) complex of the cyanobacterium, \( Synechocystis \) sp. PCC 6803 (36). From the precedents of loss of chlorophyll upon monomerization or dissociation of the Rieske protein (12) and of diminished content of \( \beta \)-carotene in the \( C. \) reinhardtii complex (Table I), it is possible that the measurements of triplet transfer to \( \beta \)-carotene in Peterman et al. (36) were made difficult by a lowered content of \( \beta \)-carotene and chlorophyll \( a \) that is a consequence of the loss of the Rieske protein in this preparation, which results in complete loss of activity. Preliminary experiments designed to detect by EPR the presence of a \( \beta \)-carotene triplet in the \( b_{6f} \) complex following laser excitation of the chlorophyll \( a \) did not reveal any component that could be unambiguously assigned to a carotenoid triplet with a decay time in the expected 0.5–10 μs time range.²

Location of \( b_{6f} \)-bound Pigments—The bound chlorophyll \( a \) molecule is inferred to be located close to the path of electron and/or proton transfer to cytochrome \( f \), from the correspondence of the rates of reduction of cytochrome \( f \) and the rise time of an electrochromic band shift of a chlorophyll \( a \) (37). This is consistent with the tendency of the chlorophyll molecules in the LHClI protein structure to be localized near the membrane interfacial domain (38). The LHClI structure also provides a precedent for glutamate residues serving as chlorophyll ligands. The carboxylate residue on the lumen side of the small hydrophobic polG, L, and M polypeptides could provide such a residue as a ligand for the \( b_{6f} \)-bound chlorophyll. These polypeptides are uniquely present, along with the bound pigments, in the cytochrome \( b_{6f} \) compared with the \( bc_1 \) complex. It has been proposed that the Chl-specific binding site is associated with the cytochrome \( b_6 \) polypeptide (39). However, because the stoichiometry of this association was approximately only 10%, the association with cyt \( b_6 \) might be a consequence of preferential and adventitious binding of the chlorophyll with the most hydrophobic polypeptide of the complex. From the present studies on the role of \( \beta \)-carotene in protecting the Chl \( a \) in the \( b_{6f} \) complex from \( O_2 \)-dependent photobleaching, it is inferred that the \( \beta \)-carotene is positioned close to the chlorophyll \( a \) and the membrane polar interface (Fig. 6).

Differences between \( b_{6f} \) and \( bc_1 \) Complex, Significance of Absence of Pigments in the Photosynthetic \( bc_1 \) Complex—The cytochrome \( b_{6f} \) complex is similar in many respects to the \( bc_1 \) complex of the mitochondrial respiratory chain and photosynthetic bacteria, to which it is known to be related in many structure-function aspects. The possibility of major differences between the complexes has recently become clear with the discovery that cytochromes \( f \) and \( c_1 \) are completely dissimilar proteins, and provide an excellent example of divergent functional evolution in membrane proteins.

A major role for oxygen scavenging in oxygenic photosyn-

² P. D. Laible, H. Zhang, M. C. Thurnauer, and W. A. Cramer, unpublished data.
The effective diffusion constant of O2 in the membrane would be of viscosities, 0.5–5 poise, of biological membranes (42). The thylakoid membrane is assumed to be at the lower end of the range of viscosities (40). A quinone, chlorophyll, and carotenoid are shown in the bilayer region. The position of the chlorophyll a shown in the excited triplet state that is believed to be quenched by β-carotene and the β-carotene ring near the interface is based on the precedent for the position of Chl a molecules in LHCl (38) and kinetic correlation of an electrochromic shift of the Chl a with cyt f reduction (37).

We infer that the oxygen produced in the PSII region of the membrane will readily diffuse because it is highly soluble in the non-polar membrane and must be present, perhaps at a somewhat lower concentration, in the region of other thylakoid membrane-bound protein complexes (Fig. 6). Here it can also inflict similar molecular damage to lipids and protein. The range of action of the 1O2 depends upon its lifetime and diffusion rate in the membrane. The lifetime of 1O2 in dioxane, whose dielectric constant (ε = 2.2) is similar to that of the lipid bilayer core, is 30 μs and is not very dependent on solvent polarity (40). If the viscosity of the relatively fluid photosynthetic membrane is assumed to be at the lower end of the range of viscosities, 0.5–5 poise, of biological membranes (42), the effective diffusion constant of O2 in the membrane would be ~10−7 cm2 s−1. The root mean square distance of O2 diffusion from its site of generation in PSII, where the O2 concentration is highest, is then ~350 Å. This distance is larger than the average distance between the PSII reaction center and the b6f complex, which has been estimated to be 200 Å (43).

The data in the present study imply that one function of β-carotene in the b6f complex is to prevent generation of singlet O2 from the photoexcited Chl a in the complex. The role of the chlorophyll itself, presumably structural, is still unclear. It is suggested that it is also needed to exert a protective role against the toxicity of photosynthetically produced O2 toward the cytochrome b6f complex. One may suggest that all protein complexes within an effective diffusion distance of PSII in the oxygenic photosynthetic membrane may require such protection.

The bound chlorophyll and β-carotene may be “evolutionary relics” that resulted from the appearance of reaction centers prior to that of quinol oxidoreductases (11). However, there is no report of bound bacteriochlorophyll or carotenoid in the cyt b6f complex isolated from photosynthetic bacteria. Therefore, it seems likely that these “relics” are utilized functionally in the oxygenic photosynthetic membrane. In the case of carotenoids, these pigment molecules are synthesized not only to assist in light harvesting but also as a “bonus” (44) to cope with the problem of oxygen toxicity and structure stabilization in both the light-dependent and “dark” integral membrane protein complexes in the membranes of oxygenic photosynthesis.

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FIG. 6. Schematic of cytochrome b6f complex, assuming a total of 12 transmembrane helices/monomer (4, cyt b6f (pink); 3, suIV (yellow); 1, cyt f (green); 1, Rieske iron-sulfur protein (red); pet G, M, and L (brown)). Approximate shapes and sizes of lumen-side extrinsic domains of cyt f and Rieske [2Fe-2S] protein are from solved structures (4, 6). A quinone, chlorophyll, and carotenoid are shown in the bilayer region. The position of the chlorophyll a shown in the excited triplet state that is believed to be quenched by β-carotene and the β-carotene ring near the interface is based on the precedent for the position of Chl a molecules in LHCl (38) and kinetic correlation of an electrochromic shift of the Chl a with cyt f reduction (37).
