Stability of Water-Soluble Chlorophyll Protein (WSCP) Depends on Phytyl Conformation

Daniel M. Palm,[†,∥] Alessandro Agostini,*,[†,§,∥] Anne-Christin Pohland,[†] Mara Werwie,[†] Elmar Jaenicke,[‡] and Harald Paulsen*,[†]

[†]Institute of Molecular Physiology, Johannes Gutenberg-University, Johannes-von-Müller-Weg 6, 55128 Mainz, Germany
[‡]Institute of Molecular Physiology, Johannes Gutenberg-University, Jakob-Welder-Weg 26, 55128 Mainz, Germany

S *Supporting Information*

ABSTRACT: Water-soluble chlorophyll proteins (WSCP) from *Brassicaceae* form homotetrameric chlorophyll (Chl)−protein complexes binding one Chl per apoprotein and no carotenoids. Despite the lack of photoprotecting pigments, the complex-bound Chls displays a remarkable stability toward photodynamic damage. On the basis of a mutational study, we show that not only the presence of the phytyls is necessary for photoprotection in WSCPs, as we previously demonstrated, but also is their correct conformation and localization. The extreme heat stability of WSCP also depends on the presence of the phytyl chains, confirming their relevance for the unusual stability of WSCP.
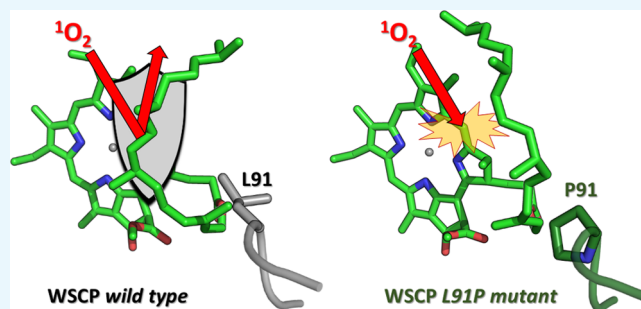
INTRODUCTION

Since the first appearance of oxygenic photosynthesis about 2.5 billion of years ago,[1,2] the chlorophyll (Chl) *a*-based photosynthetic process has profoundly shaped Earth's biosphere. Chl *a* has become a ubiquitous molecule, a signature of life that is visible from space, making Earth the "green planet". This molecule has been selected during evolution presumably because of its ability to absorb and pass on excitation energy during light-harvesting and to undergo light-driven charge separation in the photosynthetic reaction centers.[3] The molecular properties that make Chl *a* capable to perform such diverse roles are of great interest and have been widely discussed.[3,4] Primarily, the chlorin ring and its substitutions define the electronic properties of the chromophore. Particularly, the intense absorption in the red[5−7] of Chl *a* is of crucial relevance for its functions.[8] The binding of a central metal ion has been shown to contribute to the preservation of the planar conformation of the molecule,[9] and the advantages of magnesium ion over other metal ions have been recently attributed to the favorable tuning of the energy of its triplet state, with a consequent lowering of the probability of singlet oxygen photosensitization.[10]

The role of the phytyl has been scarcely investigated and mostly connected to anchoring the Chls to the transmembrane proteins to which they belong[11,12] and affecting their packing.[13] Fiedor et al.[9] highlighted that the esterification of the C-17^3 propionate also avoids the self-chelation of the central Mg^{2+}.

A class of Chl-binding complexes in which phytyls seem to play a pivotal role is that of the non-photoconvertible water-soluble chlorophyll proteins (WSCP). These proteins, found in plants belonging to the *Brassicaceae* family, are quite distinct from the majority of the Chl-binding proteins. WSCP is not involved in the photosynthetic processes, as it is not a membrane protein and is not even located in the chloroplast.[14] WSCP consists of a homotetrameric complex containing four subunits of about 20 kDa, each binding one Chl molecule (Figure 1).[15−17] A recombinant version can be reconstituted with either 4 or 2 Chls per protein tetramer.[17,18] The biological function of WSCP is still enigmatic,[14,19−21] but it is reasonable to assume that it is related to the remarkable stability of the pigment−protein complex. Tetrameric WSCP resists dissociation and denaturation even at 100 °C.[17,22−25] Additionally, the complex is astoundingly photostable,[17,21,23] even though it does not bind any carotenoids that are commonly involved in the photoprotection of Chl-binding proteins.

A close inspection of the crystal structure of *L. virginicum* WSCP wild type (Lv-wt)[15] reveals that the four phytyl chains protruding from the Chl planes interact at the center of the Chl-binding cavity (Figure 1). This hydrophobic interaction was assumed to be the driving force for triggering the tetrameric assembly of WSCP complexes,[15] since it was thought that in the absence of phytyl chains, no oligomerization of WSCP occurred.[23] However, in a previous publication, we have shown that WSCP also tetramerizes upon reconstitution of WSCP apoprotein with chlorophyllide (Chlide) *a*, a Chl *a* derivative lacking the phytyl chain.[21]
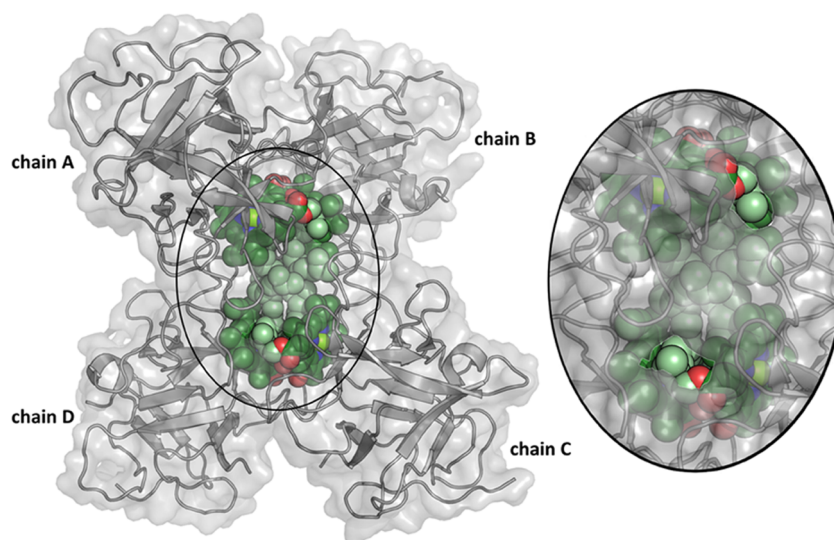
Figure 1. Structure of *Lepidium virginicum* WSCP wild type (Lv-wt) (PDB entry: 2DRE),[15] with the polypeptide chains shown in gray cartoons, the protein surface in transparent gray, and the Chls *a* in spheres. The chlorin macrocycle is colored with carbon atoms in dark green, nitrogen atoms in blue, oxygen atoms in red, and magnesium atoms in green. The carbon atoms of the phytyl chains are colored in light green. The inset on the right is a close-up view of the pores facing the Chls.

These tetrameric Chlide−protein complexes exhibited similar spectroscopic properties as their phytyl-containing counterparts.[21,24] However, complexes of Lv-wt reconstituted with Chlide *a* showed significantly reduced protection of their Chls toward photodynamic damage, as revealed by photobleaching measurements,[21] and the complexes of *Arabidopsis thaliana* WSCP reconstituted with Chlides *a* and *b* were previously shown to be less stable toward dissociation.[24] Therefore, the phytyl group appears to play a key role in the remarkably high photo- and heat stability of the WSCP complex.

The recently solved structure of the L91P mutant of Lv-wt (Lv-L91P, see Table S1), where leucine is replaced by proline in the LCPS affinity motif, revealed striking differences regarding the conformation of the phytyl chains in comparison with the wt complex (Figure 2).[26] In Lv-wt, the bulkiness of the leucine residue forces the phytyl chains toward the Chl macrocycles. The closest distance between the L91 residue and the phytyl chain is about 4 Å. When L91 is changed to the less bulky P91, more space between the Chl macrocycles and the backbone is available, allowing a reorganization of the phytyl chains. Consequently, they move away from the Chl macrocycles. A similar effect was reported by Saer et al.[27] in the reaction center of *Rhodobacter sphaeroides*, where the mutation of residue M214 was shown, by solving the crystallographic structure, to affect the conformation of the phytyl chain of one of the accessory bacteriochlorophyll *a*.

Intriguingly, in Lv-L91P, the two phytyl chains within one open-sandwich dimer adopt different conformations, with only one of the two protruding from the Chl planes toward the center and thus breaking the symmetry of the Chl dimer (Figure 2).

Due to the important role of the phytyl chains in stabilizing the WSCP complex,[21,24] the question arose whether the altered phytyl conformation in Lv-L91P-bound Chls has an impact on their photo- and heat stability. To address this question, we compared Lv-wt and its L91P mutant with regard to their photo- and heat stability. By comparing the results of the Chl-reconstituted Lv-wt mutants with those collected for Lv-wt

reconstituted with Chlide, we were able to assess the structural role of the phytyl chain and its conformation on the WSCP stability.

■ RESULTS AND DISCUSSION

Phytyl Chain Is Required to Stabilize WSCP Complexes against Thermal Degradation. To gain more insight into the role of the phytyl groups in stabilizing WSCP toward thermal degradation, Lv-wt reconstituted with the phytyl-free Chl derivatives (Chlide *a* and Chlide *b*) was investigated. To quantify the complex integrity of the different WSCPs upon heat exposure, we measured vis-circular dichroism (CD) spectra before and after a 5 min boiling treatment (Figure 3). CD spectra are good indicators for the complex integrity because they depend on both pigment−protein and pigment−pigment interactions. In agreement with a previous study on *A. thaliana* WSCP reconstituted with Chlides *a* and *b*,[24] also *L. virginicum* WSCP reconstituted with either Chlide *a* or *b* were found to be significantly destabilized, as evident from the significant loss of CD signal upon heat treatment (57 and 32% for Chlide *a*- and *b*-reconstituted complexes, respectively, Figure 3A,B) in comparison to that of the complexes reconstituted with Chls (6 and 4% for Chl *a* and Chl *b*, respectively; see Table 1).

In the case of Lv-wt, a crystallographic structure is available[15] (by contrast to *A. thaliana* WSCP) and therefore it is possible to analyze the interactions between the phytyls and their surroundings. In the complex, each of the four phytyl chains is in van der Waals contact with a cluster of hydrophobic side chains constituted by L41, L153, and W154 of the subunit binding the corresponding Chl and the side chains of the same three amino acids of the opposing WSCP subunit (A with C and B with D, see Figure 1). The contribution of the phytyl chains to these hydrophobic interactions in the core of WSCP seems to be necessary for the extraordinary stability of the Chl−protein complex, as the absence of the phytyl chains led to a severe loss of complex heat stability (Figure 3). However, Chlides lacking the phytyl chains are still able to support tetramerization of Lv-wt,[21] albeit
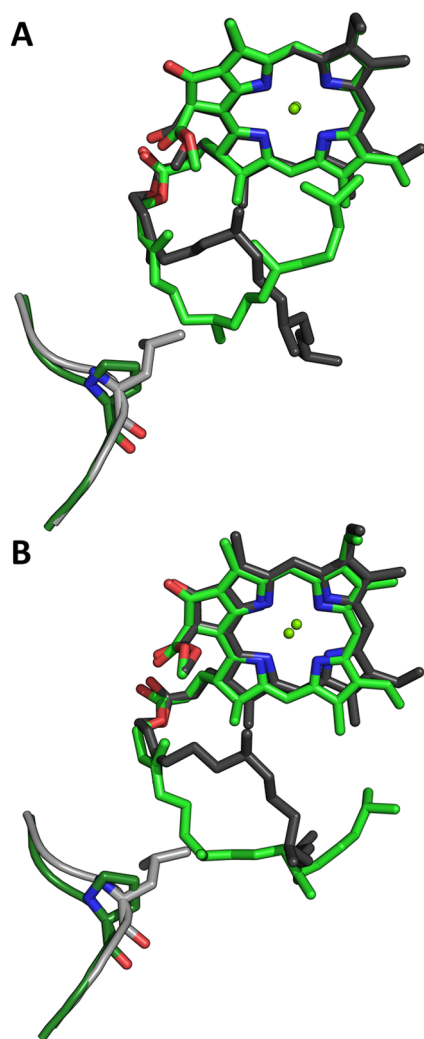
Figure 2. Details of the phytyl conformation in Lv-wt[15] (Chl *a* in dark gray, L91 residue in gray) and Lv-L91P[26] (Chl *a* in green, P91 residue in dark green). To ease the inspection, in panel (A), the interaction between the L/P91 residues (of chain A) with Chls *a* of chain B is shown. Panel (B) shows the interaction of the L/P91 (of chain B) residues with the Chls *a* of chain A.

at lower complex stability, whereas in the absence of any Chls, no tetramers form.[17] This indicates that the chlorin rings have a stabilizing effect as well.

As in Lv-L91P the phytyl conformation of the bound Chls is altered,[26] by changing the interactions between the phytyls and their surroundings, we were interested in determining whether the hydrophobic interactions exerted by these phytyls still stabilize the complexes against heat denaturation the way they do in the wt structure. As evident from Figure 4, the CD spectra of Lv-L91P Chl *a* and Lv-L91P Chl *b* did not significantly change after the boiling treatment (Figure 5A,B). They exhibited a similar stability, as reported earlier for *Brassica oleracea* WSCP wild type[17,23,25] and Lv-wt.[25] The calculated losses of signal intensity were 7% for Lv-L91P Chl *a* and 8% for Lv-L91P Chl *b* and are comparable to that of Lv-wt (see Table 1).

We conclude that the differences in the conformation of the phytyls in L91P, with respect to Lv-wt, do not seem to affect the heat stability of the complex. In Lv-L91P, only two of the four phytyl chains, each from an opposing Chl dimer, point to
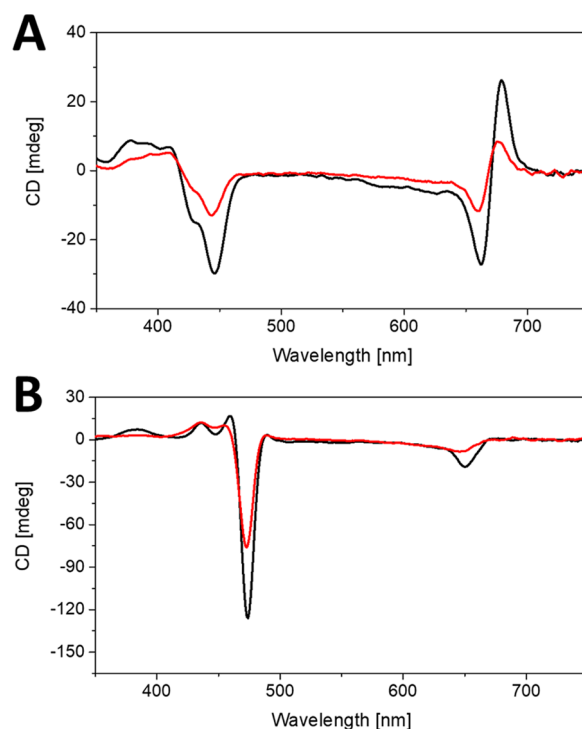
Figure 3. Heat stability of Lv-wt reconstituted with Chlide *a* (A) and Chlide *b* (B). Vis-CD spectra were recorded before (black) and after (red) a 5 min boiling treatment. The spectra were normalized to the maximum of their corresponding Chlide Q_y absorption band.

Table 1. Photo- and Heat Stability of Different WSCP Variants Reconstituted with either Chl *a* or Chl *b*[a]

	loss of CD signal (%)		F_{60}/F_0 (%)	
	Chl *a*	Chl *b*	Chl *a*	Chl *b*
Lv-wt	6	4	56[b]	54
Lv-L91V	11	5	29	33
Lv-L91A	12	6	18	27
Lv-L91P	7	8	13	25
Lv-L91G	18	9	11	24

[a]Heat stability was evaluated by comparing the integrals of vis-CD spectra (350−750 nm) before and after a 5 min boiling treatment. Photostability was quantified upon illumination of the recombinant WSCPs under medium-strong light conditions (500 μmol of photons m^{-2} s^{-1}) for 0−60 min. The samples were adjusted to the same Chl OD (0.005 at Q_y maximum). Emission maxima after illumination (F) were measured upon excitation at the Soret band maximum and compared to the initial maximum before illumination (F_0). F_{60}/F_0 displays the loss of Chl fluorescence after 60 min of illumination. [b]Agostini et al.[21]

the center of the complex. Inspection of the crystal structure of Lv-L91P reveals that each of the two protruding phytyls is in van der Waals contact with the side chains of L41, L153, and W154, similarly to the wt. Apparently, their presence in the WSCP core suffices to stabilize the Lv-L91P toward dissociation to the same extent as seen in the Lv-wt.

Side-Chain Length Modifications at Position 91 Do Not Impair Tetramerization and Heat Stability. Since apparently the side-chain length of the amino acid at position 91 strongly affects the conformation of the phytyl chains (as evident from the L91P mutation), we extended this mutational study by investigating the effect of three more mutations with decreasing side-chain lengths (valine, alanine, and glycine) at
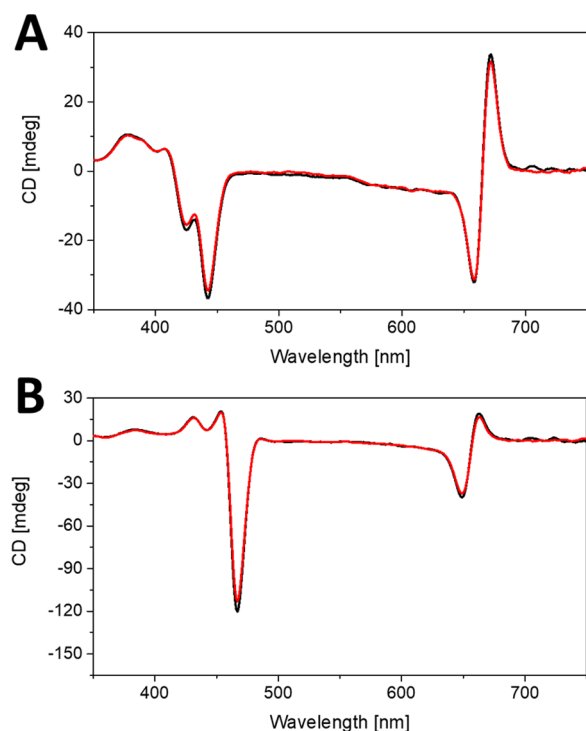
Figure 4. Heat stability of Lv-L91P reconstituted with Chl a (A) and Chl b (B). Vis-CD spectra were recorded before (black) and after (red) a 5 min boiling treatment. Spectra were normalized to the maximum of their corresponding Chl Q_y absorption band.

position 91 (L91V, L91A, and L91G, respectively; see Table S1 and Figure 5).

All four studied mutants of Lv WSCP (L91P, L91V, L91A, and L91G) were successfully reconstituted with either Chl a or b, leading to tetrameric complexes, as evaluated from size exclusion chromatography (data not shown). CD spectra have been recorded in the UV region to assess the secondary structure of the proteins, to verify if the mutations affected the overall fold of the complexes. All variants were found to be characterized by a UV-CD spectrum profile coincident to the one of the parent wt variants (Figure S1), with an intense negative band between 190 and 220 nm, in good agreement with the high content of β-strands[28] seen in the X-ray structure.[15] In this band, two negative peaks are seen at 196 and 203 nm, with the former being the predominant, as expected for WSCP complexes containing four Chls.[17] Therefore, we could exclude any detectable rearrangement of the loops and β-strands of the proteins to be caused by the investigated mutations.

The absorption and vis-CD spectra of Lv-wt and the four mutants reconstituted with Chl a were nearly identical (Figure

6A shows the L91P mutant, Figure S2A shows L91V, L91A, and L91G). This indicates that all mutated proteins bind Chls at the same Chl−protein stoichiometry, forming Chl dimers with an open-sandwich geometry. Similar data were obtained upon reconstitution of the mutants with Chl b (Figure S2B), with the exception of the L91P mutant (Figure 6B).

When Lv-L91P was reconstituted with Chl b, a significant variation in the relative intensities between the Soret and Q_y region, as well as a 2 nm red shift of the Q_y absorption was observed in comparison with Chl b Lv-wt (Figure 6B). In addition, the maximum of the Soret band absorption in Chl b Lv-L91P was shifted by 3 nm to the blue. The vis-CD spectra show differences between L91P and Lv-wt with respect to the circular dichroism in both Q_y and Soret bands of bound Chl b molecules.

As the CD signals in the Q_y are attributed to the characteristic open-sandwich conformation of the Chls bound,[29,30] the observed alterations in Chl b Lv-L91P, with a more conservative CD signal, indicate a modified Chl−Chl interaction, presumably originating from a modified Chl−protein interaction. This putatively altered Chl−Chl interaction in the case of Chl b bound to Lv-L91P has been correlated to the variation of the hydrogen-bond network involving the formyl group of Chl b by comparing the crystal structure of Chl b Lv-L91P with that of Lv-wt.[26] In Lv-wt, the formyl group of Chl b is expected to be directly hydrogen-bonded to the backbone nitrogen of L91 located in the affinity motif LCPS, whereas, in the case of the L91P mutant, this direct hydrogen bond is replaced by a water-bridged interaction with the backbone nitrogen of C92, since no hydrogen-bond donor is provided by P91.[26] We assume that this difference alters the Chl orientation in its binding site and, consequently, the Chl−Chl interaction within the open-sandwich dimer. This assumption is supported by the vis-CD spectra of the Chl b-reconstituted mutants (L91V, L91A, and L91G). In all of these mutated proteins, the residues in position 91 provide a hydrogen-bond donor like in Lv-wt and consistently they all exhibit nonconservative CD signals same as those of the wt (see Figure S2).

The heat stability of these mutants was evaluated, similar to that for Lv-L91P. Similar results were obtained for the other mutants (Figure S3 and Table 1, all calculated losses of complex integrities were clearly below 20%). Intriguingly, the Chl a-reconstituted as compared with the Chl b-reconstituted complexes seem to be somewhat less stable, as their calculated losses were roughly twice as high. As the only difference between the two pigments is the presence of a formyl group in Chl b, it is tempting to attribute the observed difference to the capability of Chl b to establish an additional H-bond with its surrounding, with a consequent increase in the stability. Congruently, it has been observed, in the case of the major
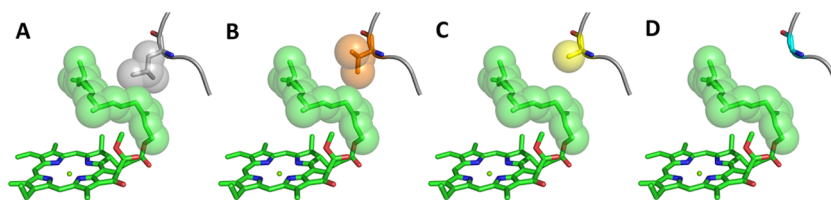
Figure 5. Comparison between Lv-wt (A), Lv- L91V (B), L91A (C), and L91G (D) (with residue 91 in gray, orange, yellow, and light blue, respectively). The structure of Lv-wt[15] is shown, with the polypeptide chain in gray cartoons and the Chls a in green. The mutations have been introduced in silico with PyMOL Mutagenesis Tool. Phytyls and the side chains of residue 91 have been highlighted in spheres.
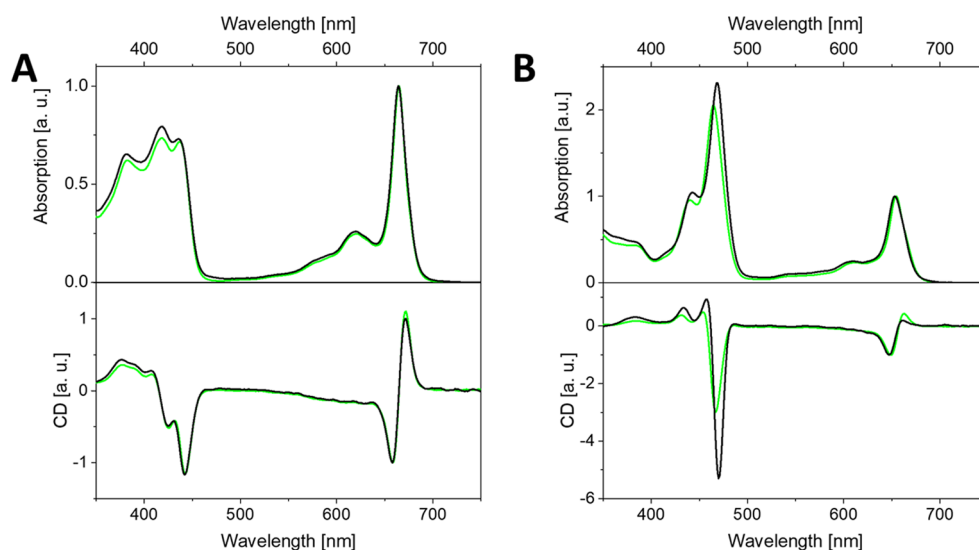
Figure 6. Spectroscopic comparison of Lv-wt (black) and Lv-L91P (green). Absorption (top) and vis-CD (bottom) spectra after reconstitution with Chl a and after reconstitution with Chl b (A and B, respectively). Absorption spectra were normalized to 1 at the Q_y maxima. Vis-CD spectra were normalized to −1 at the Q_y minima.
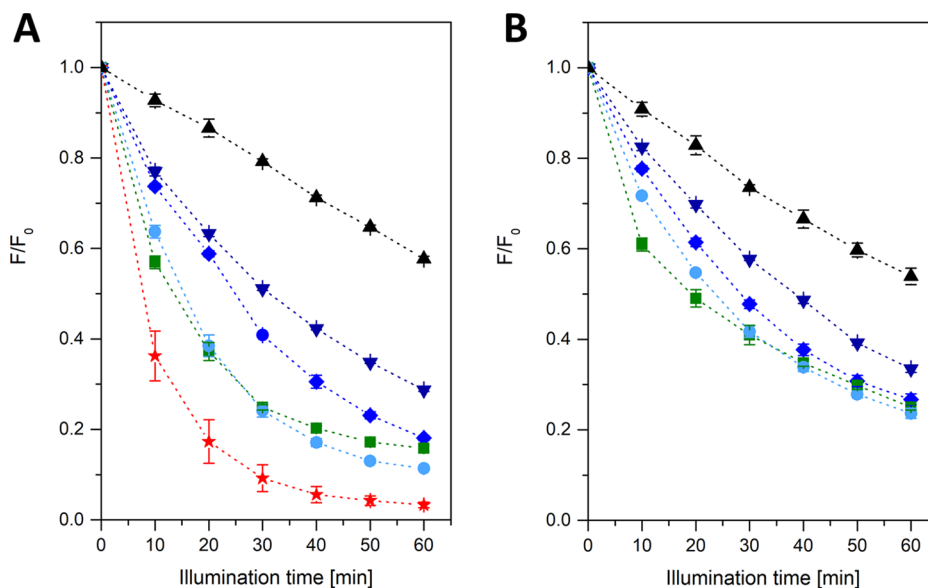

Figure 7. Photostability of Lv WSCP variants reconstituted with Chl a or Chl b (panel A and B, respectively). Lv-wt, black triangles; Lv-L91V, dark blue inverted triangles; Lv-L91A, blue diamonds; Lv-L91G, light blue circles; Lv-L91P, dark green squares. Chl a in 2% (w/v) OG (red stars) is reported for comparison in panel A.[21] All recombinant WSCPs were illuminated under medium-strong light conditions (500 μmol of photons m^{-2} s^{-1}) for 0−60 min. The samples were adjusted to the same Chl OD (0.005 at Q_y maximum). Emission maxima after illumination (F) were measured upon excitation at Soret band maximum and compared to the initial maximum before illumination (F_0). F/F_0 was plotted vs illumination time. Data are expressed as mean values ($n = 3$).

light-harvesting complex (LHCII, a plant antenna protein that binds both Chl a and b) that the loss of Chls b affects its stability more than the loss of Chls a.[31,32]

Impact of the Phytyl Chain Conformation on the Photostability. In previous studies, we have shown that Chls bound to WSCP are accessible[33] and can efficiently photosensitize oxygen to singlet oxygen ($^1O_2$)[21] but even so undergo only little photodynamic damage,[17,21,23] even in the absence of any protecting carotenoids. The underlying photoprotective mechanism that we proposed[21] is based on the capability of the phytyl chains either to shield methine 20 of the Chl macrocycle, which has been shown to be prone to the reaction with $^1O_2$,[34,35] or to limit the interaction of the oxygen with the

magnesium ion, decreasing the residency time of the singlet oxygen near the Chl macrocycle[36] and consequently decreasing its probability to react. This mechanism has been proposed on the basis of the loss of photostability of the WSCP complex when Chlide a is bound in place of Chl a, similar to the loss of heat stability presented and discussed above (Figure 3).

The role of the phytyls in the photoprotection of WSCP-bound Chls, shielding the more reactive portions of the chlorin macrocycle, relies on the presence of the Chl phytyl chains with a specific localization in the complex. Therefore, it is expected to be highly dependent on the phytyl conformations, as the phytyl chains are required to be in van der Waals contact with the portions of the macrocycle involved in the reaction
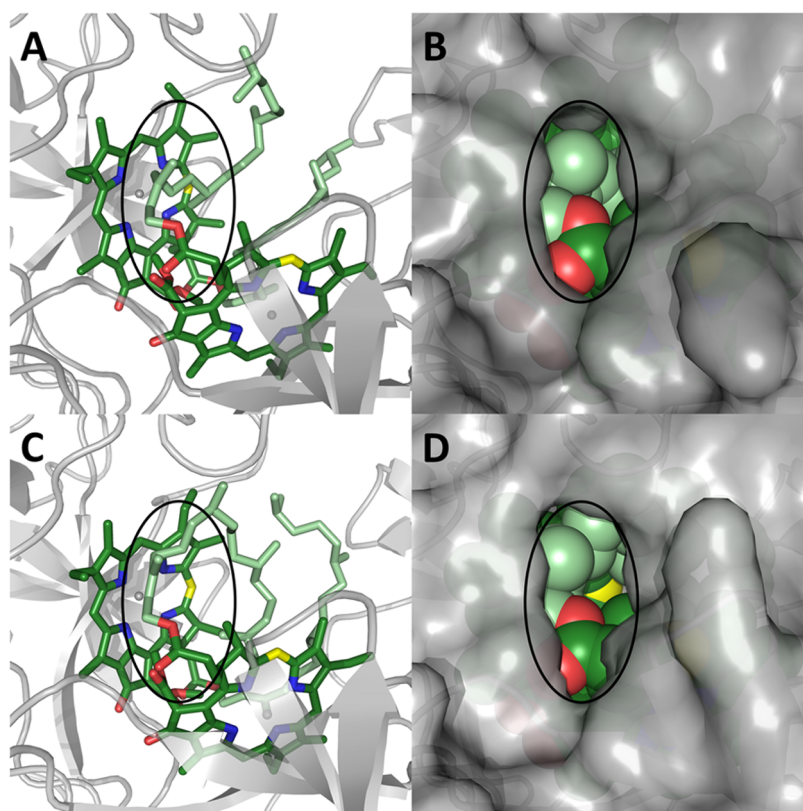
Figure 8. Comparison between the structures of Lv-wt[15] (panels A and B) and Lv-L91P[26] (panels C and D), focused on the solvent-accessible part of the Chl molecule in the cavity (highlighted with a black ellipse). The same view is reported in the four panels, with the polypeptide chains in gray cartoons (protein surface in transparent gray in panels B and D) and the Chls *a* constituting a dimer shown either in sticks (A and C) or in spheres (B and D). The chlorin macrocycle is colored with carbon atoms in dark green, the methine carbon 20 in yellow, nitrogen atoms in blue, oxygen atoms in red, and magnesium atoms in gray. The carbon atoms of the phytyl chains are colored in light green.

with $^1O_2$. As the Lv-L91P complex has been shown to exhibit a strikingly different phytyl conformation (Figure 2),[26] we have tested whether in Lv-L91P the bound Chls are still protected against photodamage. To address this question, photodynamic damage was monitored, as previously reported,[17,21,23] by measuring the decrease of fluorescence intensity upon photooxidation of the Chls. After 1 h of white light exposure at 500 μmol photons m^{-2} s^{-1}, Chls *a* bound to Lv-L91P exhibited a loss of roughly 80% of their initial fluorescence, a value that is 2-fold higher than that seen in the Lv-wt complex (Figure 7). In the same time interval of illumination, Chl *a* as a control dissolved in 2% OG (w/v) has lost almost all (98%) of its initial fluorescence, as has been shown previously.[18] Obviously, the exchange of leucine at position 91 with proline has a drastic effect on the photostability, leading to the question of whether this is a consequence of the less bulky side chain of P91 with respect to that of L91. To address this question, photobleaching measurements with Lv mutants exhibiting different side-chain lengths in position 91 (L91V, L91A, and L91G) were performed under identical experimental conditions.

By comparing the different mutants (L91P, L91V, L91A, and L91G) with regard to their ability to protect the bound Chls from photodynamic damage, it clearly appears that the loss of photostability is gradual, exhibiting an inverse correlation to the size of the side chain of the residue in position 91 (Figure 7A and Table 1). This strongly suggests that the more the bulkiness of the amino acid side chain in position 91 is reduced (in the series from L via V, A, P, to G),

the more the phytyl chain will be displaced from its position in the wt structure, leading to a gradually decreased photostability.

The same photobleaching measurements were extended to Chl *b*-reconstituted complexes (Figure 7B and Table 1), obtainining a similar trend (L ≫ V > A, P, G). Whereas Lv-wt complexes reconstituted with the two Chls have nearly identical photostability, the Chl *b*-reconstituted mutants show a slightly smaller loss of photostability when compared with the corresponding Chl *a*-containing complexes. This difference does not seem to be affected by the capability of residue to provide a hydrogen-bond donor, as the L91P follows the same trend, and the difference in photostability seems to be greater for the variants with smaller side chains of residue 91. Therefore, these results corroborate the hypothesis that the observed difference in the photostability has to be assigned to a difference in the space available for the phytyl chains to adopt their conformation.

The crystal structure of Lv-wt[15] reveals that the phytyls of bound Chls form a steric barrier between the chlorin rings and the solvent masking the portions of the macrocycle ring proposed to be involved in the reaction with $^1O_2$[34−36] (Figure 8A,B), thus protecting the pigments from photobleaching.[21] Analyzing the structure of Lv-L91P,[26] it appears that, by contrast, the reactive portions of the chlorin macrocycle seem to be accessible (Figure 8C,D). Upon reorganization of the phytyl chains of the Chls, induced by the less bulky P91 residue (see above), a channel appears to be formed providing access for the $^1O_2$ to the methine 20 of the Chl macrocycles

and to the magnesium ion as well. This may explain why the Chls are not photoprotected any more upon exchanging leucine with proline at position 91. We propose that in the series of amino acids in position 91 (L, V, A, G, P) analyzed in this study, the gradual loss in photostability is explained by the phytyl conformation being gradually altered upon the reduction of steric hindrance exerted from the amino acid side chain, leading to a gradually reduced protection of the chlorin ring that has to be ascribed to a less effective masking of the reactive portions of the macrocycle.

The modification of the available space inside the cavity, besides allowing for changes in the static structure of the phytyl chains (see Figure 2), in principle, could also cause an increase of the phytyl conformational freedom. We compared the B-factors of the atoms of the Chls bound in the two structures (Figure 9), revealing a similar pattern with a certain degree of
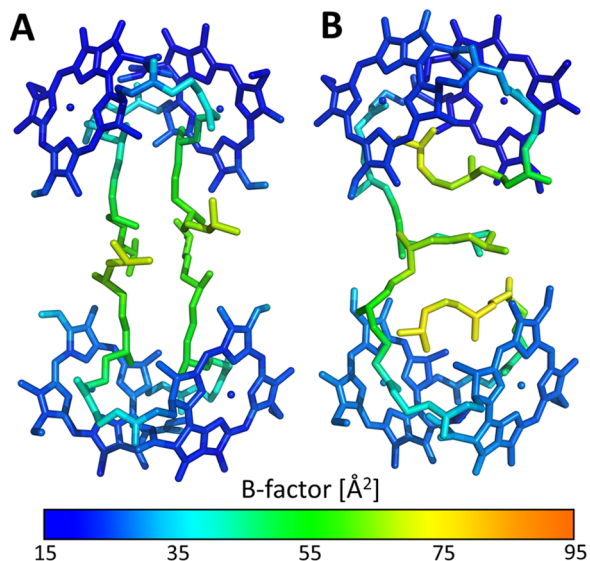
Figure 9. Comparison between the structures of Lv-wt[15] (panel A) and Lv-L91P[26] (panel B), focused on the B-factors of the atoms of the Chl molecules. The same view is reported in the two panels, with the Chls *a* shown in sticks.

flexibility in the phytyl chains in comparison with the high rigidity in the localization of the atoms of the chlorins. The phytyls in the L91P are slightly more flexible, a trait that could also have concurred to the loss of photoprotection of the chlorins.

An alternative explanation for the dependence of the photostability of the bound pigments to mutations of L91 would be that the different amino acid side chains interact differently with $^1O_2$. Since all amino acids investigated in this study have an aliphatic side chain (or a hydrogen atom in the case of glycine), their direct reaction with $^1O_2$ can be excluded. Moreover, the residues are too far away from the portion of the chlorin ring involved in the reaction with $^1O_2$[21,34−36] to be masking these parts of the chlorin ring toward $^1O_2$ access. Therefore, we conclude that photoprotection of the bound Chls in WSCP[21] depends not only on the presence of the phytyl chains but also on their correct localization, enforced by the protein surrounding, within the two adjacent Chl macrocycles in the open-sandwich dimer.

CONCLUSIONS

The biochemical and photophysical data described in this paper highlight that both phytyl−phytyl and phytyl−protein interactions in the WSCP core are involved in generating the unusual photo- and heat stability of WSCP that characterize these plant proteins, confirming that phytyl chains play a central role in the WSCP complex. In particular, we have shown that the remarkable heat stability of WSCP depends on the presence of its Chl phytyl chains that interact with one another, with both chlorin rings in the open-sandwich dimer, and with several amino acid side chains of neighboring protein subunits.

The photoprotection of Chls in WSCP relies anyway on the isoprenoids, even in the absence of carotenoids, in the form of the phytyl chains. The recently proposed phytyl-based photoprotective mechanism of WSCP is not only dependent on the presence of the phytyl moiety but even on its correct conformation. Amino acid exchanges in a single position in the apoprotein affect the localization of phytyls with regard to their chlorin rings, modifying the ability of phytyls to photoprotect these pigments. These findings highlight how interactions between Chl substructures and their protein environment determine the properties of Chl−protein complexes.

MATERIALS AND METHODS

Sample Preparation. Mutagenesis and transformation of DNA encoding Lv-wt (uniprot: O04797) and various single mutants (see Table S1) were performed as described before.[26] Recombinant protein expression and purification and its reconstitution with either Chls or Chlides was carried out as previously described.[26]

Spectroscopy. UV−vis absorption spectra of WSCP complexes in 20 mM sodium phosphate (pH 7.8) were recorded between 750 and 250 nm in a quartz cuvette (10 mm path length) with a scan speed of 200 nm min^{-1} and a bandwidth of 2 nm at room temperature (RT) using a V-550 UV/VIS spectrophotometer (Jasco).

Circular dichroism (CD) was measured with a J-810 spectropolarimeter (Jasco) equipped with a Jasco CDF-426S peltier element for temperature control. CD spectra of WSCP complexes in 20 mM sodium phosphate (pH 7.8) were monitored at 23 °C. In the visible region (750−350 nm), the following parameters were adopted: 2 mm path length, data pitch 1 nm, 100 nm min^{-1} scan speed, response time 4 s, 4× accumulation. In the UV region (300−180 nm), the parameters were as follows: 0.5 mm path length, 0.5 nm data pitch, 50 nm min^{-1} scan speed, 4 s response time, and 9× accumulation.

Steady-state fluorescence emission of WSCP complexes in 20 mM sodium phosphate (pH 7.8) was measured in a FluoroMax-2 instrument (Jobin Yvon-Spex) at RT using a cuvette with the dimensions of 5 × 5 mm^2 (slits 1 nm, increment 1 nm, integration time 1 s). Emission was monitored between 650 and 850 nm with excitation at 410 nm (Chl *a* WSCP) and 430 nm (Chl *b* WSCP).

ASSOCIATED CONTENT

ⓢ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.9b00054.

Mutations introduced in the LCPS motif of Lv-wt (Table S1), UV-CD spectra of Lv-wt, Lv-L91V, Lv-L91A, Lv-L91G, and Lv-L91P WSCP variants reconstituted with Chl *a* (Figure S1), absorption and vis-CD spectra of L91V, L91A, and L91G WSCP mutants reconstituted with Chl *a* and Chl *b* (Figure S2), heat stability of L91V, L91A, and L91G WSCP mutants reconstituted with Chl *a* and Chl *b* (Figure S3) (PDF)

Accession Codes

Lepidium virginicum WSCP: O04797 (UniProtKB), 2DRE (PDB).

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: alessandro.agostini.1@unipd.it (A.A.).
*E-mail: paulsen@uni-mainz.de. Tel: +49 6131 3924633 (H.P.).

ORCID ⓘ

Daniel M. Palm: 0000-0002-6874-3316
Alessandro Agostini: 0000-0002-8877-315X
Harald Paulsen: 0000-0003-0532-3004

Present Address

§Department of Chemical Sciences, University of Padova, via Marzolo 1, 35131 Padova, Italy (A.A.).

Author Contributions

H.P. and E.J. designed the research; A.A. and M.W. designed the WSCP mutants; D.M.P, A.A., and A.-C.P. prepared the WSCP samples and performed and analyzed the spectroscopic measurements; and D.M.P., A.A., M.W., and H.P. wrote the paper.

Author Contributions

||D.M.P. and A.A. contributed equally to this work.

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

CD,circular dichroism; Chl,chlorophyll; Chlide,chlorophyllide; WSCP,water-soluble chlorophyll protein; Lv-wt,*Lepidium virginicum* WSCP wild type

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