Characterization of different isoforms of the light-harvesting chlorophyll *a/b* complexes of photosystem II in bamboo

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Abstract

The major light-harvesting chlorophyll (Chl) *a/b* complexes of photosystem II (LHCIIb) play important roles in energy balance of thylakoid membrane. They harvest solar energy, transfer the energy to the reaction center under normal light condition and dissipate excess excitation energy under strong light condition. Many bamboo species could grow very fast even under extremely changing light conditions. In order to explain whether LHCIIb in bamboo contributes to this specific characteristic, the spectroscopic features, the capacity of forming homotrimers and structural stabilities of different isoforms (Lhcb1-3) were investigated. The apoproteins of the three isoforms of LHCIIb in bamboo are overexpressed *in vitro* and successfully refolded with thylakoid pigments. The sequences of Lhcb1 and Lhcb2 are similar and they are capable of forming homotrimer, while Lhcb3 lacks 10 residues in the N terminus and can not form the homotrimeric structure. The pigment stoichiometries, spectroscopic characteristics, thermo- and photostabilities of different reconstituted Lhcbs reveal that Lhcb3 differs strongly from Lhcb1 and Lhcb2. Lhcb3 possesses the lowest Qy transition energy and the highest thermostability. Lhcb2 is the most stable monomer under strong illumination among all the isoforms. These results suggest that in spite of small differences, different Lhcb isoforms in bamboo possess similar characteristics as those in other higher plants.

Additional key words: bamboo; isoform; light-harvesting complexes.

Introduction

Bamboo (a potential source for bioenergy), occurs naturally in tropical, subtropical, and temperate regions from sea level to 4,000 m (Isagi *et al.* 2004), and includes more than 70 genera and about 1,000 species. The genus *Phyllostachys*, which includes temperate moso and timber bamboo species, contains the ecologically and economically most important species. China has the highest bamboo diversity (626 species) and the bamboo area of more than 4 million ha (Kleinhenz *et al.* 2003). Many bamboo species are fast growing, reaching their full height within a single growth season at an average elongation rate, *e.g.* for *Phyllostachys edulis*, of over 100 cm d^{-1} (Ueda 1960), which suggests that bamboo may possess unique carbon assimilation mechanism. It would be interesting to study the light-harvesting process in bamboo, because normally in natural forests, bamboo grows in the understorey under other timber species (Saitoh *et al.* 2002), where irradiation fluctuates. Related studies on the light-harvesting process in bamboo are still missing.

Light harvesting is conducted by a number of light-harvesting complexes in the thylakoid membrane, among which the light-harvesting chlorophyll (Chl) *a/b* protein of photosystem II (LHCIIb) is the most abundant. It constitutes more than 40% of the photosynthetic membrane protein and about half of the total thylakoid pigments. LHCIIb functions, *in vivo*, as trimers consisting of various combinations of the three isoforms: Lhcb1,

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Lhcb2, and Lhcb3 (the latter only found in heterotrimers). High-resolution structural analysis of LHCIIb reveals that every monomer contains three trans-membrane α-helixes, one amphipathic α-helix and one 3_{10}-helix on the lumenal side. It combines, as cofactors, 14 Chls (8 Chl *a* and 6 Chl *b*), two luteins (Lut), one neoxanthin (Neo) and one violaxanthin (Vio) per monomer (Liu *et al.* 2004, Standfuss *et al.* 2005). LHCIIb is a multifunctional complex, and its function depends on the light condition. Besides harvesting the sunlight and transferring energy to the reaction center, LHCIIb participates in dissipating the excess of excitation energy (nonphotochemical quenching, NPQ) under the strong light conditions. NPQ can be measured *in vivo* as a reduction in the yield of Chl fluorescence. NPQ is induced by acidification of the thylakoid lumen, and is related to a conformational change of LHCIIb (Ruban *et al.* 2012).

Because of the structural similarity, it is technically impossible to purify highly homologous isoforms of LHCIIb. Recombinant LHCIIb provides a unique opportunity for studying the characteristics of each isoform of LHCIIb *in vitro*. The three isoforms of LHCIIb of *Arabidopsis* and of barley have been reconstituted *in vitro* and studied in terms of sequence and spectroscopic characteristics (Caffarri *et al.* 2004, Standfuss and Kühlbrandt 2004). Zhang *et al.* (2008, 2012) obtained the three isoforms, Lhcb1–3 of pea, and analyzed structural stabilities of monomers under elevated temperature and strong illumination, and their temperature-dependent pigment dissociation kinetics. Bamboo is fast growing even under fluctuating light conditions. The function of light harvesting might be important for the fast-growing character. The aim of this work is a structural and functional investigation of each monomeric and homotrimeric form of the three isoforms of LHCIIb in bamboo constituted *in vitro*. For this aim, we cloned and expressed apoproteins of the three isoforms of LHCIIb from *P. edulis*. After reconstitution with pigment *in vitro* and trimerization, we studied their biochemical and spectroscopic characteristics. Furthermore, their themostabilities and photostabilities are studied, and possible physiological functions of the three isoforms in bamboos are discussed.

Materials and methods

Plant materials and strains: Seeds of *Phyllostachys edulis* were sterilized and germinated on moistened tissues and then transplanted into vermiculite in growth chambers under long-day conditions (16 h light/8 h dark) at 25°C with light intensity of 150 μmol m^{-2} s^{-1}. The air humidity was about 42%. One-year-old seedlings with formed 5th leaf were selected. Fresh leaves were collected, frozen with liquid nitrogen and stored at –80°C until needed for use in experiments.

Escherichia coli DH5α was used as the recipient in routine cloning experiments. *E. coli* BL21 (*DE3*) was used for Lhcb expression *in vitro*.

Isolation of Lhcb cDNA and sequence analysis: Total RNA was isolated from leaves of *P. edulis* with Trizol (*Invitrogen*, USA) according to an isolation protocol recommended by the manufacturer. First-strand cDNA was synthesized from 500 ng of RNA using the Promega cDNA synthesis system.

Sequence analysis was carried out with the *DNAMAN* software package, and the cDNA sequences were subjected to a similarity search against the NCBI database (http://blast.ncbi.nlm.nih.gov) using the Blastx algorithm with default parameters.

Construction of expression vector for the recombinant proteins: On the basis of this assembled sequences, the fragments of Lhcbs that encodes the mature protein were re–amplified by PCR to introduce *Nde* I (forward) and *Xho* I (reverse) sites. Fragments incorporating *Nde* I and *Xho* I sites were cloned into the pET–23a vectors, respectively. The primers used to generate fragments from Lhcb1 were I mF (5′- AA<u>CATATG</u>CGCAAGAC CGGCGCCAAGC –3′) and I mR (5′- <u>CTCGAG</u>CTTG CCGGGCACGAAGTTG -3′); The primers used to generate fragments from Lhcb2 were II mF (5′- AA <u>CATATG</u>CGCCGCACCGTCAAGAGCG -3′) and II mR (5′- <u>CTCGAG</u>CTTCCCTGGGACGAAGTTG -3′); The primers used to generate fragments from Lhcb3 were III mF (5′- AA<u>CATATG</u>AGCAACGACCTGTGGTAC GGG-3′) and III mR (5′- <u>CTCGAG</u>AGACCCCGGCGCG AACTTG -3′); the *Nde* I and *Xho* I recognition sequences in the primers are underlined. The sequences were confirmed by sequencing.

After sequencing, the fragments encoding the mature proteins were introduced into the multiple cloning sites of pET–23a respectively, the recombinant plasmids carrying pET–23a– Lhcb1m, pET–23a– Lhcb2m and pET–23a– Lhcb3m were transformed into competent *E. coli* strain BL21(*DE3*) cells for protein expression.

BL21(*DE3*) cells harboring pET–23a– Lhcb1m, pET–23a– Lhcb2m, pET–23a– Lhcb3m or an empty vector (pET–23a) were cultured at 37°C, in LB liquid medium containing 100 μg ml^{-1} of ampicillin until an OD_{600} of approximately 0.6 was attained. The medium was then supplemented with 0.1 mM IPTG and the *E. coli* cells were cultured at 37°C for an additional 4 h to induce synthesis of the recombinant protein.

Preparation of recombinant LHCIIb: The Lhcb apoproteins were reconstituted with total thylakoid pigments (Chl *a/b* 3:1 and Lut:Neo:Vio 3:1:1). The mixture containing 1 μg $μL^{-1}$ apoprotein in a reconstitution buffer [2% (w/v) lithium dodecyl sulfate (LDS) (*Amresco*,

USA), 100 mM Tris-HCl (pH 9), 12.5% (w/v) sucrose, and 10 mM β–mercaptoethanol] were mixed, while agitating, with thylakoid pigments dissolved in ethanol (protein:Chl = 1:2.5). After reconstitution, the complexes were loaded to a Ni^{2+}-chelating Sepharose Fast Flow column (0.8 cm × 4 cm) (*Bio-Rad*, CA, USA) which was equilibrated with OG buffer (1% (w/v) OG (*Sigma*, USA), 0.1 M Tris-HCl (pH 9), 12.5% (w/v) sucrose), and then incubated in darkness at 4ºC for 30 min. The column was washed with 1 mL OG-buffer and 2 mL Triton X-100 buffer [0.05% (w/v) Triton X-100 (*Sigma*, USA), 0.14 mM PG (*Avanti*, USA), 0.1 M Tris-HCl (pH 7.5)]. The complexes were eluted with elution buffer [0.05% (w/v) Triton X-100, 0.14 mM PG, 10 mM Tris-HCl (pH 7.5), and 0.3 M imidazole (*Amresco*, USA)].

The reconstituted monomeric and trimeric complexes were separated by sucrose density gradients centrifugation. The material eluted from the column was loaded onto a sucrose density gradient in a 12.5 mL centrifuge tube containing 0.1–1.0 M sucrose density gradient, 2.0 mM DM (*Sigma*, USA), and 5 mM phosphate buffer (pH 7.5) and centrifuged at 230,000 × *g* and 4°C for 18 h. The bands were collected for further experiment.

Pigment analysis: The band corresponding to the monomers and trimers in the sucrose density gradient after the ultracentrifugation were further analyzed for pigment stoichiometries of the recombinant LHCIIb complexes with *Waters 600* high performance liquid chromatography (HPLC) (*Waters*, USA). The pigments were extracted with 2-butanol according to the method described in (Martinson and Plumley 1995). The 2-butanol extraction were applied to an *RP-C18 HPLC* column (*Merck*, Germany) and separated with a gradient from 70 to 100% acetone, at a rate of 1 mL min^{-1}. Pigments were quantified by comparing integrated peak areas to calibrated ones of known pigment amounts.

Absorption spectra: The samples were diluted with the dilution buffer [12.5% (w/v) sucrose, 2.0 mM DM, and 5 mM phosphate buffer (pH 7.5)] to approximate 5 µg(Chl) mL^{-1}. The absorption spectra were recorded using *Shimadzu UV-VIS 2550* spectrophotometer (*Shimadzu*, Japan) at room temperature. The wavelength step was 0.5 nm, the scan rate was 100 nm min^{-1}, the optical pathlength was 1 cm, and the spectral slit-width was 1 nm. The 4th-derivative spectra were calculated by using *Origin* (*OriginLab*, USA).

The circular dichroism (CD) spectra were recorded on

a *Jasco 815* spectropolarimeter (*Jasco*, Japan) at 10ºC. The spectra were measured from 350 to 750 nm at a scan rate of 100 nm min^{-1}. The measurements were repeated for three times and averaged.

Fluorescence emission spectra were recorded with a *Hitachi F-4500* spectrofluorometer (*Hitachi*, Japan). The samples were diluted to 0.5 µg(Chl) mL^{-1} with the dilution buffer. For measuring 77 K fluorescence emission, the samples were frozen in a sample tube by immersing the samples in liquid nitrogen. The fluorescence emission spectra were measured from 600 nm to 780 nm, with the excitation wavelength set to 480 nm, slit widths set to 5 nm for excitation and 2.5 nm for emission. The spectra were not corrected for the spectral sensitivity of the detection system. Fluorescence reabsorption is negligible.

The thermal stability of reconstituted LHCII was measured by observing the decrease in the extent of energy transfer from complex bound Chl *b* to Chl *a* upon gradual dissociation of the complexes at 37°C. The samples were diluted to 0.5 µg(Chl) mL^{-1} with dilution buffer. The decay of Chl *a* fluorescence emission (excitation and emission wavelengths of 480 and 680 nm with slit widths of 5 and 2.5 nm, respectively) was measured in a *Hitachi F-4500* spectrofluorometer (*Hitachi*, Japan) thermostated to 37°C. Measurements were taken for 18 min at 1-s intervals. The time course of the decrease of the fluorescence intensity was modeled with a two-phase exponential decay [$y = A_1 \exp(-x/T_1) + A_2 \exp(-x/T_2) + Y_0$] using the software *Origin* (*OriginLab*, USA). The quality of the fitting was evaluated using the coefficient of determination R^2. All R^2 were higher than 0.95. The decay times are presented as half-lives (T' = T ln2).

Photobleaching: The concentration of the samples was adjusted with the dilution to approximate 5 µg(Chl) mL^{-1}. The complexes were then illuminated with cold white light (4,000 µmol m^{-2} s^{-1}) from a fiber light source under stirring. After each time interval, the cuvette was removed from the light source, and the absorption spectrum was recorded with *Shimadzu UV–VIS 2550* spectrophotometer (*Shimadzu*, Japan) in the range of 600–750 nm. The time course of the photobleaching was modeled with a one-phase exponential decay [$y = A \exp(-x/T) + Y_0$] with the software *Origin* (*OriginLab*, USA). The quality of the fitting was evaluated using the coefficient of determination R^2. All R^2 were higher than 0.95. The decay times are presented as half-lives (T' = T ln2).

Results

Sequence alignment: We cloned genes for all the three isoforms of LHCIIb in bamboo. A comparison of the sequences of the three LHCIIb isoforms from *Arabidopsis* and bamboo is shown in Fig. 1. The sequences of

Lhcb1 from *Arabidopsis* and bamboo are very similar, with 91.3% identity. Compared to the sequence of *Arabidopsis* Lhcb1 (Jansson 1999), bamboo Lhcb1 missed Asn 145. When the three isoforms of bamboo are

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                                                                          Helix B
Arabidopsis Lhcb1  RKTVAKPKGPSGSFWYGSDRVKYLGPFSGESPSYLTGEFPGDYGWDTAGLSADPETFARNRELEVIHSRWAMLGALGCVF    80
Bamboo Lhcb1       RKTGAKPKATSGSPWYGPDRVIYLGPISGEPPSYLTGEFPGDYGWDTAGLSADPETFAKNRELEVIHSRWAMLGALGCVF    80
Arabidopsis Lhcb2  RRTVKS...TPQSIWYGPDRPKYLGPFSENTPSYLTGEYPGDYGWDTAGLSADPETFAKNRELEVIHSRWAMLGALGCTF    77
Bamboo Lhcb2       RRTVKS...APQSIWYGPDRPKYLGPFSEQTPSYLTGEFAGDYGWDTAGLSADPETFARNRELEVIHSRWAMLGALGCVF    77
Arabidopsis Lhcb3  .........GNDLWYGPDRVKYLGPFSVQTPSYLTGEFPGDYGWDTAGLSADPEAFAkNRALEVIHGRWAMLGAFGCIT    70
Bamboo Lhcb3       .........SNDLWYGPDRLKYLGPFSAQTPSYLTGEFPGDYGWDTAGLSADPEAFARNRALEVIHGRWAMLGALGCIT    70
            P          P   L  T                                         a  a  a      a   a  a a   a
                                                    b         a  a

          Helix B           Helix E                    Helix C
Arabidopsis Lhcb1  PELLARNG.VKFGEAVWFKAGSQIFSDGGLDYLGNPSLVHAQSILAIWATQVILMGAVEGYRVAGNGPLGEAEDLLYPGG   159
Bamboo Lhcb1       PELLARNG.VQFGEAVWFKAGSQIFSEGGLDYLGNPSLIHAQSILAIWACQVVLMGAVEGYRIAG.GPLGEVVDPLYPGG   158
Arabidopsis Lhcb2  PEILSKNG.VKFGEAVWFKAGSQIFSEGGLDYLGNPNLIHAQSILAIWAVQVVLMGFIEGYRIGG.GPLGEGLDPLYPGG   155
Bamboo Lhcb2       PEILSKNG.VKFGEAVWFKAGAQIFSEGGLDYLGNPNLVHAQSILAIWASQVVLMGFVEGYRVGG.GPLGEGLDKVYPGG   155
Arabidopsis Lhcb3  PEVLQKWVRVDFKEPVWFKAGSQIFSEGGLDYLGNPNLVHAQSILAVLGFQVILMGLVEGFRINGLDGVGEGND.LYPGG   149
Bamboo Lhcb3       PEVLQKWVRVSFKEPVWFKAGAQIFSEGGLDYLGNPNLVHAQSILAVLGFQVILMGLVEGYRINGLPGVGEGND.LYPGG   149
                      a              b                    L      b        b b      b           a

          Helix A                       Helix D
Arabidopsis Lhcb1  .SFDPLGLATDPEAFAELKVKELKNGRLAMFSMFGFFVQAIVTGKGPIENLADHLADPVNNNAWAFATNFVPGK       232
Bamboo Lhcb1       .SFDPLGLADDPEAFAELKVKELKNGRLAMFSMFGFFVQAIVTGNGPIENLADHLADPVNNNAWAYATNFVPGK       231
Arabidopsis Lhcb2  .AFDPLNLAEDPEAFSELKVKELKNGRLAMFSMFGFFVQAIVTGKGPIENLFDHLADPVNNNAWSYATNFVPGK       228
Bamboo Lhcb2       .AFDPLGLADDPDTFAELKVKELKNGRLAMFSMFGFFVQAIVTGKGPIENLFDHVADPVNNNAWAYATNFVPGK       228
Arabidopsis Lhcb3  QYFDPLGLADDPVTFAELKVKEIKNGRLAMFSMFGFFVQAIVTGKGPLENLLDHLDNPVANNAWAFATKFAPGA       223
Bamboo Lhcb3       QYFDPLGLADDPVTFAELKVKEIKNGRLAMFSMFGFFVQAIVTGKGPLENLLDHIDNPVANNAWVYATKFAPGS       223
                        L                                   a                L
                        a a a             a                a
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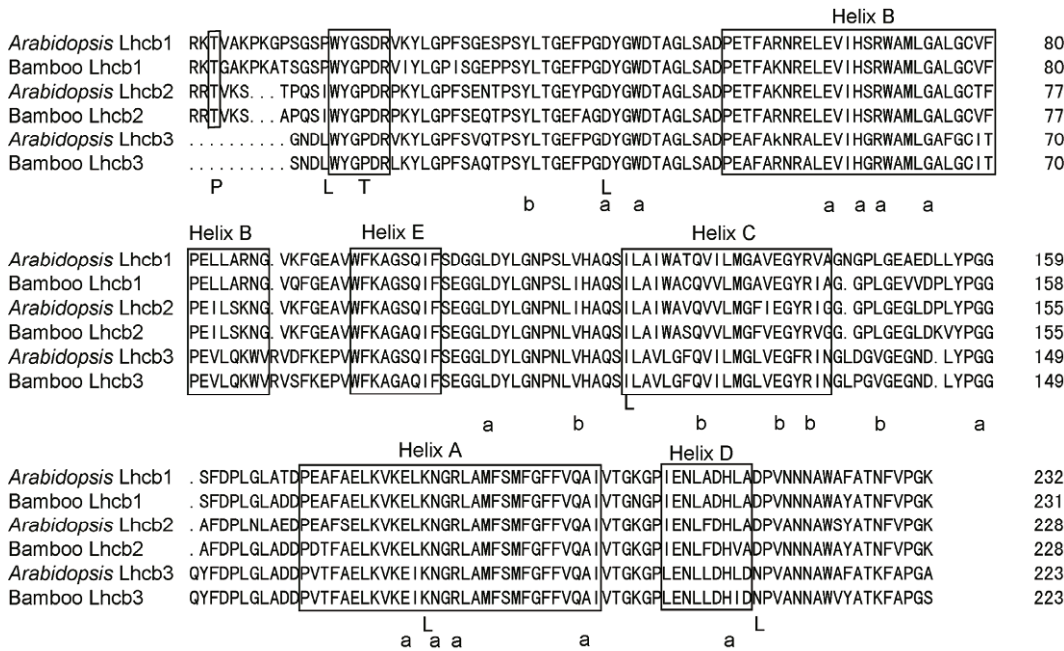
Fig. 1. Alignments of the primary sequences of Lhcb1–3 from *Phyllostachys edulis* and *Arabidopsis thaliana*. Different features are indicated: *black rectangles* – α–helix; P – phosphorylation site; T – trimerization motifs; L – lipid binding sites; a – Chl *a* binding sites; b – Chl *b* binding sites.
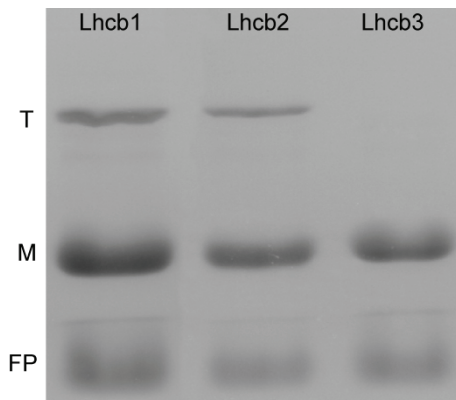

Fig. 2. Partial denaturing polyacrylamide gel electrophoretic analysis of Lhcb1–3 complexes reconstituted with pigments. T – trimer, M – monomer, FP – free pigment.

compared, the homology is even lower, with 82.62% identity. The residues involved in Chl binding are very conservative with only one exception: The ligand to Chl *b* 605, Val in Lhcb1 from *Arabidopsis* is changed to Ile in that from bamboo, while in Lhcb2, the residue Ile at the same position in *Arabidopsis* is changed to Val in bamboo. The phosphatidylglycerol binding motif (WYGPDR) (Vener *et al.* 2001) is conserved in all the three isoforms of bamboo, while, in *Arabidopsis*, Pro19 in Lhcb1 is changed to a Ser. Similarly to other higher plants, the N terminus of Lhcb3 is 10 residues shorter and lacks the phosphorylation site, which implies that this isoform could not be phosphorylated.

Refolding and trimerization: Partly denaturing gel electrophoresis proved that all the three isoforms could refold into pigment-protein complexes using detergent exchange method (Fig. 2). Only Lhcb1 and Lhcb2 could form trimers, the yield of trimer formation of Lhcb1 with the reconstitution method was much better than that of Lhcb2, probably because the method to induce trimer formation was optimized for Lhcb1.

Pigment stoichiometry: The native LHCIIb contains Chl *a*, Chl *b*, Lut, Neo, and Vio. The pigment content of the refolded monomer and trimer, calculated on the basis of mole pigment per 2 mole Lut as described in Yang *et al.* (1999), is shown in Table 1. Lhcb1 and Lhcb2 monomers bind on average 0.9 neoxanthin molecules, Lhcb3 monomers binds about 0.74 neoxanthin molecules. The Chl *a/b* ratios of refolded monomers are 1.18, 1.17, and 1.39 for Lhcb1, Lhcb2 and Lhcb3, respectively. The high Chl *a/b* ratio of Lhcb3 possibly results from the fact that Lhcb3 binds one Chl *b* less than Lhcb1 and Lhcb2.

Table 1. Pigment composition of recombinant LHCII complexes. All values are given as the average of 3–5 independent experiments ± SD.

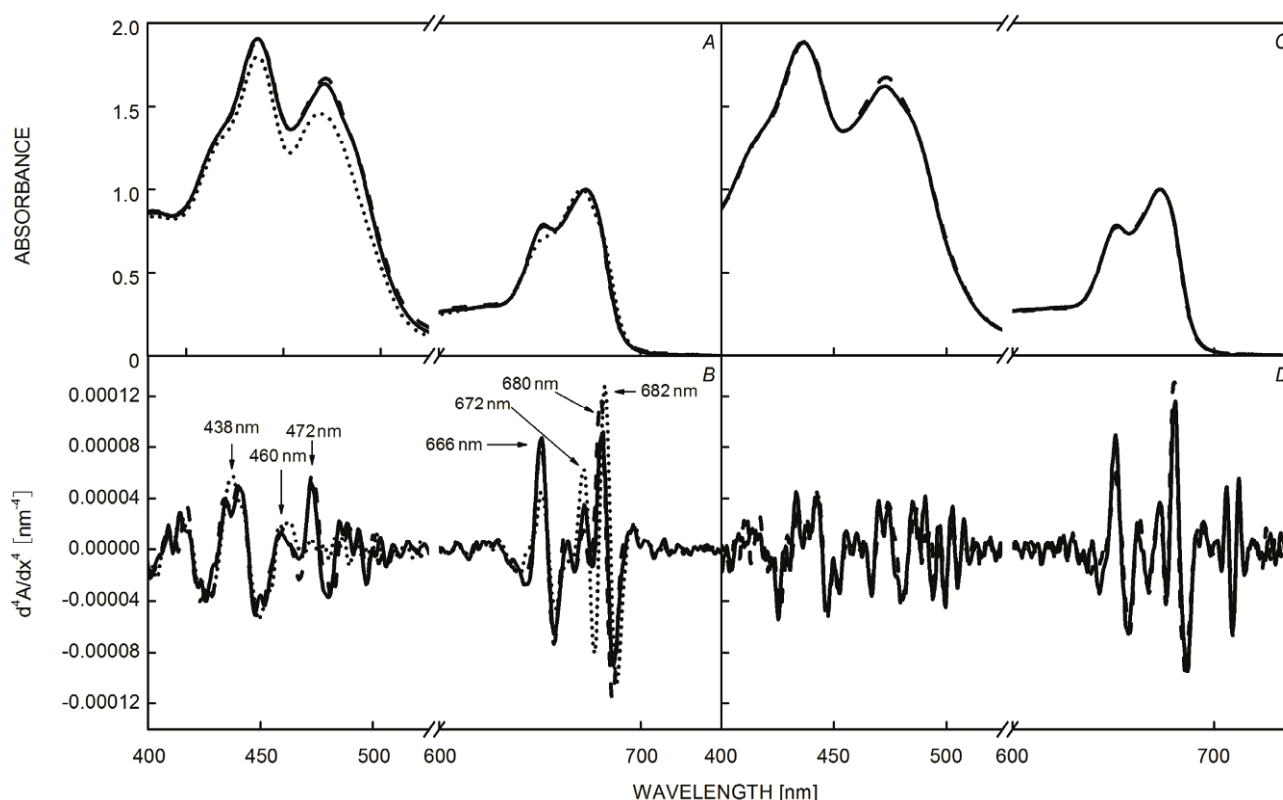| Lhcb	Chl *a*	Chl *b*	Lutein	Neoxanthin
Lhcb1M	7.02 ± 0.24	5.95 ± 0.39	2	0.91 ± 0.03
Lhcb2M	6.94 ± 0.28	5.92 ± 0.15	2	0.92 ± 0.01
Lhcb3M	7.72 ± 0.21	5.55 ± 0.14	2	0.74 ± 0.03
Lhcb1T	7.74 ± 0.15	6.48 ± 0.19	2	0.94 ± 0.02
Lhcb2T	7.53 ± 0.13	6.55 ± 0.07	2	0.91 ± 0.01

Fig. 3. Absorption spectra of monomeric (*A,B*) and trimeric (*C,D*) Lhcb isoforms at room temperature and their fourth-derivation spectra. The spectra were normalized at red-most absorbance maximum. Lhcb1 (*solid line*), Lhcb2 (*dashed line*) and Lhcb3 (*dotted line*).

The Chl *a/b* ratio of Lhcb1 and Lhcb2 trimers was roughly the same, namely, 1.19 and 1.15 respectively.

The absorption spectra of each isoform of LHCIIb measured at room temperature are presented in Fig. 3*A*, normalized to the red-most Chl *a* absorption in the Qy region. The maxima in the Qy region were around 672 nm and 651 nm, and in the Soret region around 436 nm and 472 nm, for all the isoforms. The fourth derivative of the absorption spectra (Fig. 3*B*) revealed three components peaking at 666 nm, 672 nm, and 680 nm, attributed to Chl *a* Qy absorption transitions. The red-most component (680 nm) was 2 nm red-shifted in Lhcb3. In the Soret region, the peak at 438 nm in Lhcb3 was split into two peaks (435 and 439 nm) for both Lhcb1 and 2; the peak at 472 nm for Lhcb1 and Lhcb2 was missing in the fourth derivative of the absorption spectra of Lhcb3.

The CD spectra in the visible range reflect sensitively the intramolecular pigment-pigment interactions. CD spectra of the refolded monomeric and trimeric LHCIIb isoforms are shown in Fig. 4. The spectra of monomeric Lhcb1 and Lhcb2 are almost identical. The CD signal of Lhcb3 is very similar to those of the other two isoforms, except that Lhcb3 has one obvious peak at 470 nm, which reflects the differences among pigment contents or
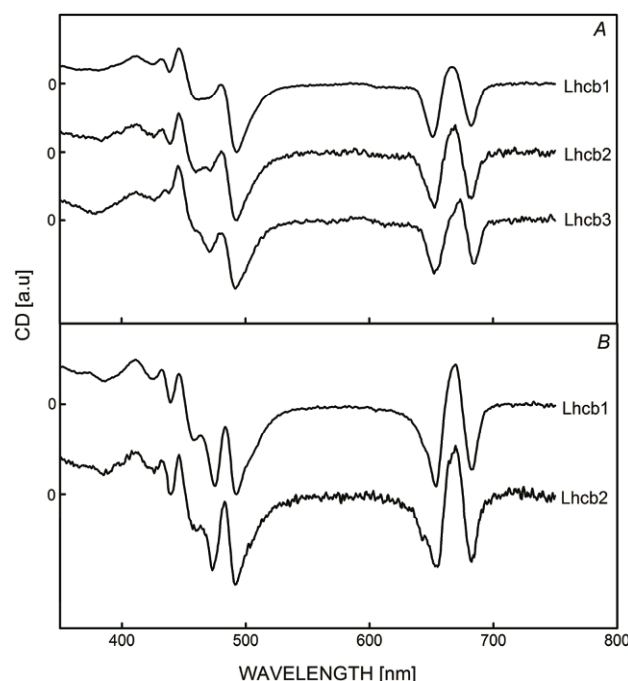

Fig. 4. Circular dichroism (CD) spectra of monomeric (*A*) and trimeric (*B*) Lhcb isoforms measured at room temperature. The spectra were normalized to the same Chl concentration. The spectra are vertically shifted for clarity.
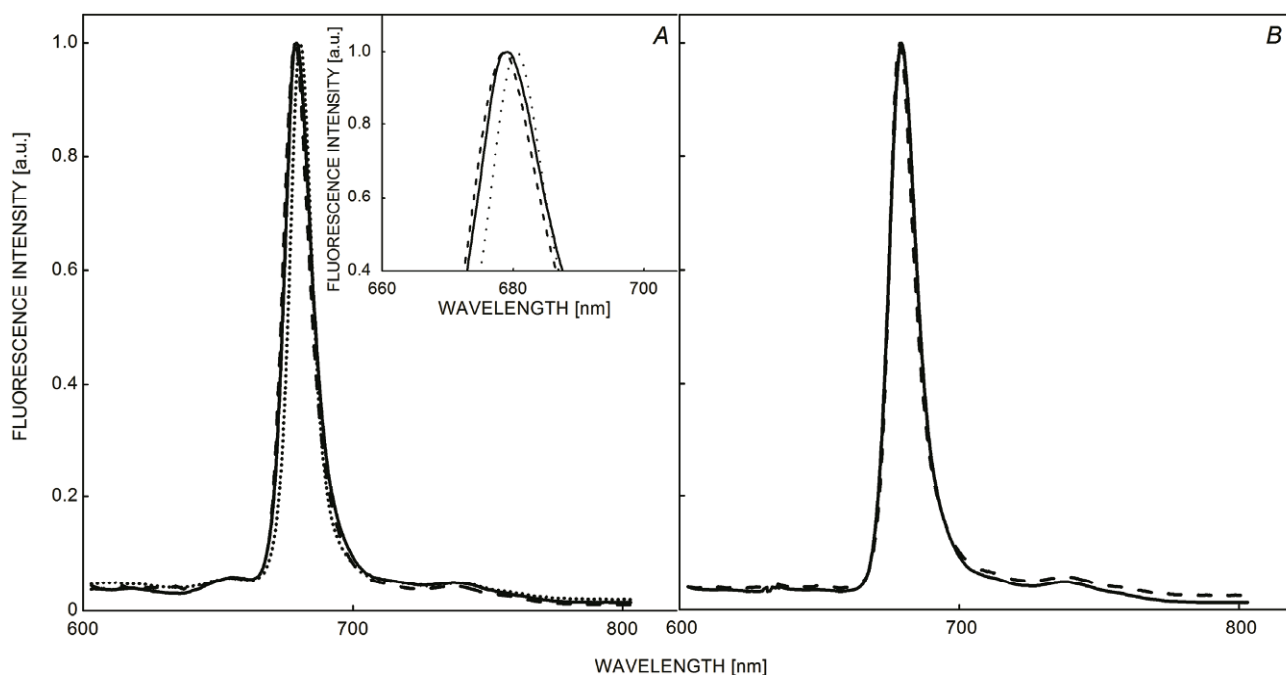
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Fig. 5. Fluorescence emission spectra of monomeric (*A*) and trimeric (*B*) Lhcb isoforms. Lhcb1 (*solid line*), Lhcb2 (*dashed line*) and Lhcb3 (*dotted line*). The samples were excited at 480 nm. Fluorescence spectra were normalized to the maximum. *The inset* is an enlarged view of the emission spectra around 680 nm to show the emission peaks of different monomeric Lhcb isoforms.

Table 2. Parameters of the two-phase exponential decay functions describing stability of reconstituted complexes toward thermal denaturation [asymptote Y_0, amplitudes (A_1 and A_2) and half-lives (Second) (T'_1 and T'_2) of two phases]. All values are given as the average of 3–5 independent experiments ± SD.

Lhcb	T'_1	A_1	T'_2	A_2	Y_0
Lhcb1M	51.6 ± 2.5	0.14 ± 0.02	751 ± 23	0.51 ± 0.04	0.33 ± 0.05
Lhcb2M	53.3 ± 1.3	0.15 ± 0.03	762 ± 13	0.48 ± 0.06	0.36 ± 0.02
Lhcb3M	43.6 ± 1.9	0.05 ± 0.02	699 ± 11	0.48 ± 0.03	0.45 ± 0.03
Lhcb1T	45.7 ± 2.1	0.14 ± 0.03	552 ± 24	0.23 ± 0.04	0.61 ± 0.02
Lhcb2T	42.2 ± 1.2	0.12 ± 0.02	529 ± 26	0.33 ± 0.06	0.54 ± 0.04

pigment interactions. The CD spectra of the trimers are characterized by a strong negative peak around 472 nm, which is absent in the monomers.

Fluorescence emission spectra: The energy transfer from Chl *b* to Chl *a* in LHCIIb can be monitored by the fluorescence emission spectra with an excitation wavelength of 480 nm (Fig. 5). The emission peaks of Lhcb1 monomer and trimer are both at 679 nm and those of Lhcb2 monomers and trimers are blue-shifted about 0.4 nm, to 678.6 nm. The emission peak of Lhcb3 monomer, at 681 nm, differs clearly from Lhcb1 and Lhcb2.

Thermal stability: The thermal stabilities of different LHCIIb isoforms were investigated by monitoring the time course of the decrease of Chl *b*-sensitized Chl *a* fluorescence emissions at 37°C, because only within the intact complexes can the excitation energy on Chl *b* be transferred to Chl *a*. The data were fitted to a two-phase exponential decay (Table 2). The Y_0 value indicates the proportion of complexes in which the energy transfer from Chl *b* to Chl *a* is unaffected by the treatment; the A_1 and A_2 values indicate the amount of energy transfer lost in the two components, T'_1 and T'_2 are the half-lives of two different components. It was shown that Lhcb1 and Lhcb2 had similar thermal stabilities. Among the three monomeric isoforms, Lhcb3 had the highest thermal stability, because it showed the least fluorescence decay in the first phase. Both Lhcb1 and Lhcb2 trimers had higher Y_0 values (at about 0.6) compared with their monomeric forms, which is in agreement with former observations (Wentworth *et al.* 2003).

Photostability: Under strong light conditions, over-excitation of Chls can invoke active oxygen species, which are dangerous to Chls since the Chls will then be oxidized and damaged very quickly. The photostability of
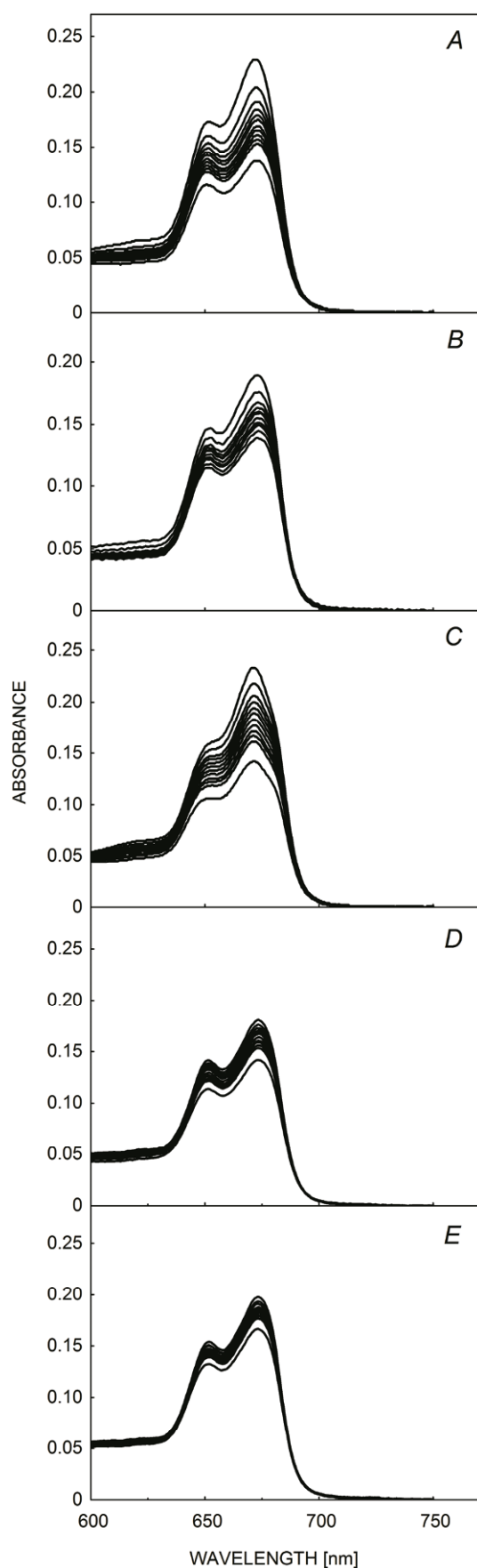
Fig. 6. Changes of the absorption spectra during photobleaching process. *A*: Lhcb1M, *B*: Lhcb2M, *C*: Lhcb3M, *D*: Lhcb1T, *E*: Lhcb2T. M – monomer, T – trimer. The spectra were recorded at 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, and 25 min under the strong illumination and the spectra are gradually decreasing upon time of exposition.

different monomers and trimers of LHCIIb were investigated by monitoring the decrease in Chl absorption of the complexes in the Qy region (600–700 nm) under strong illumination according to the method described by Liu *et al.* (2008). Fig. 6 presents the changes of the absorption spectra of the three monomers (Lhcb1–3) and two trimers (Lhcb1 and Lhcb2) of LHCIIb. Fig. 7 presents the time course of the photobleaching of LHCIIb under strong illumination. A first-order exponential decay was found as a good approximation of the observed data. The asymptote Y_0 indicates the amount of pigment protected from bleaching. The amplitude A indicates the amount of pigment photobleached in the reaction, and T' is the half-life of the photobleach in different LHCIIb species. Table 3 presents these parameters for the three Lhcb monomers and Lhcb1 and Lhcb2 homotrimers. The data show that the trimers were more resistant to strong light than the monomers. Lhcb3 possessed the strongest stability in high light among the monomers, with Y_0 value of 0.73 compared to 0.67 and 0.69 for Lhcb1 and Lhcb2, respectively.

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Discussion

Bamboo is a special kind of plant, which is distributed mostly in Asia. Most bamboo species are fast growing, suggesting that they might possess high photosynthetic activity. Besides, the living environments for the bamboos usually include fluctuating light conditions because most bamboo species grow as understorey plants (Saitoh et al. 2002). How bamboo sustains greater growth rate under fluctuating light conditions is an interesting question. Photosynthesis begins with the process of light harvesting, conducted by a number of Chl-binding light-harvesting proteins in the thylakoid membrane among which LHCIIb is the most abundant in the higher plant.

As in other higher plants, LHCIIb in bamboo consists of various combinations of three different isoforms encoded by Lhcb1–3 genes (Peng et al. 2010). It has been suggested that the three isoforms of LHCIIb have specific functions in harvesting and utilizing solar energy (Caffarri et al. 2004, Standfuss and Kühlbrandt 2004, Zhang et al. 2008). Studies on the characteristics of each isoform of LHCIIb in bamboo showed that the sequence identity of all the three isoforms from bamboo was 82.62%. The differences are located mostly in the loops and terminal regions, which is in agreement with the former observations on other species (Caffarri et al. 2004, Standfuss and Kühlbrandt 2004, Zhang et al. 2008). The lumenal loop has been suggested to be a functionally significant motif which may be involved in the NPQ process in Lhcb1 trimers (Liu et al. 2008). All three isoforms can bind pigments, in vitro, and yield pigment-protein complexes with similar absorption (Fig. 3), fluorescence emission (Fig. 5), and CD spectra (Fig. 4), similar to other well studied species such as Arabidopsis (Standfuss and Kühlbrandt 2004), barley (Caffarri et al. 2004), and pea (Zhang et al. 2008), suggesting that all complexes are authentic LHCIIs. The function of LHCIIb depends on the type and number of bound pigments and

their conformations. The sequences of transmembrane region of all the three isoforms of bamboo are highly homologous and all pigment-binding residues are conserved, which implies a similar pigment organization which was confirmed by CD spectra (Fig 4). Nevertheless, Lhcb3 shows some significant differences compared to Lhcb1 and Lhcb2, especially in the fluorescence and absorption properties. Lhcb3 absorbed and emitted light at a wavelength 2 nm longer than the other two isoforms did. In the CD spectrum (Fig. 4), Lhcb3 possesses a prominent peak at 470 nm, compared to the other two isoforms.

We also measured the thermostabilities (Table 2) and photostabilities (Figs. 6,7 and Table 3) of the three types of monomers and two types of trimers to explore the function of these complexes. Lhcb2 has been proved to become phosphorylated three times more rapidly than Lhcb1 (Islam 1987, Jansson and Gustafsson 1990). Our results have also showed that Lhcb2 is the most stable monomer in high light, indicating that the complexes containing Lhcb2 monomer might be an important player in modulating the energy balance in the bamboo thylakoid membrane under the fluctuating light conditions which bamboos have to encounter frequently in nature. There are contradictory conclusions about the function of Lhcb3. Lhcb3 is suggested to remain associated with PSII in different light regimes and not involved in light adaptation of photosynthesis because the absorption spectra of Lhcb3 indicate a clear red shift of the longer wavelength in Chl a molecules and the emitted light is clearly red-shifted as well, but with Lhcb3 mutant, Lhcb3 was found to play roles in modulating the rate of state transitions in Arabidopsis (Damkjaer et al. 2009) which is an important light adaptation. Caffarri et al. (2004) and Standfuss and Kühlbrandt (2004) concluded that Lhcb3 might act as an intermediary of the excitation energy
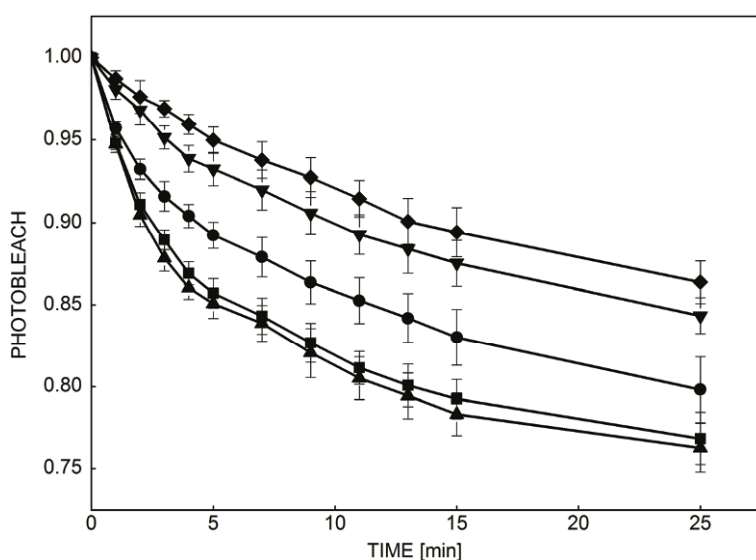

Fig. 7. Time course of the photobleaching processes. The decay curves show the whole pigment absorptions, measured upon integration of the absorption spectra in the Qy region, relative to the initial value (the initial value was set to 1). Each point represents the average of 5 individual experiments ± SD. Lhcb1M: (■), Lhcb2M: (●), Lhcb3M: (▲), Lhcb1T: (▼), Lhcb2T: (◆).

Table 3. Parameters of the one-phase exponential decay functions describing the decrease in the chlorophyll absorption due to photobleaching (asymptote Y_0, amplitude A, and half-life of the decay T'). All values are given as the average of 3–5 independent experiments ± SD.

Lhcb1	Y_0	A	T' [min]
Lhcb1M	0.78 ± 0.011	0.20 ± 0.013	3.5 ± 0.13
Lhcb2M	0.81 ± 0.012	0.17 ± 0.012	4.5 ± 0.14
Lhcb3M	0.77 ± 0.011	0.21 ± 0.014	3.2 ± 0.15
Lhcb1T	0.86 ± 0.012	0.13 ± 0.014	7.5 ± 0.13
Lhcb2T	0.88 ± 0.013	0.11 ± 0.015	8.4 ± 0.14

transfer between heterotrimers of Lhcb1/Lhcb2/Lhcb3 and the PSII core, but spectroscopic results from transient absorption and time-resolved fluorescence measurements did not prove it (Palacios *et al.* 2006). We revealed that

Lhcb3 of bamboo differs significantly in its biochemical and spectral properties, with the highest thermostability compared to the Lhcb1 and Lhcb2 isoforms in bamboo, which might make the trimers containing Lhcb3 more efficient and stable even at higher temperatures. The different combinations of Lhcb isoforms would cooperate and conduct the regulation between energy absorption and excess energy dissipation, ultimately maintain an appropriate amount of stable energy input for the light reaction in bamboo.

In summary, the studies on the biochemical and spectroscopical properties of all the Lhcb monomers and homotrimers in bamboo have not revealed significant differences in the structures and functions in these complexes compared to other well-studied species such as *Arabidopsis* (Standfuss and Kühlbrandt 2004), barley (Caffarri *et al.* 2004), and pea (Zhang *et al.* 2008).

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