Role of Gln1029 in the Photoactivation Processes of the LOV2 Domain in *Adiantum* Phytochrome3[†]

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ABSTRACT: Phototropin (phot) is a blue-light receptor in plants. The molecule has two FMN (flavin mononucleotide)-binding domains named the LOV (light−oxygen−voltage) domain, that is a subset of a PAS (per−arnt−sim) superfamily. Illumination of phot−LOV domains produces a covalent C(4a) flavin−cysteinyl adduct, which is called the S390 intermediate state. According to the crystal structures of the LOV2 domain of *Adiantum* phytochrome3 (phy3), a fusion protein of phot containing the phytochrome chromophoric domain, in the unphotolyzed and S390 states, and the side chain of Gln1029 switches hydrogen bonds with the FMN chromophore. Gln1029 is the hydrogen-bonding donor of the C(4)═O group of FMN in the unphotolyzed state, whereas Gln1029 is the hydrogen-bonding acceptor of the N(5)−H group of FMN in S390. In this paper, we measured the light-induced structural changes in the Q1029L mutant protein of phy3−LOV2 by means of low-temperature FTIR spectroscopy, and the obtained spectra are compared with those of the wild type. Low-temperature UV−visible spectroscopy of Q1029L detected only one intermediate state, S390, at 77−295 K, as well as the wild type. The C(4)═O stretch of FMN at 1710 cm^{-1} is shifted to 1723 cm^{-1} in Q1029L, presumably because of the lack of hydrogen bonds between Gln1029 and FMN. Upon formation of S390, the C(4)═O group hydrogen bond is weakened in both wild type and Q1029L. These observations are fully consistent with the X-ray crystal structures of the unphotolyzed and S390 states. On the other hand, the C(4)═O stretch of FMN and amide-I vibrations are temperature-independent in Q1029L, in contrast to wild type, in which highly temperature-dependent FTIR spectra are detected. Amide-I vibrations of Q1029L at room temperature are similar to those of the wild type at 77−150 K but not at room temperature. These facts imply that the Q1029L mutant protein lacks progressive protein structural changes following flavin−cysteinyl adduct formation in the wild type, which eventually alter structures of β sheet and α helix in the protein moiety. Hydrogen-bonding interaction of Gln1029 with the FMN chromophore likely plays an important role in the protein structural changes of phy3−LOV2.

Plants use visible light as a signal from their environments as well as an energy source for photosynthesis. Plants sense red/far-red and blue light by phytochrome and blue-light receptors (*1*), respectively. One of major blue-light receptors, phototropin (phot), regulates tropic response (*2*), relocation of chloroplast (*3*), and opening of stomata (*4*), that are deeply involved in improving the efficiency of photosynthesis. Higher plants have two isoforms of phot, phot1 and phot2, that share these responses by their different sensitivities to light (*5*). In addition to phot1 and phot2, *Adiantum* has a fusion protein of phot in its C terminus with the chromophoric domain of phytochrome in its N terminus, which is named phytochrome 3 (phy3) (*6*) and acts as a red-light sensor for *Adiantum* to survive in the canopy of forests (*7*).

Phototropins are composed of about 1000 amino acid residues and two prosthetic flavin mononucleotide (FMN)[1] molecules. The N-terminal half has two chromophoric domains, which noncovalently bind FMN molecules, while the C-terminal half includes Ser/Thr kinase motifs. The two chromophoric domains (ca. 100 residues) are named LOV1 and 2, because they have highly homologous structures to those of the LOV (light, oxygen, and voltage sensing) domain (*8*, *9*), which is a subset of the PAS (per−arnt−sim) superfamily. Light enhances autophosphorylation of phot molecules both *in vivo* and *in vitro* (*10*, *11*). Although the involvement of the autophosphorylation in the signal transduction and the presence of a substrate(s) for the kinase activity have not been shown, photon energy absorbed by the FMN chromophores regulates the kinase activity through

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[1] Abbreviations: FMN, flavin mononucleotide; LOV, light−oxygen−voltage; PAS, per−arnt−sim; FTIR, Fourier transform infrared; UV, ultraviolet.

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unknown molecular processes occurring in both the chromophore and the protein moieties.

The initial step of the blue-light sensing is the photochemical reaction of FMN in LOV domains. Various techniques have shown that the primary reaction in phot is an adduct formation between FMN and a nearby cysteine (*12*−*16*). After FMN light absorption, intersystem crossing leads to the formation of a triplet excited state absorbing at 660 nm (L660), appearing with a time constant of 3 ns in *Adiantum* phy3−LOV2 and oat phot1−LOV2 (*17*). This process is followed by an adduct formation, which accompanies the formation of the S390 intermediate with a time constant of 4 μs in oat phot2−LOV2 (*14*) and with 0.9 and 4 μs in *Chlamydomonas* phot−LOV1 (*18*). Swartz et al. proposed an ionic reaction pathway for the adduct formation between FMN and the deprotonated cysteine in the excited triplet state (*14*). However, our FTIR study revealed that the cysteine is protonated in the unphotolyzed state (*19*). Instead, Kay et al. proposed a radical-pair mechanism through the protonated cysteine based on EPR and electron−nuclear double resonance studies (*20*). The molecular mechanism of the adduct formation remains unsolved.

One of the characteristic features in the photoreaction of LOV domains is that it leads to the formation of only one ground-state product (S390), which is very different from other biological light-sensor proteins (*14*). Nevertheless, previous low-temperature FTIR spectroscopy studies suggested the presence of progressive protein structural changes following flavin−cysteinyl adduct formation in the photoactivation processes of phy3−LOV2 (*21*). This fact suggests that the protein changes its structure progressively, whereas these changes do not affect the electronic state of FMN. Therefore, the question arises how large these protein changes are. X-ray crystallography of S390 of *Adiantum* phy3−LOV2 (*16*) and *Chlamydomonas* phot−LOV1 (*22*) has indicated very similar protein structure in S390 and in the unphotolyzed state (D447), particularly at the protein surface. This observation questions the mechanism by which LOV domains transmit signals following light perception. Nevertheless, our FTIR studies suggested global protein motion based on progressive spectral changes in the amide-I region that probes the peptide backbone structure (*21*). These studies indicated a secondary structural change of turn at low temperatures, while that of the sheet was observed at high temperatures (*21*). Thus, we concluded that local structural changes at the turn moiety lead to global structural changes involving sheet and helical structures. Protein structural changes in the crystal may take place to lesser extent mainly nearby chromophores relative to the solution (*23*), and various techniques are required to evaluate the molecular mechanism of the light-signal transduction in LOV domains.

X-ray crystallography studies of S390 revealed an interesting motion of an amino acid. In the unphotolyzed state, Gln1029, located at the βE sheet, forms a hydrogen bond with the C(4)═O group of FMN, where Gln1029 is the hydrogen-bonding donor (Figure 1a). After light absorption and formation of S390, the side-chain oxygen of Gln1029 forms a hydrogen bond with the N(5)−H group of FMN, where Gln1029 is the hydrogen-bonding acceptor (Figure 1b). Thus, Gln1029 experiences side-chain rotation accompanying adduct formation between FMN and Cys966. The switch of the Gln1029 hydrogen-bonding partner should be important in forming the stable structure of S390 and may trigger the global structural changes of the sheet. In fact, glutamine at this position is highly conserved among LOV domains (among 36 LOV domain proteins) (*24*).

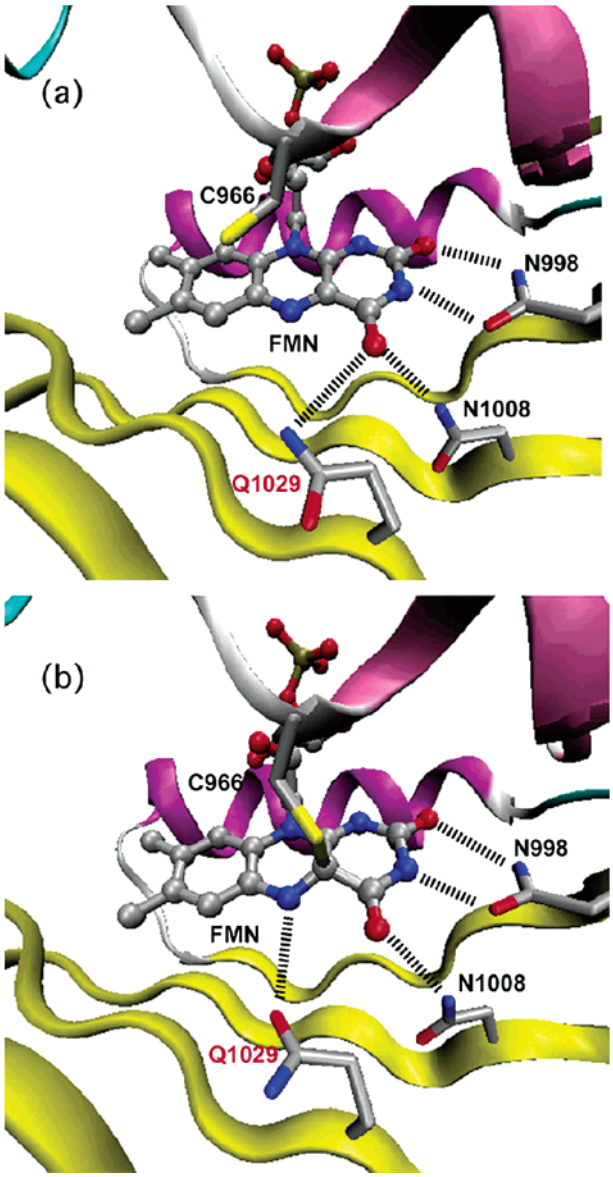

FIGURE 1: Structure of the chromophore-binding domain of Phy3−LOV2 in the unphotolyzed state (a) and in the S390 intermediate state (b) from PDB entry 1G28 and 1JNU, respectively. FMN is shown by a ball-and-stick drawing, and C966, N998, N1008, and Q1029 are shown by a stick drawing, where the colors of the atoms are according to the CPK model. The ribbon string represents the peptide backbone structures, where helices, turns, and sheets are colored by red, white, and yellow, respectively. Dotted lines represent possible hydrogen bonds. Q1029 is located at the βE sheet, and the side-chain nitrogen forms a hydrogen bond with the C(4)═O group of FMN in the unphotolyzed state (a). In S390, the side-chain oxygen of Q1029 forms a hydrogen bond with the N(5)−H group of FMN, which requires the rotational motion of Q1029 to switch the hydrogen-bonding partner.

In this paper, we measured the light-induced structural changes of the Q1029L mutant of *Adiantum* phy3−LOV2 by means of low-temperature UV−visible and FTIR spectroscopy and compared the IR spectra with those of the wild type. The C(4)═O stretch of FMN at 1710 cm^{-1} is shifted

to 1723 cm^{-1} in Q1029L, presumably because of the lack of hydrogen bonding between Gln1029 and FMN. Upon formation of S390, the hydrogen bond of the C(4)═O group is weakened in both the wild type and Q1029L. These observations are fully consistent with the X-ray crystal structures of the unphotolyzed and S390 states. On the other hand, FTIR spectra such as the C(4)═O stretch of FMN and amide-I vibrations are temperature-independent in Q1029L, while highly temperature-dependent FTIR spectra are obtained for the wild type. Amide-I vibrations of Q1029L at room temperature are similar to those of the wild type at 77−150 K but not at room temperature. The role of Gln1029 in the photoactivation processes of phy3−LOV2 is discussed based on the present FTIR observations.

MATERIALS AND METHODS

The pCAL−Phy3−LOV2Q1029L mutant was generated using site-directed mutagenesis by overlap extension using the polymerase chain reaction (PCR) with mutagenic primers. The mutagenic primers were 5′-CATCGGAGTTCTACAG-GAGAT-3′ and 5′-ATCTCCTGTAGAACTCCGATG-3′. The underlines indicate the positions of substituted bases. The flanking primers were pCAL-N5 5′-GAATTTCAT-AGCCGTCTCAGC-3′ and T7 5′-GTAATCGACTCAC-TATAGGGC-3′. The PCR products were cloned to the *Eco*RI−*Xho*I site of the pCAL vector after digestion with *Eco*RI and *Xho*I. The desired mutation was confirmed by DNA sequencing. Overexpression and purification of CBP−phy3−LOV2 were carried out as described previously (*21*). The purified protein was dialyzed against a 1 mM K/phosphate buffer (pH 7) and supplied to the film preparation.

UV−visible and infrared spectra of the hydrated films were measured using V-550DS (JASCO) and FTS-7000 (Bio-Rad) spectrophotometers, respectively, as described previously (*21*). Low-temperature spectra were measured by using a cryostat (Optistat DN, Oxford, U.K.) and a temperature controller (ITC 4, Oxford, U.K.) with liquid nitrogen as the coolant. The detailed experimental conditions are described previously (*21*).

RESULTS AND DISCUSSION

UV−Visible Spectra of the Q1029L Mutant and Wild-Type Proteins. Figure 2 shows light minus dark difference UV−visible spectra of the wild type (upper trace) and Q1029L mutant (lower trace) proteins of phy3−LOV2 measured at room temperature. The spectrum of the wild type is almost identical to those reported previously (*21*). The spectrum of Q1029L is very similar to that of the wild type, indicating that S390 is formed in the Q1029L mutant. Almost identical difference spectra were obtained for Q1029L at lower temperatures, indicating that only one photointermediate in the electronically ground state, S390, is formed in the wide temperature range from liquid nitrogen to ambient (data not shown). Although S390 can be formed at all of the temperatures measured for Q1029L, only a part of the unphotolyzed state was photoconverted to S390 at low temperatures, similar to the wild type (data not shown) (*21*). Two negative peaks in the wild type at 451 and 475 nm exhibit downshifts to 442 and 468 nm, respectively, in Q1029L. In contrast, two positive peaks in the wild type at 304 and 397 nm are preserved in Q1029L.

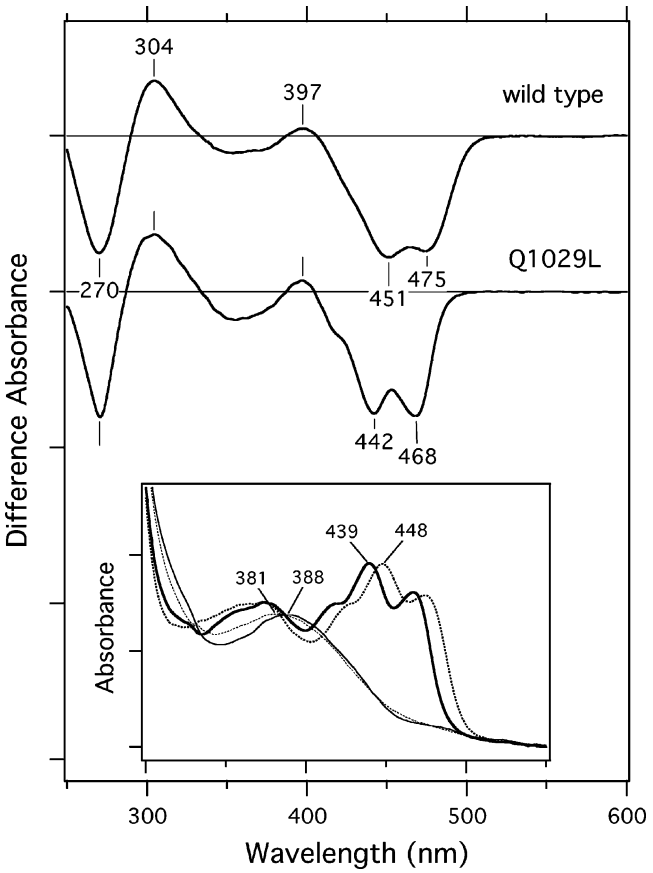

FIGURE 2: Light minus dark difference spectra for the wild type and Q1029L mutant proteins of phy3−LOV2 in the UV−visible region. The spectra are recorded at 295 K. One division of the *y* axis corresponds to 0.003 absorbance units. The inset shows absorption spectra of the Q1029L (thick solid line) and wild type (thick dotted line) proteins of phy3−LOV2 in unphotolyzed states. Calculated spectra of S390 in Q1029L (—) and the wild type (···) are also shown.

To further examine the absorption properties, we have compared absolute absorption spectra (inset of Figure 2). Spectra of the unphotolyzed states of the wild type or Q1029 possess λ_{max} at 427, 448, and 474 nm or 418, 439, and 467 nm, respectively, indicating that the mutation at position 1029 affects the electronic state of FMN in the unphotolyzed state. A lack of hydrogen bonding between Gln1029 and FMN in the unphotolyzed state (Figure 1a) seems to promote the relocation of the electron on FMN, resulting in a spectral blue shift of 9 nm. Further, we have calculated the absolute absorption spectra of S390 by using the difference spectra in Figure 2 to eliminate spectral fine structures in the 400−500 nm region because of the unphotolyzed state. Resulting absorption spectra of S390 possess λ_{max} at 381 and 388 nm for the wild type and Q1029L, respectively (inset of Figure 2). The S390 intermediate absorption maximum of the mutant is red-shifted in contrast to the unphotolyzed state. It is thus likely that the lack of the proton-donating group to FMN results in the shorter wavelength shift of the unphotolyzed state, while the lack of the proton-accepting group from FMN leads to a longer wavelength shift of S390. This observation suggests that the hydrogen-bonding interaction between FMN and Gln1029 plays an important role in determining their electronic absorption spectra.

Low-Temperature FTIR Spectra of the Q1029L Mutant and Wild-Type Proteins. Figure 3 shows light minus dark

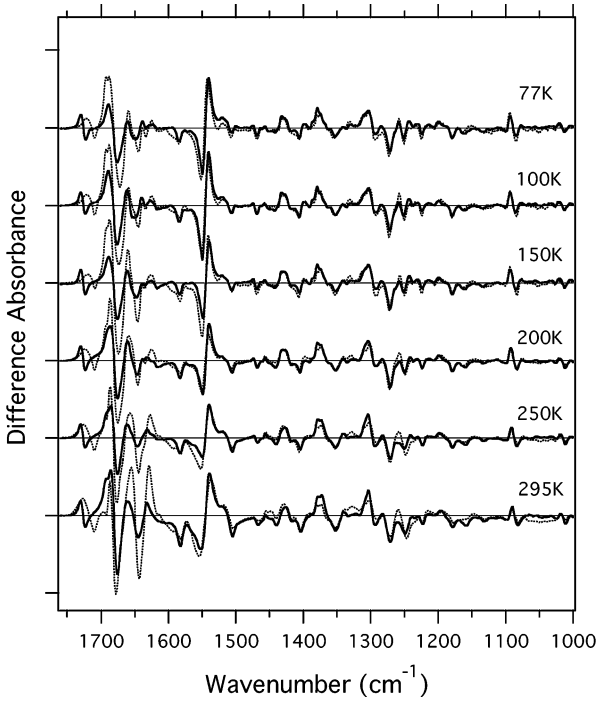

FIGURE 3: Light minus dark infrared difference spectra for the wild type (···) and Q1029L mutant (—) proteins in the 1760−1000 cm^{-1} region. The spectra of the wild type are reproduced from ref *21*. Spectra are measured at 77, 100, 150, 200, 250, and 295 K (from top to bottom). One division of the *y* axis corresponds to 0.005 absorbance units. Note that spectra are normalized to show the same photoconversion of D447 to S390 as judged by the UV−visible absorption.

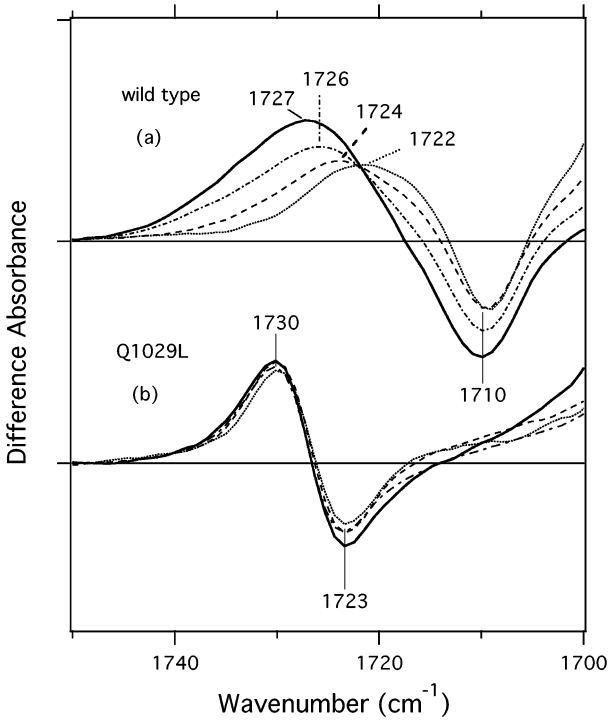

FIGURE 4: Light minus dark infrared difference spectra for the wild type (a) and Q1029L mutant (b) proteins in the 1750−1700 cm^{-1} region. Spectra are measured at 100 (···), 200 (- - -), 250 (-·-·-), and 295 K (—). One division of the *y* axis corresponds to 0.002 absorbance units.

difference IR spectra of the wild type (dotted line) and Q1029L (solid line) in the 1760−1000 cm^{-1} region. It is noted that identical hydrated films are used for UV−visible (Figure 2) and FTIR (Figure 3) measurements and FTIR spectra are normalized using UV−visible spectra to show the same photoconversion of the D447 state to S390. In the 1600−1000 cm^{-1} region of Figure 3, both spectra are almost superimposable at all temperatures measured from 77 to 295 K, except for the slightly spectral deviation at 1350−1250 cm^{-1} above 200 K. Furthermore, negative peaks owing to the S−H stretching vibrations were observed for Q1029L at all temperatures (data not shown). These data indicate that Gln1029 forms a flavin−cysteinyl adduct, S390, as low as 77 K, similar to the wild type and that S390 is the only photointermediate detectable in such a temperature range. On the other hand, the spectra in the 1760−1600 cm^{-1} region differ significantly between the wild type and Q1029L.

Figure 4 shows difference IR spectra in the 1750−1700 cm^{-1} region measured at 100, 200, 250, and 295 K. Positive and negative bands are observed between the wild type (a) and Q1029L (b) in a different manner. Previous resonance Raman spectroscopy of *Chlamydomonas* phot−LOV1 showed that the band at 1710 cm^{-1} was assigned to the stretching vibration of the C(4)═O group of FMN (*25*). In case of the wild type, a negative band is observed at 1710 cm^{-1} regardless of temperatures (Figure 4a), presumably originating from the C(4)═O stretch of FMN. Upon the formation of S390, positive bands appear at the higher frequency side, which are affected by temperature variations. The positive peak tends to appear at a higher frequency as the temperature is higher, from 1722 cm^{-1} at 100 K to 1727 cm^{-1} at 295 K. A similar peak pair was observed at 1724(+)/1712(−) cm^{-1} for *Chlamydomonas* phot−LOV1 (*25*) and at 1725(+)/1711(−) cm^{-1} for oat phot1−LOV2 (*26*) at room temperature. Thus, it appears common that the C(4)═O stretch of FMN in the LOV domains is at about 1710 and 1725 cm^{-1} in the unphotolyzed and S390 states, respectively.

According to the X-ray crystallographic structure of the unphotolyzed state (Figure 1a), the C(4)═O group of FMN forms two hydrogen bonds with Asn1008 and Gln1029 serving as donors. The O−N distances are 3.42 and 3.52 Å with Asn1008 and Gln1029, respectively. In contrast, in the S390 state, only one hydrogen bond is established between Asn1008 and the C(4)═O group of FMN (Figure 1b), in which the O−N distance is 3.43 Å. Thus, the hydrogen-bond weakening of the C(4)═O group in S390 detected by FTIR spectra is consistent with the X-ray crystallographic structure of S390. Variable frequencies at different temperatures for S390 (but not for the unphotolyzed state) imply multiple conformations in the isoalloxazine ring vicinity of FMN in S390 induced by different temperatures. Figure 4a indicates the presence of an isosbestic point at about 1723 cm^{-1} for the positive bands, suggesting two conformations for the S390 state in view of the FMN C(4)═O stretch.

The spectral feature in this frequency region is highly different in Q1029L relative to the wild type. Figure 4b shows that the peak pair is at 1730(+)/1723(−) cm^{-1} at all temperatures. In the unphotolyzed state, the 1723 cm^{-1} band in Q1029L is 13 cm^{-1} upshifted relative to the wild type, indicating that the hydrogen bond of the C(4)═O group is weaker in Q1029L. This observation is also consistent with the X-ray structure of the unphotolyzed state. Figure 1a indicates that the hydrogen bond between the C(4)═O group of FMN and Gln1029 is lost because of the Q1029L

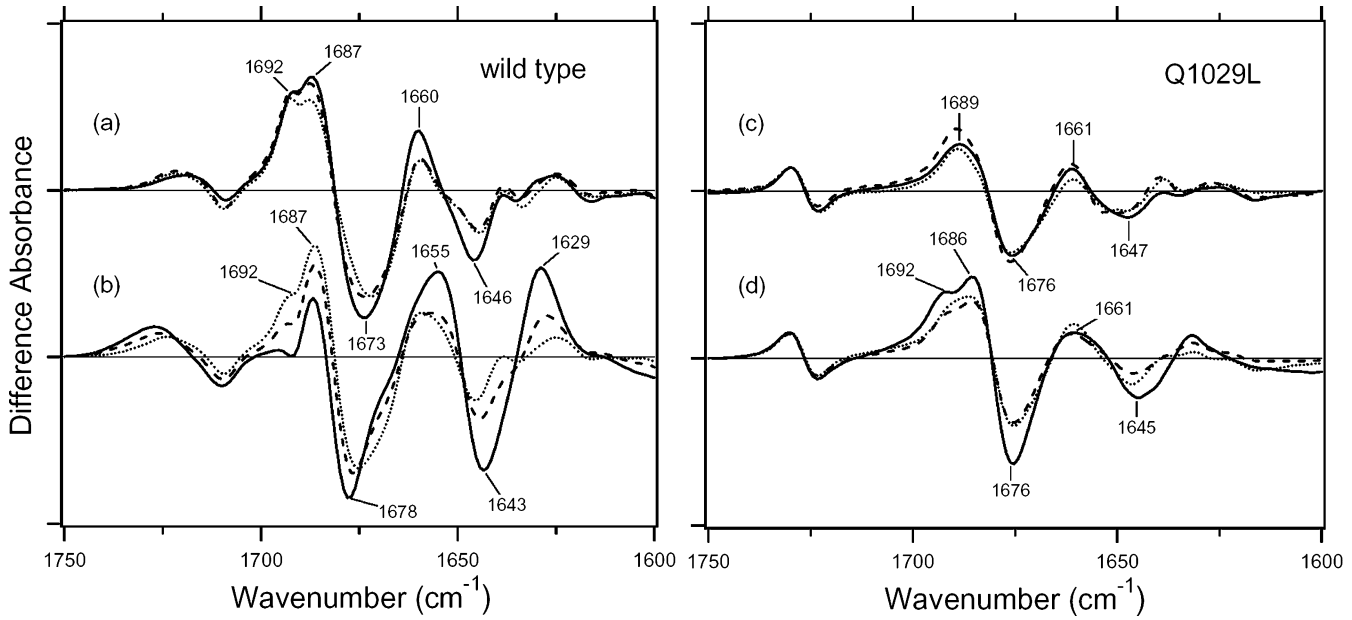

FIGURE 5: Light minus dark infrared difference spectra for the wild type (a and b) and Q1029L mutant (c and d) proteins in the 1750−1600 cm^{-1} region. Upper and lower spectra correspond to those at 77−150 K (a and c) and at 200−295 K (b and d). Spectra are measured at 77 K (•••, in a and c), 100 K (- - -, in a and c), 150 K (—, in a and c), 200 K (•••, in b and d), 250 K (- - -, in b and d), and 295 K (—, in b and d). One division of the *y* axis corresponds to 0.006 absorbance units.

mutation, causing weakening of the C(4)═O hydrogen bonding of the C(4)═O group to be weakened. Upon formation of S390, the C(4)═O stretch appears at 1730 cm^{-1}, exhibiting a spectral upshift by 7 cm^{-1} similar to the wild type. The distance between the oxygen atom of the C(4)═O group and the nitrogen atom of Asn1008 is identical in both unphotolyzed and S390 states by the wild type (3.42 and 3.43 Å, respectively). If such distances are preserved for the Q1029L mutant, the weakened hydrogen bond of the C(4)═O group in Q1029L originates from either (i) a change in geometry of the hydrogen bond or (ii) a different electronic state of the FMN chromophore between the unphotolyzed and S390 states. On the other hand, its frequency is not affected by the temperature (Figure 4b). We thereby infer that the S390 state of the Q1029L mutant does not exhibit multiple conformations, unlike the wild type.

Figure 5 shows light minus dark difference IR spectra of wild type (a and b) and Q1029L (c and d) in the 1750−1600 cm^{-1} region. The spectra of the wild type, reproduced from the previous study (*21*), are highly affected by temperature variations. In contrast, the spectra of Q1029L are less temperature-dependent. It is also noted that the spectral amplitude is generally reduced in Q1029L relative to the wild type. Because all difference IR spectra are normalized by UV−visible absorption spectra, reduced spectra suggest that structural changes are smaller in Q1029L than in the wild type. This frequency region is known to be characteristic of an amide-I vibration that probes the C═O stretch of the peptide backbone, although the C═O stretching vibrations of the FMN chromophore are also involved at about 1710 and 1670 cm^{-1} (C(4)═O and C(2)═O stretches, respectively). These observations suggest that normal structural changes in wild-type phy3−LOV2 are lacking in the Q1029L mutant protein, particularly in the peptide backbone alterations.

The spectra of the wild type at 77−150 K (Figure 5a) have peaks at 1692(+), 1687(+), and 1673(−) cm^{-1}, which are attributable to the amide-I vibrations of the turn structure. In addition, they have peaks at 1660(+) and 1646(−) cm^{-1}, which are attributable to the amide-I vibrations of the helical structure. On the basis of the large spectral changes at about 1690 cm^{-1}, we have concluded that the peptide backbone alteration takes place mainly in the turn structure at 77−150 K, which also contains that of the α helix (*21*). At 200−295 K, the spectra of the wild type have peaks at 1692(+), 1687(+), 1678(−), 1655(+), 1643(−), and 1629(+) cm^{-1}. The positive new band at 1629 cm^{-1} can be assigned to the amide-I vibrations of the *β*-sheet structure. Because the turn-specific 1692 cm^{-1} band is significantly reduced and the *β*-sheet-specific 1629 cm^{-1} band is strongly enhanced at high temperatures, we concluded that the structural change in the turn structure is restored at room temperature, while the structural changes in the sheet structure as well as helices newly appear (*21*). Local structural changes around the turn structure can be transferred to the helix and sheet at room temperature, despite the fact that a unique electronic structure is present for the FMN chromophore (S390).

Parts c and d of Figure 5 show the spectra of Q1029L at 77−150 and 200−295 K, respectively. At 77−150 K, there are peaks at 1689(+), 1676(−), 1661(+), and 1647(−) cm^{-1} in the amide-I region. Compared with those of the wild type, they lack the positive peak at 1692 cm^{-1} (parts a and c of Figure 5). Interestingly, the positive 1692 cm^{-1} band appears at 200−295 K in Q1029L (Figure 5d), whereas the band disappears at such temperatures in the wild type. Unlike the wild type, the appearance of the *β*-sheet-specific 1629 cm^{-1} band is also lacking in Q1029L. The consequence of the spectrum of Q1029L at 295 K is similar to that of the wild type at low temperatures. This fact indicates the important structural role Gln1029 plays in the photoactivation process of phy3−LOV2 and suggests as well that the Q1029L mutant prohibits the proceeding of structural changes from turns to helices and sheets that is observed in the wild type.

Unlike the unphotolyzed state (Figure 1a), the hydrogen bond of the C(4)═O group in S390 (Figure 1b) seems to be less influenced by the mutation at position 1029, presumably because of the lack of the direct hydrogen bond. The present FTIR studies have indicated indeed that the frequencies in S390 are located in the 1730−1720 cm^{-1} range (Figure 4). Nevertheless, temperature dependence of the C(4)═O stretch in S390 was highly different in the wild type and Q1029L. The C(4)═O stretch of S390 in Q1029L exhibits a temperature-independent unique frequency at 1730 cm^{-1} (Figure 4b). In contrast, those of the wild type exhibit an isosbestic point (Figure 4a), suggesting the presence of two frequency states as the C(4)═O group, at 1727 and about 1720 cm^{-1}, which are not observed in Q1029L. As shown in the amide-I spectra region of the wild type (parts a and b of Figure 5), such an observation for the C(4)═O group probably originates from multiple conformations of the protein. In particular, a protein conformation at 77−150 K that mainly alters the turn structure corresponds to the C(4)═O stretch at about 1720 cm^{-1}. Another protein conformation at room temperature that alters sheet and helix structures corresponds to the C(4)═O stretch at 1727 cm^{-1}. In Q1029L, the amide-I spectra at room temperature appear similar to those of the wild type at 77−150 K, suggesting that only the turn structure is altered. The upshifted C(4)═O stretch in Q1029L (1730 cm^{-1}) from the wild type (∼1720 cm^{-1}) under a similar protein environment may originate from absence of the hydrogen bond between FMN and Gln1029, where both the N−H and C═O stretching modes could be coupled.

Role of Gln1029 in the Photoactivation Processes of the LOV2 Domain in Adiantum Phytochrome3. In the present paper, we have studied light-induced structural changes in the Q1029L mutant of the LOV2 domain of *Adiantum* phytochrome3 using low-temperature UV−visible and FTIR spectroscopy. Similar to the wild type, adduct formation took place in the Q1029L mutant protein and only one intermediate, S390, was observed at 77−295 K. Spectral analysis of the C(4)═O stretching vibration of the FMN chromophore in the >1700 cm^{-1} region provided fully consistent pictures to that of the X-ray crystal structures; the side chain of Gln1029 is rotated, and the hydrogen-bonding interaction is switched from the C(4)═O group to the N(5)−H group of FMN. On the other hand, the FTIR spectral comparison between wild type and Q1029L at 77−295 K revealed that Q1029L lacks progressive protein structural changes detected in the wild type, which eventually alter the structures of the β sheet and α helix. It is noted that the ring moiety of the FMN chromophore is sandwiched by a helical loop that contains Cys966 and antiparallel β sheets that contain Gln1029 (Figure 1). It is therefore likely that no structural change in the β sheet for Q1029L originates from the lack of the hydrogen bond between FMN and Gln1029 in S390. In other words, Gln1029 probably plays an important role in transducing the light signal from the chromophore to the β sheet.

Recently, Harper et al. reported the three-dimensional NMR structure of the LOV2 domain of oat phototropin1 (*27*). They have shown the presence of an α helix outside the PAS core, and the interaction between the extra α helix and the PAS core is loosened upon illumination by partial unfolding. Interestingly, the β sheet, which contains the mutated glutamine (Gln1029 in *Adiantum* phytochrome3), is located between the FMN chromophore and the helix. Because the present LOV2 construct contains the region of the extra α helix and the sequence in *Adiantum* phytochrome3 is similar to that in oat (*Avena sativa*) phototropin1, an altered interaction may take place between the β sheet and the extra α helix for the LOV2 domain of *Adiantum* phytochrome3. As a consequence, the signaling pathway could be from FMN to the extra α helix through the β sheet that contains Gln1029. It is however noted that the FTIR study of the LOV domains with or without the extra α helix is necessary for a better understanding of the mechanism of the light-signal conversion in phototropin, which is our future focus.

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