Probing the structure of Lhca3 by mutation analysis

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Abstract

Lhc proteins constitute a family of transmembrane proteins which share homology in sequence and similarity in the general organisation although members can be strongly differentiated such as in the case of PsbS and ELIPs. In this work, we report on the structure of Lhca3, a pigment-protein subunit component of the antenna system of higher plants Photosystem I, through the effect of point mutations in critical sites. Based on the structure of PSI-LHCI (Ben Shem et al., PDB file 1QZV remark 999) it has been suggested that Lhca3 may have different folding as compared to other members of the Lhc family. In particular, it was proposed that the two central helices may be swapped and chlorophylls in sites 1013 and 1023 are not present. This different folding would imply that the chlorophylls coordinated to the two central helices have different ligands in Lhca3 with respect to the other Lhc complexes. The structural model was tested by substituting the putative binding residues with residues unable to coordinate chlorophyll and the spectroscopic properties of the individual pigments were used as structural probes. The results indicate that Lhca3 folds in the same way as the other antenna proteins. Moreover, the low-energy absorption form originates from interaction between chlorophylls in sites 1015 and 1025, like for the other PSI antenna subunits. Evidence is also shown for the presence in Lhca3 of chlorophylls in sites 1013 and 1023.

Keywords: Photosystem I; Light-harvesting complex; Lhca3; Mutant; Fluorescence; Red form

Higher plant Photosystem I, the plastocyanin/ferredoxin oxido-reductase, is composed of a core complex and of an outer antenna system, consisting of four major polypeptides, the products of the genes Lhca1–4 [1,2]. A breakthrough in the study of the PSI-LHCI was the resolution of the structure of this supercomplex at 4.4 Å [3]. This represents the largest structure of a membrane complex resolved up to now and it provides essential information about the organisation of the complexes and the localisation of most Chl molecules. It was shown that Lhca1–4 subunits are bound on one side of the core in agreement with a previous model [4], and that a single copy of each polypeptide is present per P700. Moreover, it was shown that several Chl molecules are bound at the interface between the reaction centre and the antennas, possibly favouring the energy transfer to P700 [3,5–7].

Lhca proteins belong to the Lhc multigenic family, which also includes the Lhcb antenna complexes of Photosystem II. These pigment–protein complexes are characterised by three transmembrane helices sharing high similarity. Comparison of the Chl organisation in LHClII, a complex whose structure has been analysed in detail [8,9], and most Lhca complexes reveals a high level of homology, consistent with the conservation of all Chl binding residues [10]. One exception seems to be Lhca3: based on the analysis of the electron density map, it has been suggested that the two central helices might be swapped ([3], PDB file 1QZV). In the remark 999 of the 1QZV structure it is reported: “in Lhca3 only, the transmembrane helix closest to helix C is not the longer of the two tilted transmembrane helices (labelling as in Kuhlbrandt et al. 1994 [29]). This might indicate that in Lhca3 the helix closes to helix C is not the first helix (from the N-terminus) but the third one. The electron density map is not conclusive here but seems to suggest that this might be the case.
It this is true and Lhca3 folds differently, then the numbering of residues in Lhca3 must change accordingly”. Moreover, it appears that in the Lhca3 structure the Chls in sites 1013 and 1023 are missing while a “new” Chl (1041) in an intermediate position [3] was resolved. In this work we have checked these suggestions by mutation analysis. In the following the Chl nomenclature by Ben-Shem et al. [3] is used. For clarity, the correspondence to the nomenclature of LHCBII [8] is reported in brackets.

Each Chl coordinated to an Lhc complex has specific spectroscopic properties due to interactions with the protein and neighbouring pigments. Mutation analysis has been performed on two Lhcβ complexes [11–13] and three Lhca complexes [14–16] revealing conservancy of the chromophore properties in several binding sites. For example, the interacting dimer Chl 1012 (612)/Chl 1022 (611) displays its main absorption at 681–682 nm in all complexes analysed so far, as can be inferred from mutation of the ligand for Chl 1012. Lhca protein subfamily is characterised by the presence of low-energy absorption forms yielding fluorescence emission above 700 nm. In the case of Lhca1, 2 and 4 it was shown that these forms originate from an excitonic interaction involving Chl a molecules in sites 1015 (603) and 1025 (609) [14–16]. In this work we integrate the model obtained for Lhca3 from X-ray crystallography with the results from mutation analysis, using the spectroscopic properties of the individual chlorophylls as structural probes, in order to get details on the overall structure of the complex and on the spectroscopic properties of the individual chromophores coordinated to it. We found evidence for the presence of Chls in binding sites 1013 and 1023 in Lhca3. Moreover, the Chls responsible for the low energy absorption in Lhca3 are the same as found in the other Lhca complexes. We conclude that Lhca3 shares its overall structural organisation with the other members of the Lhca subfamily.

1. Materials and methods

1.2. Sample preparation

cDNA of Lhca3 from Arabidopsis thaliana [17] was mutated with the QuickChange© Site directed Mutagenesis Kit, by Stratagene©. WT and mutants apoproteins were isolated from the SG13009 strain of E. coli DM40000 Site directed Mutagenesis Kit, by Stratagene©. WT and mutant apoproteins were isolated from the SG13009 strain of E. coli and reconstituted in vitro adding pigments [23]. The refolded complexes were purified from the excess of pigments used in the reconstitution by sucrose gradient ultracentrifugation and ion exchange chromatography. All mutants yielded stable reconstituted monomeric products except in the case of R64L/E184V and E59V/R189L. For mutant Q201L a faint green band was obtained in the sucrose gradient, but the complex was highly instable and unable to survive the following purification steps.

The pigment content of the complexes is reported in Table 1. We have to mention that the number of Chls coordinated to reconstitute Lhca3 is lower than in the native complex [3]. This is probably due to the fact that the binding of few peripheral Chls is stabilised by interactions between two different complexes, while

Reconstitution and purification of protein–pigment complexes were performed as described in [19] with the modification reported in [20].

1.3. Protein and pigment concentration

HPLC analysis was done as in [21]. The chlorophyll to carotenoid ratio and the Chl a/b ratio were measured independently by fitting the spectrum of acetone extracts with the spectra of individual purified pigments [22].

1.4. Spectroscopy

The absorption spectra at RT and 77 K were recorded using a SLM-Aminco DK2000 spectrophotometer, in 10 mM HEPES pH 7.5, 60% glycerol and 0.06% n-dodecyl-β-D-maltopyranoside. The wavelength sampling step was 0.4 nm, the scan rate 100 nm/min, the optical pathlength 1 cm. Fluorescence emission spectra were measured using a Jasco FP-777 spectrofluorimeter and corrected for the instrumental response. The samples were excited at 440, 475 and 500 nm. The spectral bandwidth was 5 nm (excitation) and 3 nm (emission). Chlorophyll concentration was about 0.02 μg/ml in 60% glycerol and 0.03% n-dodecyl-β-D-maltopyranoside.

2. Results

The cDNA encoding Lhca3 from Arabidopsis thaliana was mutated at the putative Chl binding sites by substituting nucleophilic residues with apolar amino acids, which cannot coordinate the central Mg of the Chls. The list of mutations performed is reported in Table 1 (Chl binding sites nomenclature from [8] and [3]). In Fig. 1 a schematic representation of the structure of Lhca3 from Ben-Shem et al. [3] is reported, the Chls in color are the target of the mutations. WT and mutant apoproteins were overexpressed in E. coli and reconstituted in vitro adding pigments [23]. The refolded complexes were purified from the excess of pigments used in the reconstitution by sucrose gradient ultracentrifugation and ion exchange chromatography.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Putative Chl binding site</th>
<th>Chl tot</th>
<th>Chl a/b</th>
<th>Chl a</th>
<th>Chl b</th>
<th>Violax</th>
<th>Lutein</th>
<th>β-Car</th>
<th>1607 M. Mozzo et al. / Biochimica et Biophysica Acta 1757 (2006) 1607–1613</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>(12)</td>
<td>10</td>
<td>6.2±0.8</td>
<td>8.6±0.15</td>
<td>1.4±0.15</td>
<td>0.5±0.04</td>
<td>1.9±0.2</td>
<td>0.36±0.25</td>
<td></td>
</tr>
<tr>
<td>R64L/E184V (1)</td>
<td>1011–610</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>N107V (3)</td>
<td>1012–612</td>
<td>8</td>
<td>4.7±0.4</td>
<td>6.6±0.1</td>
<td>1.4±0.1</td>
<td>0.5±0.01</td>
<td>1.4±0.12</td>
<td>0.38±0.14</td>
<td></td>
</tr>
<tr>
<td>Q201L (9)</td>
<td>1013–613</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>E59V/R189L (3)</td>
<td>1014–602</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>N62F (3)</td>
<td>1015–603</td>
<td>8</td>
<td>7.3±0.3</td>
<td>7.0±0.04</td>
<td>0.96±0.04</td>
<td>0.26±0.004</td>
<td>1.71±0.04</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>H126V (4)</td>
<td>1023–614</td>
<td>9</td>
<td>5.5±0.6</td>
<td>7.6±0.12</td>
<td>1.4±0.12</td>
<td>0.62±0.08</td>
<td>1.77±0.17</td>
<td>0.34±0.25</td>
<td></td>
</tr>
<tr>
<td>E126V/R129L (6)</td>
<td>1025–609</td>
<td>8</td>
<td>9.2±3.2</td>
<td>6.3±0.15</td>
<td>0.73±0.16</td>
<td>0.33±0.04</td>
<td>1.52±0.26</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>E118V (5)</td>
<td>1026–606</td>
<td>9</td>
<td>5.9±0.8</td>
<td>7.7±0.18</td>
<td>1.32±0.18</td>
<td>0.38±0.08</td>
<td>1.88±0.15</td>
<td>0.1±0.09</td>
<td></td>
</tr>
</tbody>
</table>

The putative Chl binding sites affected by the mutation are indicated, as identified from sequence homologies. Nomenclature from [8] and [3] are reported for the Chls. In brackets are reported the number of reconstitutions performed for each complex.
the reconstituted complex is in monomeric form. As expected, given the high Chl \( a/b \) ratio of the WT (17; 24), most mutations affected Chl \( a \) binding. Only two mutants, namely E126V/R129L and N62F, have a higher Chl \( a/b \) ratio, indicating loss of Chl \( b \).

The Chl/Car ratio of the WT was 3.6, suggesting the presence of 3 carotenoid molecules per 10±1 Chls in agreement with previous results [17,24]. This value was different in all mutants with the exception of N187V: lower values were observed for mutant H216F, suggesting a preferential loss of Chl, while all other complexes showed an increase in this value implying a more severe carotenoid loss. Three carotenoid species were found associated to the WT: lutein, which represents the main xanthophyll, violaxanthin and \( \beta \)-carotene. The carotenoid composition was affected in most of the mutants: complete loss of \( \beta \)-carotene and reduction of violaxanthin content were observed in mutants N62F, E126V/R129L and E118V. Reduced content of lutein was observed upon mutation at site N187V.

In mutant N187V, H216F and E118V the number of the Chls lost was estimated assuming a constant amount of Chl \( b \). This assumption is supported by the analysis of the absorption spectra of these complexes, which showed no changes in the Chl \( b \) region as compared to the WT (Fig. 3). In this view, Lhca3–N187V loses two Chls \( a \), mutants H216F and E118V one each. For E126V/R129L and N62F complexes, which lose Chl \( b \), the pigment to protein stoichiometry was estimated based on the carotenoid content. Both mutants show complete loss of \( \beta \)-carotene and strong reduction of violaxanthin, which suggests that one of the three carotenoid binding sites is empty. Normalisation to two carotenoids gives a Chl/protein ratio of 8 in both complexes.

2.1. Fluorescence emission spectra

The major spectroscopic characteristic of Lhca3 is the presence of spectral forms which emit around 724 nm (17). By mutating the ligands of the individual Chls and measuring the fluorescence emission spectra of the mutated complexes it is possible to detect which are the pigments responsible for the red emission. The fluorescence spectra of WT and mutated complexes were recorded at 77 K where the contribution of the red forms is enhanced (Fig. 2). At 77 K the fluorescence emission spectrum of Lhca3-WT is characterised by a maximum at 724 nm and an additional band around 689 nm. Two mutants, namely E126V/R129L and N62F, lose the 724 nm component and show a maximum at 682.5 nm. Mutant E118V still maintains the emission at 724 nm, although the maximum of the spectrum is shifted to 689 nm. Mutants H216F and N187V have emission spectra identical to the WT in the red region, but the latter shows reduced amplitude in the 680–690 nm region, suggesting that this mutation is affecting a Chl, which participates to the 690 nm emission. Only two mutants lose the red emission band, thus indicating a specific and local effect of the mutations.

2.2. Absorption

The absorption of individual pigments in protein is tuned by the environment and thus the spectroscopic characteristics of the pigments can give information about the environment (protein, neighbour pigments, etc.) of each chromophores. To investigate the properties of the individual Chl in Lhca3, the
absorption spectra of all mutants were measured at 77 K and they are reported in Fig. 3. Mutants E126V/R129L and N62F lose completely the red absorption tail, in accordance with the fluorescence data. All other complexes exhibit absorption above 700 nm, although in the case of the Lhca3 –E118V complex the intensity is strongly reduced as compared to the WT. In agreement with the pigment analysis, the only two mutations affecting the Chl absorption region are E126V/R129L and N62F. Clear loss of absorption around 660 and 670 nm is observed in mutant H216F, while the Lhca3 –N187V complex is depleted in absorption around 680 nm.

3. Discussion

Based on the electron density map, it has been suggested that Lhca3 may fold differently as compared with the other Lhc complexes, with the helix closest to helix C not being helix B, but helix A. However, the structure also shows that the positions of most of the Chls in Lhca3 are conserved as compared to the other Lhc complexes [3]. If this suggestion is correct, then the ligands for the Chls coordinated to residues of helices A and B, namely Chls 1011 (610), 1012 (611), 1013 (613), 1014 (602) and 1015 (603) should be swapped with respect to the ligands in all other Lhc complexes. For clarity the Chl ligands in the two possible conformations are reported in Table 2. Moreover, the Lhca3 structural model does not show Chls in sites 1013 (613) and 1023 (614), but a new Chl, 1041, located in an intermediate position. For this study we have used the Lhca3 gene of Arabidopsis thaliana, while the structure has been obtained on PSI-LHCI from pea. The proteins from pea and Arabidopsis shows 91.8% identity and most of the substitution are homologous, moreover, all the putative Chl binding residues are conserved, thus strongly suggesting that the overall structure and the pigment properties are conserved between the two species.

In order to probe the chromophore coordination in Lhca3, mutation analysis of the putative Chl binding sites has been performed. It has been shown that the spectroscopic properties of several chromophores are conserved throughout the Lhc family: the interacting dimer Chl 1012 (612)/Chl 1022 (611) displays the main absorption at 680–681 nm in all complexes so far analysed, as can be inferred by mutation of the ligand for Chl 1012 [12,14]. Similarly, in Lhca complexes, Chl 1015 (603) was shown to be involved in pigment–pigment interaction with Chl 1025 (609), leading to the low-energy absorption forms [20,25,26]. If the folding of Lhca3 is different from that of the other Lhc complexes, one can expect that a mutation at residue N187 (ligand for Chl 1012 according to sequence alignment) does not affect Chl 1012, but instead Chl 1015, while a mutation at N62 would influence Chl 1012. Considering that the Chl organisation and the primary structure are very similar in the Lhc complexes we can thus expect to see the “signature” of Chl 1012 upon mutation at site N62 and the “signature” of Chl 1025 upon mutation at N187.

3.1. Mutation N187V

In Lhc complexes, Chl 1012 (612) is usually coordinated to a N (or H) in the helix A. Mutation at this site in Lhc complexes leads to the loss of the absorption at 680–681 nm (values for room temperature). This red absorption originates from an excitonic interaction which involves Chl 1012 and Chl 1022 as shown by mutation analysis and calculations based on the structure of LHCII [12,22,27].

In Lhca3, the N187V mutation leads to the loss of 2 Chl a molecules. In the absorption difference spectrum at low temperature, two bands are present at 666 nm and 679.5 nm (Fig. 4) (the difference at RT shows the maximum at 681.5 nm),

Table 2

<table>
<thead>
<tr>
<th>Ligands</th>
<th>“Alignment-based” conformation</th>
<th>“Swapped” conformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R64L/E184V</td>
<td>1011-A1-610</td>
<td>A4-1014-602</td>
</tr>
<tr>
<td>N187V</td>
<td>1012-A2-612</td>
<td>A5-1015-603</td>
</tr>
<tr>
<td>Q201L</td>
<td>1013-A3-613</td>
<td>A1-1011-610</td>
</tr>
<tr>
<td>E126V/R129L</td>
<td>1014-A4-602</td>
<td>A2-1012-612</td>
</tr>
<tr>
<td>E118V</td>
<td>1015-A5-603</td>
<td>A3-1013-B2-614</td>
</tr>
<tr>
<td>H216F</td>
<td>1023-B3-614</td>
<td>B4-1024-609</td>
</tr>
<tr>
<td>E126V/R129L</td>
<td>1025-B5-609</td>
<td>B5-1025-609</td>
</tr>
<tr>
<td>E118V</td>
<td>1026-B6-606</td>
<td>B6-1026-606</td>
</tr>
</tbody>
</table>

In the “alignment-based” conformation, the binding sites identified in LHCII structures [29] and [8] have been located in Lhca3 by sequence alignment. The “swapped” conformation, instead, indicates the correspondence following the hypothesis that helices A and B are swapped in Lhca3 [3]. The Chl nomenclature from [3,29] and [8] is reported.
which are likely to represent the two absorption contributions of the dimer, as supported also by circular dichroism measurements (data not shown). Considering isoenergetic monomers, the interaction energy would be 149 cm\(^{-1}\) [28], a value very similar to what was calculated for LHCII based on its structure [8]. Moreover, the spectrum in Fig. 3 is very similar to the spectrum of the same mutant in LHCII (see Remelli et al. [12] Fig. 5A) with the difference that the Chl 1 contribution is not present here.

This result suggests that N187 coordinates Chl 1012 also in Lhca3. The second Chl lost as a consequence of this mutation is most probably accommodated in site 1022, which is located in close proximity of Chl 1012. We can conclude that in Lhca3 both these sites coordinate a Chl \(a\) molecule responsible for the 666/680 nm absorption.

3.2. Mutations N62F, E126V/R129L and E118V

It has been shown that in Lhca1, Lhca2 and Lhca4 the red-shifted forms originate from pigment–pigment interaction involving Chls in sites 1015 and 1025 [14,15,26]. In the case of Lhca3 it was demonstrated that the substitution of N62 with H abolished the red forms without any loss of Chls, as in Lhca4 [25]. If the folding of Lhca3 is different from the one of the other Lhc complexes, the N62H mutation should not have affected the Chl in site 1015, as suggested, but the Chl in site 1012. This would imply that the second Chl participating to the interaction leading to the red forms in Lhca3 could not be Chl 1015, which is 24.7 Å distant from Chl 1012 [3], but should be Chl 1022, which is at 9.3 Å. If this is correct, then the mutation at site 1025, ligand E126, should not influence the red forms. This is not the case: the mutation of E126 completely abolished the red forms, as well as the mutation at site N62, thus suggesting that the Chls coordinated by these residues are located nearby.

In Lhca 1,2 and 4 complexes it has been observed that mutation of the glutamate in helix C, originally described as ligand for Chl 1026, has an effect on the red forms [14–16]. It was proposed that this residue is necessary to stabilise the conformation yielding to the low-energy absorption. Mutation of this residue (E118) in Lhca3 leads to the loss of one Chl \(a\) molecule (likely Chl 1026) and to a strong reduction in the red forms, although the emission at 724 nm is still present. This residue is located on the C helix and its involvement in the red forms of Lhca3 strongly supports the view that the structure of Lhca3 is very similar to the one of the other antenna complexes.

We can conclude that in Lhca3 N62 coordinates Chl 1015 and that in Lhca3 the red emission forms originate from an interaction between Chls 1015 and 1025.

3.3. Mutations at Q201L and H216F

In the Lhca3 structure Chls 1013 and 1023 were not resolved: at their place a “new” Chl in intermediate position (1041) was modelled [3]. However, sequence alignment shows that the putative ligands for these two Chls, respectively Q201 and H216, are conserved in Lhca3 [10], raising the question whether these two Chls are present in Lhcb3. Mutations of both these residues were performed. Unfortunately, it was not possible to obtain a stable complex carrying the mutation of site Q201, indicating that this residue is important for protein stabilisation. Identical results were obtained for Lhca4 [16], in which Chl 1013 is present accordingly to the X-ray structure.

Pigment analysis indicates that the H216F mutation in Lhca3 induces the loss of one Chl \(a\) molecule, strongly suggesting that this residue is coordinating a Chl \(a\). In principle this Chl can also be 1041 and this would mean that the structure of the D helix in Lhca3 is different with respect to the structure of all other Lhc complexes. However, the sequence in this region is conserved throughout the Lhc family. The absorption difference spectra for the H216F mutant upon normalisation to the Chl content is reported in Fig. 5. The spectrum is qualitatively similar to the one of the same mutant in LHCII [12] and Lhca4 [16] and it shows at least three contributing bands with maxima at around 660, 670 and 680 nm (Fig. 5). Due to the localisation of the mutation in a peripheral domain of the complex, neither
long range nor protein destabilisation effects are expected, in line with the results on other Lhc complexes [11,12,14,16], leading to the conclusion that the loss of the Chl coordinated by H216 has an effect on neighbouring pigments. Looking at the structure of Lhca3, Chl 1041 is completely isolated from the rest of the pigments and in the structure of the other complexes the only Chl 1023’s neighbour is Chl 1013, supporting the idea that both Chls are present in Lhca3.

In conclusion, H216 is a Chl ligand in Lhca3 and it coordinates a Chl a molecule, which then corresponds to Chl 1023 in the other Lhc complexes. About Chl 1013, the proposal that this Chl is present also in Lhca3 is based on indirect evidences: (1) the mutation at the putative ligand for this Chl inhibits the folding of the complex, as well as in Lhca4 where this Chl is present. (2) Mutation at site H216 shows an effect on a second Chl molecule and the most obvious candidate is Chl 1013. We thus suggest that the Chl organisation in the D helix domain is conserved in Lhca3 and Chl a molecules are present in sites 1013 and 1023. The two ER bridges — R64/E184 and E59/R189 (putative ligands for Chl 1011 and 1014).

It has been proposed that the structure of LHCCI is stabilised by two ionic pairs between helices A and B [29]. Moreover, the residues involved in the ionic bridge are also the ligands for Chls 1011 (610) and 1014 (602) and they are conserved in all Lhc family members. Reconstituted Lhcb1 complex was obtained in the absence of either one or the other ionic pair, although the stability of the complex carrying the mutation of the 1011 ligand was significantly lower [12]. It was concluded that the E (helix A) R (helix B) ionic pair plays a more prominent role in the structure stabilisation when compared to E (helix B) R (helix A) pair. This result was confirmed by mutation analysis of Lhcb4, Lhca1 and Lhca4 in which the same mutation inhibited folding [11,14]. In this respect Lhca3 behaves differently: both mutations are lethal for the protein, suggesting that in this complex all four residues play a fundamental role in protein stabilisation.

3.4. Carotenoid organisation

The X-ray structure of Lhca complexes within PSI-LHCl does not reveal the position of the carotenoids, but information about the carotenoid organisation in Lhca3 can be obtained from mutation analysis. β-carotene is specifically lost upon mutation of the ligands for Chls 1015, 1025 and 1026. In the structure of LHClI [8] these Chls are located near to the xanthophylls in site 621, thus suggesting that this site accommodates β-carotene in Lhca3. In the same mutants also a reduction of violaxanthin was observed, which suggests mixed occupancy for the 621 site. A decrease in lutein content was observed upon mutation of site 1012, which is located in close proximity of lutein 620 in LHClI, indicating that in Lhca3, as in all Lhc complexes analysed so far, the 620 site is occupied by a lutein. Based on these results and on the pigment composition of the complex it can also be concluded that the third carotenoid binding site accommodates lutein and violaxanthin.

5. Conclusions

In this work we have integrated the structural data obtained for Lhca3 in the PSI–LHCl complex [3] with spectroscopic measurement, using the chlorophylls as structural probes, to get details about the organisation of the complex. A model of the structure of Lhca3 based on the X-ray data [3] and on the results of this work is presented in Fig. 6. The absorption properties of several Chls were determined: The red forms originate from an excitonic interaction involving Chl a molecules in sites 1015 and 1025. A second excitonic couple is formed by Chls in sites 1012 and 1022 which are responsible for the absorption at 666/682 nm. Data presented here show that Lhca3 has a general folding very similar to all other members of the Lhc family and that the same residues are responsible for the coordination of the Chls. Furthermore, mutational analysis suggests that both Chls 1013 and 1023, although not visible in the structure, are present in the complex. However, it should be noticed that Lhca3 is the only protein where the mutations at both ionic bridges between helices A and B inhibit the folding, suggesting that Lhca3 has some structural peculiarity as compared to the other Lhc complexes.

Acknowledgments

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