# Light-Induced Changes in the Chemical Bond Structure of Light-Harvesting Complex II Probed by FTIR Spectroscopy†

Hans Rogl,*,‡ Werner Kühlbrandt,‡ and Andreas Barth§

*Max-Planck-Institut für Biophysik, Heinrich-Hoffmann-Strasse 7, D-60528 Frankfurt am Main, and Institut für Biophysik, Johann Wolfgang Goethe Universität, Theodor Stern Kai 7, Haus 74, D-60590 Frankfurt am Main, Germany*

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ABSTRACT: Light-harvesting complex II (LHC-II) regulates the light energy distribution between photosystem I and II in plants. This process is mediated by phosphorylation of the LHC-II protein, which depends on the oxidation state of photosynthetic electron carriers. In addition to this regulatory mechanism, it has recently been proposed that light can directly induce a conformational change in isolated LHC-II. To provide biophysical evidence for such a conformational change in the protein, we studied infrared absorbance changes in isolated LHC-II upon exposure to light flashes. Compared to the signals obtained with other proteins that exhibit well-characterized conformational changes, the signal in the LHC-II difference spectra is very weak. The position of the difference bands coincides with the main IR absorption bands of chlorophyll. We conclude that there are no detectable light-induced changes in the LHC protein structure and attribute the observed IR signals to light-induced chlorophyll degradation.

The light-harvesting complex of photosystem II (LHC-II)[1] is a trimeric, integral membrane pigment−protein complex in the thylakoid membrane of plant chloroplasts. The LHC-II monomer consists of a 25 kDa polypeptide which binds a minimum of seven chlorophyll (Chl) *a* and five Chl *b* molecules, and several different carotenoids, including two luteins and one neoxanthin (*1*). It is known that two different lipids are specifically associated with the trimeric complex (*2*). LHC-II is probably the most abundant membrane protein in plants. Its main function is to collect solar energy and to transmit it to the photosynthetic reaction centers, thus increasing their effective absorption cross section. This function is subject to well-controlled regulatory processes, as an excess uptake of light energy may damage the reaction centers which carry out charge separation and (in the case of photosystem II) water oxidation, the essential light reactions of photosynthesis. A certain pool of LHC-II is tightly associated with photosystem II, but another fraction moves between the two photosystems, thereby regulating the distribution of light energy between them (*3*, *4*). This process is mediated by LHC-II phosphorylation which in turn depends on the oxidation state of the plastoquinone pool. The cytochrome $b_6f$ complex senses the oxidation state of the plastoquinone pool and presumably regulates the activity of the LHC-II kinase (*5*, *6*). Recently, it has been postulated that an additional factor plays an important role in enhancing the adaptation of the photosynthetic apparatus to different light regimes. Upon illumination, the conformation of LHC-II changes; it becomes a better substrate for the kinase (*7*) and is phosphorylated more easily. Obviously, this mechanism requires a light-induced change in the three-dimensional protein conformation. We employed Fourier transform infrared (FTIR) difference spectroscopy to investigate the suggested light-induced changes in the LHC-II structure.

Infrared spectroscopy is based on the absorption of radiation when the frequency of incident infrared light coincides with that of a molecular vibration. The vibrational frequencies depend on the structure of the molecule and its environment. Therefore, infrared spectroscopy is a sensitive monitor of structural changes. Changes in protein backbone conformation and in the backbone hydrogen bonding pattern as well as environmental or protonation changes of amino acid side chains can be detected at the level of individual amino acid residues (*8*−*13*). This is also true for cofactors such as chlorophyll and bacteriochlorophyll, whose infrared spectra are sensitive to hydrogen bonding, redox state, and aggregation (*14*, *15*, *22*, *26*). Reaction-induced infrared difference spectroscopy is used to increase the sensitivity so that conformational changes can be detected at a single-amino acid level. The reaction is triggered in the infrared cuvette, for example, by starting a photoreaction with a light flash. In the study presented here, we have investigated possible conformational changes in LHC−II upon illumination with visible light. Small changes are indeed observed, but our data suggest that this is due to photodegradation of chlorophyll rather than to a conformational change in the protein.

## MATERIALS AND METHODS

Trimeric LHC-II was isolated from pea seedlings as described previously (*16*, *17*). Samples were concentrated

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* To whom correspondence should be addressed. Present address: CIML, Parc Scientifique de Luminy, F-13288 Marseille, France. Phone: ++33 491 26 94 77. Fax: ++33 491 26 94 77. E-mail: hans.rogl@gmx.net.

‡ Max-Planck Institut für Biophysik.

§ Johann Wolfgang Goethe Universität. Present address: Department of Biochemistry and Biophysics, The Arrhenius Laboratories for Natural Sciences, Stockholm University, S-106 91 Stockholm, Sweden.

[1] Abbreviations: Chl, chlorophyll; COBSI, change of backbone structure and interaction; FTIR, Fourier transform infrared spectroscopy; IR, infrared; LHC-II, light-harvesting complex II.

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by salt precipitation, a procedure also used to prepare two-dimensional crystals of LHC-II. LHC-II solubilized in the detergent Triton X-100 was precipitated by diluting the detergent below the critical micelle concentration and adding 300 mM KCl at room temperature. The pellet was washed with water and then taken up in a minimal volume of 0.3% dodecyl maltoside in 20 mM phosphate buffer (pH 7.5), prepared in $^1H_2O$ (water) or $^2H_2O$ (deuterium oxide). For $^2H_2O$ preparations, the sample was washed three times in phosphate-buffered $^2H_2O$ before being solubilized with detergent. The resulting highly viscous solution had a Chl concentration of 10−20 g/L.

Chlorophylls and carotenoids were extracted from spinach leaves with acetone and diethyl ether. Carotenoids were prepared by alkali treatment, and Chl *a* and *b* were separated by reverse phase high-performance liquid chromatography (*18*).

*Infrared Difference Spectroscopy.* Samples for infrared spectroscopy were prepared by placing the LHC-II solution into an 8 $\mu$m deep trough of a $CaF_2$ window which was covered with a second flat window. The LHC-II concentration in the $^2H_2O$ samples was twice that in the $^1H_2O$ samples. The optical density of the Chl $Q_y$ absorption peak in the visible region ranged between 0.5 and 1. Pigments were dissolved in acetone and dried under a nitrogen stream on a $CaF_2$ window.

Infrared difference spectra were recorded at 20 °C with a modified Bruker IFS 66 rapid scan Fourier transform infrared spectrometer equipped with a HgCdTe detector of selected sensitivity and purged with dry air. First, reference spectra of the protein before illumination were recorded. Second, an intense light flash was applied (duration of 300 $\mu$s, 370 mJ/cm$^2$ at the sample) with a xenon flash tube. Finally, time-resolved infrared spectra were recorded with a maximum time resolution of 60 ms. Since we did not observe a time dependence for the band intensities, spectra recorded during the first 70 s ($^1H_2O$ samples) or during the first 35 s ($^2H_2O$ samples) were averaged to improve the baseline stability. In both cases, data from seven flashes on two samples were averaged. The two $^2H_2O$ samples were incubated for 2 h or 3 months in $^2H_2O$ without any detectable change in their spectra. The flash light was filtered with GG400 and KG3 filters to prevent heating of the sample and photodamage of the protein by UV light.

Infrared absorbance spectra were recorded with the same spectrometer. For $^1H_2O$ samples, a water spectrum was subtracted using a flat line between 2500 and 2000 cm$^{-1}$ as the criterion for the correct subtraction.

## RESULTS AND DISCUSSION

Figure 1 shows infrared difference spectra of LHC-II in $^1H_2O$ and $^2H_2O$ that were induced by an intense light flash in the visible spectral range. No significant difference was observed between difference spectra obtained with the first flash and with subsequent flashes onto a given sample; therefore, we averaged data from experiments on two samples with either three or four flashes for each of the spectra in Figure 1. Similar spectra were obtained using an OG570 filter between the flash and the sample, which blocks the light below 570 nm where carotenoids absorb. This shows that light absorption by carotenoids is not essential for the observed infrared signals, indicating that the observed processes can be initiated by photoabsorption by Chl. We first discuss the difference bands in the spectral regions characteristic of protein backbone absorption which are sensitive to conformational changes. We then examine alternative assignments and discuss the bands in other regions of the spectra.

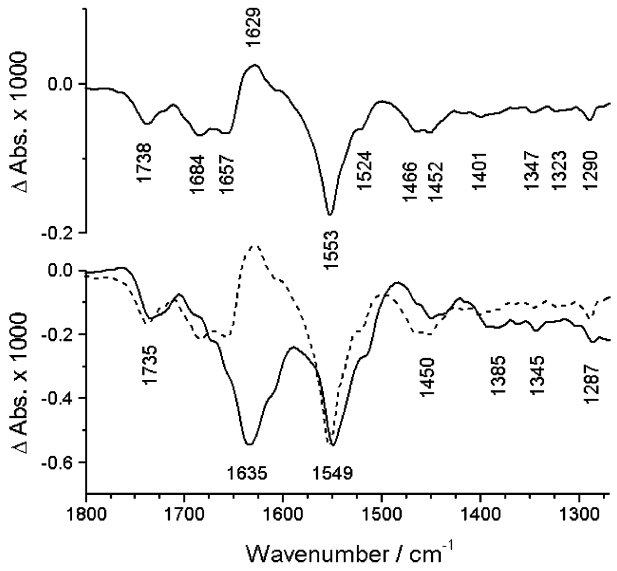


FIGURE 1: Light-induced infrared difference spectra of LHC-II recorded at 20 °C: (top) difference spectra of samples in $^1H_2O$ and (bottom) difference spectrum of samples in $^2H_2O$ (—) and in $^1H_2O$ (- - -). The latter was multiplied by a factor of 3.1 to normalize the band amplitude near 1550 cm$^{-1}$. The labels refer to the spectra depicted with solid lines.

*Amide I Region.* The amide I vibration of the protein backbone absorbs in the region from 1700 to 1610 cm$^{-1}$, mainly due to a C═O vibration. The absorption coefficient of the amide I vibration is large compared to most other infrared absorption coefficients, and the amide I band is therefore the strongest band in IR spectra of proteins. Difference spectra of protein reactions usually show the largest absorbance changes in this region, reflecting a conformational change in the protein. Only proteins with large cofactor contributions such as bacteriorhodopsin have strong additional bands in spectral regions where the cofactor absorbs. The measured absorbance changes in the light-induced LHC-II $^1H_2O$ difference spectrum are very small. The amplitude of the largest change is only 0.3% of the total absorption (band at 1635 cm$^{-1}$ in the $^2H_2O$ difference spectrum compared to the maximum of the absorbance spectrum in the amide I region). This is lower by a factor of 3 than the strongest difference band at 1553 cm$^{-1}$.

It is unlikely that the small IR signals in the amide I region of the difference spectrum are actually caused by amide I vibrations for the following reasons. There is a dramatic change between the $^1H_2O$ and $^2H_2O$ spectrum, which is untypical for amide I bands. Band shifts from 0 to −15 cm$^{-1}$ are expected, depending on the extent of $^1H-^2H$ exchange and possibly on the type of secondary structure. Also, in a typical protein difference spectrum, there would be a sequence of minima and maxima in the amide I region (*20*). Instead, only a broad minimum with several shoulders is observed for LHC-II in $^2H_2O$. These two observations are inconsistent with a protein conformational change.

However, since we cannot rule out completely the possibility that the small difference signals in this region are due to a minor rearrangement of the polypeptide, we briefly estimate the extent of a protein conformational change consistent with the intensity of the difference bands if indeed they were due to amide I vibrations. Absorbance changes in the amide I region have been used to quantify the net structural change with the help of the COBSI (change of backbone structure and interaction) index (*19*) which allows a comparison of conformational changes between different proteins. It is derived by dividing the integrated absolute absorbance change between 1700 and 1610 $cm^{-1}$ of the difference spectrum by the integrated total absorbance of the protein absorbance spectrum. It should be noted that the COBSI index is only meaningful if the spectra in the amide I region are dominated by signals of the amide I vibration. The COBSI index calculated for the $^1H_2O$ spectrum in Figure 1 is $1.5 \times 10^{-4}$. In the case presented here, this figure needs to be corrected for the contribution of the pigment to the absorbance spectrum of LHC-II in the amide I region. The COBSI index should be calculated only with the protein contribution to this spectral region. We have estimated the chlorophyll contribution to the total absorbance in the amide I spectral region to be 35% by comparing the ratio of the integrated absorption of a Chl spectrum and a LHC-II spectrum in two spectral regions. In the 1775−1700 $cm^{-1}$ region of the LHC-II spectrum, only Chl absorbs, whereas in the 1700−1610 $cm^{-1}$ region, there is absorption of Chl and protein. From this, the protein contribution to the amide I region of the absorption spectrum is estimated to be 65%, and the COBSI index needs to be multiplied by a factor of 1.5 so that the corrected COBSI index for the protein alone is close to $2.3 \times 10^{-4}$ (assuming that the protein conformational change affects only the peptide IR signals and not the pigments). This value has to be compared to protein-only COBSI indices that arise from 100% secondary structure changes in unpigmented proteins or peptides. These are typically between 0.2 and 0.6 (*10*), i.e., larger by a factor of more than 1000 compared to those we observe for LHC-II. On this basis, we estimate that only 20% of a single amino acid would participate in a net secondary structure change if the signals in the amide I region were to be assigned to the amide I vibration. The very low COBSI index indicates that there is no significant rearrangement of protein secondary structure in LHC-II that could be induced by a xenon flash. The COBSI index gives an estimate for the extent of conformational change in a "what ... if" scenario: it assumes that the difference bands in the amide I region are caused by an alteration of the protein amide I absorption. Below we develop a different assignment of the signals observed here, and in this case, the COBSI index cannot be applied.

*Amide II Region.* The second strongest band in protein IR absorption spectra is the amide II band which is caused mainly by the N−H bending and C−N stretching vibrations of peptide groups. The N−H bending contribution makes this band sensitive to $^1H-^2H$ exchange, which shifts the amide II band from ∼1550 to ∼1480 $cm^{-1}$. Bands in the amide II region are observed in the light-induced LHC-II difference spectra in $^1H_2O$ and $^2H_2O$. A closer comparison reveals that $^1H-^2H$ exchange shifts the whole band by only −4 $cm^{-1}$ to 1549 $cm^{-1}$. This rules out an assignment of this band to the amide II vibration, since one would expect a shift to ∼1480 $cm^{-1}$ for those regions of the protein that exchange protons, but no shift for regions that do not. When the absorption spectrum in $^2H_2O$ is examined (not shown), the difference band near 1550 $cm^{-1}$ coincides with a sharp absorption band that is typical for Chl absorption and which therefore does not undergo the characteristic shift expected for an amide II band in $^2H_2O$. It is conceivable that this Chl absorption band and the observed difference band are of the same origin (see below).

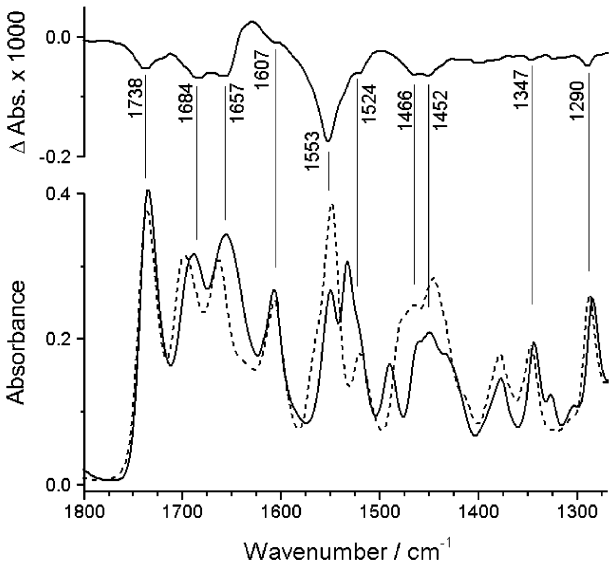


FIGURE 2: Comparison of the light-induced LHC-II infrared difference spectrum with infrared absorbance spectra of Chl *a* and Chl *b*: (top) LHC-II difference spectrum in $^1H_2O$ and (bottom) absorbance spectrum of Chl *a* (—) and Chl *b* (- - -). The Chl *a* spectrum was multiplied by a factor of 2 for better comparison.

In concluding this discussion of spectral regions of protein backbone absorption, we have shown that there are no bands in the light-induced difference spectra that would be characteristic of protein amide II vibrations, and that an assignment of difference bands in the amide I region to amide I vibrations is unlikely. Thus, there is no evidence for a significant protein conformational change in the light-induced difference spectra of LHC-II. We therefore investigated the possibility that the signals arise from Chl.

*Infrared Absorbance Spectra of Chl a and Chl b.* The bottom panel of Figure 2 shows absorbance spectra of Chl *a* and Chl *b* films. The band positions of the absorbance bands are listed in Table 1. Our spectra of dry Chl *a* and Chl *b* films are in excellent agreement with previously reported spectra of anhydrous films (*22*) and with Chl spectra obtained at millimolar concentrations in a water-saturated $CCl_4$ solution (*23*). Of particular interest are the carbonyl stretching vibration bands between 1755 and 1600 $cm^{-1}$ since they are often used as probes of the local environment (*14*). A polar environment and hydrogen bonding lead to downshifts of these bands by up to 60 $cm^{-1}$ with respect to the band position in a nonpolar environment.

It is well-known that Chl dimers and oligomers can form by ligand interaction of the 9-keto oxygen of one Chl molecule with the central $Mg^{2+}$ of another (*24*, *25*). In Chl *b* oligomers, the 3-formyl C═O groups are also involved in interactions with $Mg^{2+}$ (*22*, *24*, *26*). The formation of Chl oligomers in these systems affects predominantly the infrared bands of those C═O groups that interact with $Mg^{2+}$, in

Table 1: Absorption Bands in Infrared Spectra of Chl *a* and Chl *b* Films

| Chl *a* band position ($cm^{-1}$) | Chl *b* band position ($cm^{-1}$) | assignment and additional information about the band |
|---|---|---|
| 1735 | 1736 | 7c- and 10a-ester C═O group (*22*, *25*) |
| | | band position depends on the environment, expected range of 1710−1755 $cm^{-1}$ (*14*) |
| 1689 | 1698 | 9-keto (*22*, *24*), strongly coordinated to the $\pi$-system of the macrocycle (*25*) |
| | | band position depends on the environment, expected range of 1650−1710 $cm^{-1}$ (*14*) |
| 1656 | | 9-keto group coordinated to $Mg^{2+}$ of a second chlorophyll (*22*, *24*, *27*) |
| | 1664 | 3a-formyl and $Mg^{2+}$-coordinated keto groups (*22*, *24*) |
| | | band position depends on the environment, expected range of 1620−1660 $cm^{-1}$ (*14*) |
| 1608 | 1607 | Chl *a*, skeletal vibration (*24*, *25*) attributed to a methine bridge (*14*) |
| | | Chl *b*, skeletal vibration (*24*, *26*) attributed to a methine bridge (*14*), and $Mg^{2+}$-coordinated formyl group of oligomers (*24*, *26*) |
| | | Chl *a* and Chl *b*, sensitive to $Mg^{2+}$ coordination, near 1610 $cm^{-1}$ for five-coordinated $Mg^{2+}$ and near 1598 $cm^{-1}$ for six-coordinated $Mg^{2+}$ (*27*) |
| 1551 | 1549 | chlorin C═C, C═N vibration, characteristic of chlorin, not observed for porphyrins and tetrahydroporphyrins (bacteriochlorophyll) (*26*) |
| 1534 | | porphyrin, pyrrole C═C, C═N vibration (*26*), associated with aggregation (*27*) |
| | 1520 | porphyrin, pyrrole C═C, C═N vibration (*26*) |
| 1491 | | porphyrin, pyrrole C═C, C═N vibration (*26*) |
| 1450 | 1446 | $CH_3$ asymmetric bending vibration (*26*) |
| 1378 | 1379 | $CH_3$ symmetric bending vibration (*26*) |
| 1344 | 1346 | porphyrin, pyrrole C═C, C═N vibration (*26*) |
| 1285 | 1288 | tetrapyrrole bending (*26*) |

particular the 9-keto band near 1695 $cm^{-1}$ and the 3-formyl band near 1665 $cm^{-1}$. These bands shift by −35 and −55 $cm^{-1}$, respectively, upon $Mg^{2+}$ coordination (*22*, *24*, *27*). Our IR spectra of dry Chl films clearly indicate the presence of both Chl monomers and oligomers.

*Comparison of the Light-Induced Infrared Difference Spectra of LHC-II with Infrared Absorbance Spectra of Chl a,Chl b, and a Carotenoid Mixture.* The top panel of Figure 2 shows again the light-induced infrared difference spectrum of LHC-II in $^1H_2O$. Obviously, all bands in the difference spectrum coincide with Chl *a* and Chl *b* absorption bands. In addition, the strongest band in the $^1H_2O$ spectrum (at 1553 $cm^{-1}$) is outside the amide I region, which is typical of a strong chromophore contribution to the spectrum. We therefore attribute the bands in the light-induced difference spectrum to a photoreaction of Chl *a* and Chl *b*. On this basis, we assign the bands of the $^1H_2O$ spectrum according to Table 1 as follows: 1738 $cm^{-1}$ for non-hydrogen-bonded 7c- and 10a-ester C═O groups, 1684 $cm^{-1}$ for the 9-keto C═O group, weakly hydrogen bonded (these bands are found for anhydrous films at 1695 $cm^{-1}$ for Chl *a* and at 1700 $cm^{-1}$ for Chl *b*) (*22*), 1657−1635 $cm^{-1}$ for the Chl *b* 3a-formyl C═O group, weakly till moderately hydrogen bonded (absorbs at 1663 $cm^{-1}$ in anhydrous films) (*22*) or a hydrogen bonded 9-keto C═O group, 1553 and 1347 $cm^{-1}$ for skeletal C═C and C═N vibrations, 1466 and 1452 $cm^{-1}$ for asymmetric $CH_3$ bending, and 1290 $cm^{-1}$ for tetrapyrrole ring bending vibrations. These assignments are based on our model spectra of Chl films; they are backed by independent assignments of bands at similar positions for LHC-II: a band at 1553 $cm^{-1}$ has been observed and assigned identically by Peterman et al. (*27*) via high-resolution fluorescence spectroscopy exciting the Chl *a* of LHC-II. Ruban et al. (*28*) have observed a band at 1639 $cm^{-1}$ using resonance Raman spectroscopy and assigned it to a hydrogen-bridged C═O group of Chl *b*. As suggested by Ruban et al., it may occur exclusively in aggregated LHC-II, which may be the reason we observe it in the more concentrated sample of the $^2H_2O$ spectrum.

We did not observe a correlation of the light-induced difference bands with the main bands of a carotenoid preparation (data not shown). This is in good agreement with the fact that the light-induced difference bands of LHC-II are also observed when the excitation of the LHC-II carotenoids was blocked by an OG-570 filter.

*Photodegradation of Chl.* The observation of the 7c- and 10a-ester C═O band at 1738 $cm^{-1}$ shows that most of the photoreactive Chl molecules are in a hydrophobic environment. The signals therefore do not arise from Chls in an aqueous environment but rather from Chls in protein binding pockets. The strongest signal in the $^1H_2O$ difference spectrum is a characteristic chlorin band at 1553 $cm^{-1}$ which is not observed for porphyrins or tetrahydroporphyrins (in particular bacteriochlorophyll) (*26*). The corresponding C═C or C═N vibrations are therefore related to the asymmetric chlorin ring system of the chlorophylls in which only ring IV is reduced. It is worth noting that the difference spectra are inconsistent with a photooxidation of Chl *a* forming a Chl $a^+$ cation as in photosystem I. This would give rise to positive bands at 1719 and 1355 $cm^{-1}$ (*30*) which are not found in our spectra. Barzda et al. (*39*) have proposed Chl cations as long-lived quenchers in LHC-II. However, these experiments have been performed with intense laser flashes ~3 orders of magnitude higher in power than our xenon flash. The results are therefore not directly comparable to our study. Interestingly, an "irreversibly" generated quencher with an indefinite lifetime has been observed by Barzda et al. (*39*) which could be consistent with Chl degradation.

The amplitude of the band near 1550 $cm^{-1}$ in the light-induced difference spectra corresponds to only 0.7% of its amplitude in the absorption spectrum. If it is assumed that the absorption near 1550 $cm^{-1}$ (assigned to a vibration typical of chlorins) is zero for the photodegradation products, this number indicates that one flash photodegrades 0.7% of the Chl molecules. It is instructive to make a rough estimate of how many Chl molecules are excited per flash. In the lifetime of excited Chl (5 ns), $5 \times 10^{12}$ photons/$cm^2$ will pass through the sample, 90% of which are absorbed at the given

absorbance of ~1. At this absorbance, the sample has a Chl concentration of $6 \times 10^{15}$ molecules/cm$^2$ (extinction coefficient Chl in LHC-II, 100 mM$^{-1}$ cm$^{-1}$ which equals $10^8$ cm$^2$ mol$^{-1}$); therefore, the fraction of excited Chl molecules at any given time can be estimated to be 0.1%. The duration of our flash is ~$10^5$ times the lifetime of excited Chl. Therefore, within one flash (300 $\mu$s), each Chl molecule will be excited ~100 times.

The estimate of 0.7% Chl degradation per flash is in good agreement with photodegradation in dead phytoplanktonic cells, where 20% of the Chl is degraded upon applying a surface irradiance of 0.6 Einstein m$^{-2}$ (*31*). If photons of 550 nm are assumed, this corresponds to 13 J cm$^{-2}$. Although it may be argued that the site of Chl degradation in dead phytoplankton is not necessarily LHC-II, the estimate from our experiment extrapolated to 13 J cm$^{-2}$ would give the similar value of 22%. The Chl degradation inferred from our *in vitro* experiments is therefore comparable to that observed under more natural conditions.

*Is There a Light-Induced Conformational Change in LHC-II?* Repeated flashes on a LHC-II sample produced each time the same light-induced difference spectrum, without any obvious decay during the period of observation after the flash (4 min). The signal was not saturated by nine flashes, amounting to a total light energy of 3.3 J cm$^{-2}$. This energy input can be compared to the power of sunlight which ranges from 10 to 100 mW cm$^{-2}$. The energy of nine flashes therefore corresponds roughly to the amount of solar energy received by LHC-II on the time scale of minutes.

Several effects of LHC-II illumination have been described: enhanced LHC-II phosphorylation (*7*), dissociation of chiral LHC-II macroaggregates (*32*) accompanied by monomerization (*33*), and polypeptide fragmentation (*34*).

In our experiments, we addressed a possible role of a light-induced conformational change in the LHC-II protein in these processes by repetitive flashes and difference FTIR spectroscopy, with a total dose of photons similar to that reported in ref *7*. If there was a light-induced conformational switch in the LHC-II protein, it would have been detected by our sensitive method. However, our results clearly indicate that a defined switch in the three-dimensional protein conformation between two (or more) energy minima is not involved. Splitting of peptide bonds is expected to give a strong positive band near 1600 cm$^{-1}$ (among others) for the $COO^-$ antisymmetric stretching vibration, but was not observed. Thus, we cannot confirm polypeptide degradation (*34*), which may be due to the different time of observation in the two experiments. Whereas polypeptide degradation was analyzed after several hours of continuous illumination (*34*), our spectra were recorded within 1 min of the light flash.

Instead of confirming a light-induced conformational change or direct photolysis of peptide bonds, our results indicate light-induced Chl degradation. It is therefore conceivable that Chl degradation products play a role in the above-mentioned light-induced effects. For a better comparison of those results with our findings, it is useful to summarize the experimental conditions used in each case. Whereas the dissociation of macroaggregates has obviously been observed on an aggregated sample, the light-induced monomerization, phosphorylation, and peptide fragmentation have been observed with solubilized LHC-II as well as in a nonsolubilized sample, e.g., thylakoid membranes. Because of the high protein concentration that is necessary for IR measurements, our sample contains most likely both aggregated and solubilized LHC-II. A major difference between our experimental setup and the ones described before is the use of a xenon flash versus continuous illumination. Although we are not in a light intensity range that gives rise to singlet−singlet annihilation or Chl triplet formation, each of which has been observed with strong laser flashes (*35*), it seems highly likely that we create considerably more carotenoid triplets than in the continuous illumination schemes of refs *7* and *32*−*34*. Those are known to quench excited Chl states in a radiation-less manner (*36*), whereas they would otherwise decay by Chl fluorescence. Whether it is the Car triplet decay pathway or the presence of excited-state Chl that would be responsible for the light-induced effects observed in refs *7* and *32*−*34* has so far not been investigated. Our results clearly indicate that a strong flash illumination does not give rise to a protein conformational change that could be observed on the time scale of minutes. It seems therefore likely that slower photochemical processes are responsible for the light-induced effects that have been observed previously.

## CONCLUSION

Careful evaluation of the amide I and amide II regions of the infrared difference spectrum of LHC-II does not provide evidence for a specific, light-induced change in the structure of the main light-absorbing pigment protein in plants, LHC-II. First, the difference band in the amide II region cannot be assigned to the amide II vibration because of the small shift of 4 cm$^{-1}$ in $^2H_2O$ after incubation for 3 months. The difference bands in the amide I region for samples in $^1H_2O$ are very small and, if assigned to the amide I vibration, would only account for a net secondary structure change of statistically less than one amino acid residue. The effects of $^1H-^2H$ exchange are not typical of amide I bands, and the spectrum in the amide I region in $^2H_2O$ is untypical of protein difference spectra.

Second, all bands in the light-induced difference spectrum coincide with bands in the absorption spectrum of Chl *a* and *b*, strongly suggesting an assignment of these bands to chlorophyll. Chlorophylls in solution are well-known to undergo photodegradation (*37*, *38*). The fact that the major negative IR band in the $H_2O$ spectrum is characteristic of chlorins seems to indicate a specific partial degradation process. Our results show that a light-induced increase in the extent of LHC-II phosphorylation (*7*) and dissociation of LHC-II macroaggregates (*32*) do not correspond to a change in protein conformation that could be observed in a solubilized protein sample.

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