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Cover page

Title: Substitution of deoxycholate with the amphiphilic polymer amphipol A8-35 improves the stability of large protein complexes during native electrophoresis

Running Title: Improved Stability with Clear Native-PAGE

Corresponding Author: Atsushi Takabayashi

Institute of Low Temperature Science, Hokkaido University, N19 W8 Kita-Ku, Sapporo 060-0819, Japan

E-mail: takabayashi@pop.lowtem.hokudai.ac.jp

Tel: +81-75-706-5493; Fax: +81-75-706-5493

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Title: Substitution of deoxycholate with the amphiphilic polymer amphipol A8-35 improves the stability of large protein complexes during native electrophoresis

Running Title: Improved Stability with Clear Native-PAGE

Shinsa Kameo[1], Michiki Aso[1], Ryo Furukawa[1], Renon Matsumae[1], Makio Yokono[2], Tomomichi Fujita[3], Ayumi Tanaka[1], Ryouichi Tanaka[1], Atsushi Takabayashi[1]

[1]Institute of Low Temperature Science, Hokkaido University, N19 W8 Kita-ku, Sapporo 060-0819, Japan

[2]Innovation Center, Nippon Flour Mills Co., Ltd., Atsugi 243-0041, Japan

[3]Faculty of Science, Hokkaido University, N10 W8 Kita-ku, Sapporo 060-0810, Japan

Abbreviations: BN, blue-native; CBB, Coomassie brilliant blue; CN, clear-native; α-DDM, α-dodecyl maltoside; DOC, sodium deoxycholate; LHC, light-harvesting complex; polyacrylamide gel electrophoresis (PAGE)

Abstract

Native polyacrylamide gel electrophoresis (PAGE) is a powerful technique for protein complex separation that retains both their activity and structure. In photosynthetic research, native-PAGE is particularly useful given that photosynthetic complexes are generally large in size, ranging from 200 kD to 1 MD or more. Recently, it has been reported that the addition of amphipol A8-35 to solubilized protein samples improved protein complex stability. In a previous study, we found that amphipol A8-35 could substitute sodium deoxycholate (DOC), a conventional electrophoretic carrier, in clear-native (CN)-PAGE. In this study, we present the optimization of amphipol-based CN-PAGE. We found that the ratio of amphipol A8-35 to α-dodecyl maltoside (α-DDM), a detergent commonly used to solubilize photosynthetic complexes, was critical for resolving photosynthetic machinery in CN-PAGE. In addition, LHCII dissociation from PSII-LHCII was effectively prevented by amphipol-based CN-PAGE compared with that of DOC-based CN-PAGE. Our data strongly suggest that majority of the PSII-LHCII *in vivo* forms $C_2S_2M_2$ at least in *Arabidopsis* and *Physcomitrella*. The other forms might appear owing to the dissociation of LHCII from PSII during sample preparation and electrophoresis, which could be prevented by the addition of amphipol A8-35 after solubilization from thylakoid membranes. These results suggest that

amphipol-based CN-PAGE may be a better alternative to DOC-based CN-PAGE for the study of labile protein complexes.

[Introduction]

Oxygenic photosynthetic organisms possess two photosystems, PSI and PSII, which are comprised of core and peripheral antenna complexes. The core antenna complex harvests light energy and transfers it to the reaction center where charge separation and the first electron transfer step occur. In comparison, the peripheral light-harvesting antenna associated with the PSII core harvests light energy and transfers the excitation energy to the core complex, where charge separation and the first electron transfer step occurs. Heat dissipation of the excess energy primarily occurs in the peripheral antenna complex. The core complexes of both PSI and PSII are highly conserved among photosynthetic organisms, while peripheral antenna systems are quite diverse (Blankenship 2010; Croce and van Amerongen 2014; Neilson and Durnford 2010; Nelson and Junge 2015). The diversity of the peripheral antenna system should be key to the adaptation of photosynthetic organisms to a wide range of environments.

The peripheral antenna complexes of green plants, including green algae and land plants, are comprised of light-harvesting complexes (LHCs). Light harvesting

complex proteins bind chlorophyll *a*, chlorophyll *b*, and carotenoids, which are indispensable for multiple LHC antenna functions. The LHCs can be associated with PSI and PSII to form PS supercomplexes (Caffarri et al. 2014; Gao et al. 2018; Kouril et al. 2012; Pan et al. 2019). Notably, the number of LHCs associated with PSI and PSII can be dynamically changed in response to environmental changes (Pan et al. 2019). Dynamic structural changes in the size of the peripheral antenna of photosystems may reveal the adaptive mechanisms of photosynthetic species.

Blue-Native (BN)-PAGE is a powerful technique that can be used to resolve protein complexes. For the analysis of photosynthetic complexes from various photosynthetic organisms, BN-PAGE has been used to separate the components of the thylakoid membranes that are solubilized with mild detergents such as dodecyl maltoside (α-DDM) or digitonin (Järvi et al. 2011; Wittig et al. 2006). Since BN-PAGE allows for the native structure and activity of protein complexes to be retained during electrophoresis, broad downstream BN-PAGE applications have been reported, such as proteomic identification, pigment composition evaluations, spectroscopic analyses, and the reconstitution of protein structures using electron microscopy (Wittig et al. 2006). As such, multiple post-BN-PAGE applications have furthered the analysis of photosynthetic machinery from various perspectives.

92 Coomassie brilliant blue (CBB) dye is used in BN-PAGE to negatively charge
93 protein complexes, which enables them to be resolved at neutral pH (Wittig et al. 2006).
94 The presence of CBB on the protein surface reduces the dissociation risk due to protein-
95 protein interactions when detergents are present (Wittig et al. 2006). However, the blue
96 color of the CBB dye inhibits some downstream experiments, including absorption
97 spectra measurements of the resolved photosynthetic complexes. In addition, CBB dye
98 can act as a competitive quencher of chlorophyll fluorescence in LHCII (Yokono,
99 2015). In such cases, CBB is substituted with sodium deoxycholate (DOC), a colorless
100 compound that negatively charges proteins. This form of PAGE is referred to as clear
101 native (CN)-PAGE (Järvi et al. 2011; Wittig and Schägger 2005). However, the use of
102 DOC as an electrophoresis carrier may promote dissociation due to protein-protein
103 interactions, which is not a problem when CBB is used (Witting and Schägger 2005).
104 Thus, an alternative to DOC in CN-PAGE is needed.
105 Amphipols are a new class of amphipathic polymers that enable membrane
106 protein complexes to be solubilized while enhancing their stability in detergent-free
107 solutions (Popot et al. 2011). In particular, amphipol A8-35 has been widely used to
108 stabilize membrane proteins (Popot et al. 2011). In addition, a pioneering study with

Chlamydomonas showed that amphipol A8-35 was able to stabilize PSII-LHCII supercomplexes during sucrose density gradient centrifugation (Watanabe et al. 2019).

In addition to its stabilizing effect on membrane proteins, amphipol A8-35 can negatively charge protein complexes under conditions of neutral pH due to its negatively charged surface. These characteristics suggest that amphipol A8-35 may be a good alternative to DOC. We recently tested the ability of an amphipol-based CN-PAGE to resolve photosynthetic protein complexes with *Physcomitrella* (Furukawa et al. 2019). In the current study, we demonstrated that the substitution of DOC with amphipol A8-35 improved the stability of the photosynthetic machinery, especially for the PSII-LHCII supercomplexes during CN-PAGE separation. The resolution of amphipol-based CN-PAGE was comparable to that of DOC-based CN-PAGE. Our results strongly suggest that amphipol-based CN-PAGE is a better alternative to DOC-based CN-PAGE with the potential for a wide variety of downstream applications.

[Results]

Optimization of the ratio of amphipol A8-35 to α-DDM on CN-PAGE

The first step of amphipol-based CN-PAGE is the addition of amphipol A8-35 instead of DOC to maintain the solubility of the protein complex. Owing to the slow

127 dissociation of amphipols from membrane proteins (Popot et al. 2011), the addition of
128 amphipol A8-35 to the cathode buffer is not necessary during electrophoresis (Furukawa
129 et al. 2019). Since amphipol A8-35 is much more expensive than DOC, it is also cost-
130 effective to avoid the addition of amphipol A8-35 to the cathode buffer. However, no
131 optimization of the A8-35:α-DDM ratio for a given protein sample has yet to be
132 reported.

133 To optimize the A8-35:α-DDM ratio, we evaluated the changes in *Arabidopsis*
134 photosynthetic machinery band patterns as the amphipol A8-35 concentration varied.
135 The concentration of α-DDM was fixed at 1% to solubilize *Arabidopsis* thylakoid
136 protein complexes. After electrophoresis, several pigment-protein complexes were
137 observed, including the PSII-LHCII supercomplexes, PSI-LHCI and LHCII (Fig. 1).
138 Band identification was conducted using 2D-SDS-PAGE with silver staining (Fig. S1)
139 according to the band patterns of previous studies (i.e., Järvi et al. 2011; Takabayashi et
140 al. 2011). Although the band patterns were very similar between 1% and 0.5% amphipol
141 A8-35, slight differences were observed. Specifically, the mobility of PSI-LHCI and
142 LHCII trimers showed greater impairment with 0.5% amphipol A8-35 than with 1%
143 amphipol A8-35 (Fig. 1). This impaired mobility suggests that the quantity of amphipol
144 A8-35 bound to the photosynthetic machinery was not saturated when 0.5% amphipol

145 A8-35 was employed. However, when the amphipol A8-35 concentration was lower
146 than 0.25%, apparent differences were present among the band patterns. Specifically,
147 the LHCII trimers and PSI-LHCI bands were poorly resolved. These results demonstrate
148 that amphipol A8-35:α-DDM ratio in a given sample is important for resolving protein
149 complexes by amphipol-based CN-PAGE.

150 Since surfactants and amphipols competitively bind to the protein surface
151 (Popot et al. 2011), a low amphipol A8-35:α-DDM ratio may inhibit the binding of
152 amphipol A8-35 to the surface of the protein. To evaluate this hypothesis, we reviewed
153 changes in the band patterns as the α-DDM concentration increased from 1-4% while
154 the amphipol A8-35 concentration was fixed at 1%. As expected, the resolution
155 worsened as the α-DDM concentration increased (Fig. 2). Finally, we reviewed band
156 patterns as the α-DDM concentration increased with a fixed amphipol A8-35:α-DDM
157 ratio. When the ratio of amphipol A8-35 to α-DDM was fixed, the band patterns
158 presented little variability, even when the α-DDM concentration increased from 1 to 4%
159 (Fig. 3). These results indicated that amphipol A8-35:α-DDM ratio was important for
160 ensuring sufficient resolution when amphipol A8-35 was not added to the cathode
161 buffer. It is noteworthy that the separation pattern of the photosynthetic complexes in
162 amphipol-based CN-PAGE did not significantly worsen even when high concentration

α-DDM (4%) was used for solubilization (Fig. 3). This strongly suggests that the dissociation of LHCII from PSII by high concentration α-DDM occurs extensively during electrophoresis, which can be prevented by the addition of amphipol A8-35 after solubilization.

Optimization of the timing of adding amphipol A8-35 to a solubilized protein sample

In a previous study, amphipol A8-35 was added to solubilized protein samples after centrifugation. In this study, we optimized the timing of amphipol A8-35 addition to protein samples. It should be noted that although α-DDM is a mild detergent, it promotes the dissociation of LHCII from PSI-LHC and PSII-LHCII (Järvi et al. 2011). Thus, we hypothesized that the early addition of amphipol A8-35 to a protein sample would reduce the risk of LHCII dissociation.

We noted that the photosystems were hardly solubilized by α-DDM when amphipol A8-35 was included in the thylakoid membrane solution. Similarly, the amounts of solubilized photosystems were greatly reduced when A8-35 was added immediately after mixing the samples with α-DDM (Fig. 4a). However, a two minute incubation period, after mixing the thylakoid sample with α-DDM prior to the addition

of A8-35, resulted in a considerable amount of solubilized photosystem as when A8-35 was added after centrifugation (Fig. 4b). These data strongly suggest that amphipol A8-35 inhibits the solubilization of the membrane protein complexes in thylakoids by α-DDM, unless there is enough incubation time before adding A8-35 to the sample. In addition, we found that the band patterns were similar regardless of whether amphipol A8-35 was added to the protein samples either before or after centrifugation (Fig. 4). These results suggest that LHCII dissociation rarely occurred during the short 5-min centrifugation (Fig. 4).

Comparison of the band patterns of *Arabidopsis* and *Physcomitrella* thylakoid protein complexes between DOC-based CN-PAGE and amphipol-based CN-PAGE using amphipol A8-35

To compare amphipol-based CN-PAGE using amphipol A8-35 with DOC-based CN-PAGE, we resolved α-DDM-solubilized *Arabidopsis* thylakoid protein complexes using the two methods (Fig. 5). The identification of the resolved bands by DOC-based CN-PAGE was performed by 2D-SDS-PAGE followed by silver staining (Fig. S1), following the methodology of Furukawa et al. (2019). We found that the PSI-LHCI, LHCII trimer, LHC monomers, and free pigments were well resolved by both

methods (Fig. 5). Since free pigments appeared yellow in color, they likely contained more carotenoids than chlorophylls. On the other hand, we found notable differences in the resolution of the PSII-LHCII supercomplexes (Fig. 5). The three PSII-LHCII forms were observed with DOC-based CN-PAGE. The largest PSII-LHCII form was $C_2S_2M_2$. As the number of attached LHCII decreases, the $C_2S_2M_2$ form changes to C_2S_2M and then to C_2S_2 (Caffarri et al. 2009; Pan et al. 2019; Su et al. 2017; van Bezouwen et al. 2017; Wei et al. 2016). The most commonly observed PSII-LHCII forms in both methods were $C_2S_2M_2$ and C_2S_2M. In addition, the band intensity of $C_2S_2M_2$ seemed more prominent than that of C_2S_2M on amphipol-based CN-PAGE. In contrast, the C_2S_2 band was clearly observed in DOC-based CN-PAGE, whereas this band was hardly observed in amphipol-based CN-PAGE. The greater quantity of C_2S_2 in DOC-based CN-PAGE compared to that of amphipol-based CN-PAGE was likely owing to the dissociation of LHCII from the larger PSII-LHCII forms. These results strongly suggest that LHCII detachment from PSII-LHCII was low when amphipol A8-35 was employed with CN-PAGE.

The same trend was observed for CN-PAGE with *Physcomitrella* thylakoid protein complexes. A PSII-PSI megacomplex, PSII-LHCII supercomplexes, PSI-LHCI, LHCII trimer, and LHCII monomer were resolved (Fig. 6). To identify the resolved

bands, we performed 2D-CN/SDS-PAGE followed by silver staining (Fig. S2). Although the overall band patterns were similar between DOC-based CN-PAGE and amphipol-based CN-PAGE, a notable difference was observed between these methods (Fig. 6). Two PSII-LHCII bands were clearly observed with DOC-based CN-PAGE, whereas one PSII-LHCII band was clearly observed with amphipol-based CN-PAGE. The additional PSII-LHCII band found in the DOC-based CN-PAGE had a lower molecular weight, which suggests that LHCII partially dissociated from PSII-LHCII. The low molecular weight form of PSII-LHCII may have possibly been C_2S_2M. However, the PSII-LHCII supercomplex forms in *Physcomitrella* have not yet been fully elucidated. Therefore, we were unable to identify the low molecular weight form of PSII-LHCII as C_2S_2M in this study. These results indicated that amphipol A8-35 was superior to DOC when preventing LHCII dissociation from PSII-LHCII in CN-PAGE. Furthermore, these data strongly suggest that majority of the PSII-LHCII *in vivo* forms $C_2S_2M_2$ in *Arabidopsis* and *Physcomitrella*. The other forms of PSII-LHCII are likely to have emerged as a result of the dissociation of LHCII from PSII during sample preparation and electrophoresis.

[Discussion]

The ratio of amphipol A8-35 to α-DDM in a sample is important to resolve membrane protein complexes

In this study, we optimized amphipol-based CN-PAGE protocols. Since amphipol A8-35 is negatively charged, amphipol A8-35-bound membrane protein complexes can migrate during electrophoresis at neutral pH. On the other hand, CBB and DOC must be added to the cathode buffer for BN-PAGE and DOC-based CN-PAGE, respectively. This difference is based on the chemical properties of amphipol A8-35. Amphipol A8-35 tightly and noncovalently binds to the hydrophobic surfaces of membrane protein complexes and shows an extremely slow dissociation rate (Popot et al. 2011). Owing to this property, amphipol A8-35 was not added to the cathode buffer during electrophoresis. However, amphipol A8-35 dissociates from membrane proteins in the presence of competing surfactants, such as α-DDM. This is likely the reason why using a proper amphipol A8-35:α-DDM ratio is important to achieve good protein complex resolution (Figs 1-3).

If a sufficient amount of amphipol A8-35 is added to the cathode buffer, the amphipol A8-35:α-DDM ratio in the loading sample may not be important. However, the economic cost of amphipol-based CN-PAGE is much higher when using amphipol A8-35 in the cathode buffer.

Amphipol A8-35 can efficiently prevent the dissociation of LHCII from PSII-LHCII supercomplexes during CN-PAGE

We found that the amphipol-based CN-PAGE method was better able to separate PSII-LHCII supercomplexes while retaining their intact forms than the CN-PAGE method when using *Arabidopsis* and *Physcomitrella* thylakoid membranes (Fig. 5 and 6). Specifically, greater dissociation of LHCII from the PSII-LHCII supercomplexes seemed to occur both with *Arabidopsis* and *Physcomitrella* when using DOC-based CN-PAGE. These results are consistent with those of a previous report in which amphipol A8-35 was found to prevent the dissociation of LHCII from the PSII-LHCII supercomplexes with *C. reinhardtii* during sucrose density gradient separation (Watanabe et al. 2019). It is noteworthy that membrane protein complexes are typically much more stable with amphipols compared to their stability in detergent solutions (Popot et al. 2011). Thus, amphipol A8-35-PSII-LHCII complexes in detergent-free states are likely to be more stable than PSII-LHCII complexes in α-DDM solutions or DOC solutions. As such, amphipol-based-CN-PAGE facilitates the retention of intact PSII-LHCII forms during sample preparation and electrophoresis.

Our findings that the C_2S_2 M_2 was the major form of the PSII-LHCII on amphipol-based CN-PAGE (Figs 5 and S1) strongly suggest that PSII-LHCII should be essentially a $C_2M_2S_2$ *in vivo*, and other forms are likely to emerge owing to the dissociation of LHCII from PSII during the experimental procedure. Accordingly, amphipol-based CN-PAGE can be a useful tool to reveal the dynamics of the PSII-LHCII supercomplexes in response to environmental changes.

Potential applications of amphipol-based CN-PAGE

Previous reports have shown that amphipols typically enhance protein stability but do not interfere with protein function (Popot et al. 2011). These properties permit a wide variety of applications using amphipols, including protein structural and functional analyses. In addition, BN-PAGE and DOC-based CN-PAGE can separate protein complexes while retaining their structure and function, allowing for a wide variety of downstream applications using these methods (Wittig et al. 2006; Wittig and Schägger 2005). Specifically, BN-PAGE and CN-PAGE have often been used to separate membrane protein complexes with mild detergents, including α-DDM and digitonin. Here, we found that amphipol-based CN-PAGE can be applied to separate membrane protein complexes solubilized by α-DDM and digitonin (Fig. S3). It is noteworthy that

digitonin is the only known detergent that can retain the PSI-LHCI-LHCII complex after solubilization (Järvi et al. 2011). Due to these advantages, amphipol-based CN-PAGE is likely to have wide-ranging applications. In particular, the advantage of CN-PAGE over BN-PAGE is that CBB does not interfere with spectroscopic and pigment analyses. Since these analyses are important for understanding photosystem properties, CN-PAGE with amphipol A8-35 is preferable to BN-PAGE. In addition, given the stabilizing effect of amphipol A8-35, structural and functional analyses following amphipol-based CN-PAGE may yield better results than those of either DOC-based BN- or CN-PAGE. Nonetheless, further studies are needed to verify the potential of amphipol-based CN-PAGE.

[Materials and Methods]

Plants and growth conditions

Arabidopsis thaliana ecotype Columbia plants were grown in soil for 6 weeks at 23°C with a 14:10 h (light:dark) photoperiod that employed 70 µmol photons m^{-2} s^{-1}. Protonemata from *Physcomitrella patens* were cultured for 4 days on a layer of cellophane overlaid on BCDAT (BCD medium supplemented with 1 mM $CaCl_2$ and 5 mM di-ammonium [+]-tartrate) that was solidified with 0.8% (w/v) agar at 25°C under

306 continuous light conditions (40 μmol photons m^{-2} s^{-1}), following the methodology of
307 Furukawa et al. (2019).
308
309 **Separation of thylakoid protein complexes by CN-PAGE**
310 *Arabidopsis* thylakoid membrane isolation followed the methodology of Järvi et al.
311 (2011). *Physcomitrella* thylakoid membrane isolation was performed according to the
312 methodology of Furukawa et al. (2019). Unless otherwise noted, the thylakoid
313 membranes were solubilized with 1% α-DDM in a solubilization buffer (50 mM
314 imidazole/HCl, pH 7.0, 20% glycerol) with 10 mM sodium fluoride and a protease
315 inhibitor cocktail for plant cell lysate (Sigma, St. Louis, MO, USA) at 4°C and
316 centrifuged at 22,500 × *g* for 5 min at 4°C.
317 For amphipol-based CN-PAGE, amphipol A8-35 was added to the supernatants
318 at a final concentration of 1%. The supernatants with amphipol A8-35 were separated on
319 4-13% polyacrylamide gradient gels at 4°C using anode (50 mM imidazole/HCl, pH
320 7.0, 4°C) and cathode buffers (50 mM Tricine and 15 mM imidazole/HCl, pH 7.0, 4°C),
321 according to the methodology of Furukawa et al. (2019). Amphipol A8-35 was not
322 added to the cathode buffer of amphipol-based CN-PAGE. For DOC-based CN-PAGE,
323 0.05% DOC and 0.02% α-DDM were added to the cathode buffer following the

methodology of Järvi et al. (2011). Native Mark Unstained Protein Standard (Thermo Fisher Scientific, Rockford, IL, USA) was used as a marker of molecular size.

2D-SDS-PAGE followed by immunoblot analysis and silver staining

Proteins in the CN-PAGE gel strip were denatured in a solubilization buffer (1% SDS and 50 mM DTT) for 30 min at 25°C and separated on a 14% acrylamide gel containing 4 M urea using the Laemmli system following the methodology of Furukawa et al. (2019). Silver staining was performed using the Pierce Silver Stain kit (Thermo Fisher Scientific, _Rockford, IL, USA), according to the manufacturer' s protocol. Precision Plus Protein All Blue Standards (Bio-Rad, Hercules, CA, USA) was used as a marker of molecular size. The separated proteins were transferred to a PolyScreen polyvinylidene fluoride membrane (PerkinElmer Life Sciences, MA, USA). The PsaD, PsbB, and Lhcb2 subunits were detected using specific antibodies and a Western Lightning Plus-ECL kit (PerkinElmer Life Sciences). All antibodies, including anti-PsaD (AS09 461), anti-PsbB (AS04 038), and anti-Lhcb2 (AS01 003) were purchased from Agrisera (Vännäs, Sweden).

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[Figure legends]

Fig. 1 Changes in the band pattern of amphipol-based CN-PAGE with amphipol A8-35. *Arabidopsis* thylakoid protein complexes were solubilized by 1% α-DDM. After centrifugation, amphipol A8-35 was added to the supernatants for final concentrations of 1%, 0.5%, 0.25%, and 0.125%. The samples were separated by 4-13% acrylamide gels. No amphipol A8-35 was added to the cathode buffer.

Fig. 2 Changes in the band pattern of amphipol-based CN-PAGE as the concentration of α-DDM varied. *Arabidopsis* thylakoid protein complexes were solubilized by 1%, 2%, and 4% α-DDM. After solubilization, amphipol A8-35 was added to the supernatants at a final concentration of 1%. The samples were loaded and separated by 4-13% acrylamide gels. No amphipol A8-35 was added to the cathode buffer.

Fig. 3 The changes in the band pattern of the amphipol-based CN-PAGE with the same ratio of amphipol A8-35 to α-DDM. *Arabidopsis* thylakoid protein complexes were solubilized by 1%, 2%, and 4% α-DDM. After solubilization, amphipol A8-35 was added to the supernatants for final concentrations of 1%, 2%, and 4%, respectively. The ratio of amphipol A8-35 to α-DDM was the same among the samples. The samples were loaded and separated by 4-13% acrylamide gels. No amphipol A8-35 was added to the cathode buffer.

Fig. 4 Band patterns of amphipol-based CN-PAGE when amphipol A8-35 was added to the samples either before or after centrifugation. *Arabidopsis* thylakoid protein complexes were solubilized by 1% α-DDM. (a) Immediately after mixing the samples with α-DDM, amphipol A8-35 was added to the supernatants either before or after centrifugation. (b) After mixing with α-DDM and the subsequent incubation for 2 min, amphipol A8-35 was added to the supernatants either before or after centrifugation. The samples were loaded into and separated by 4-13% acrylamide gels. No amphipol A8-35 was added to the cathode buffer.

Fig. 5 *Arabidopsis* thylakoid protein complexes resolved by DOC-based and amphipol-based CN-PAGE. *Arabidopsis* thylakoid protein complexes were solubilized by 1% α-DDM and resolved by the DOC-based (A) and amphipol-based (B) CN-PAGE. After solubilization, amphipol A8-35 was added to the supernatants at a final concentration of 1% (B). The samples were loaded and separated by 4-13% acrylamide gels. The cathode buffer of the DOC-based CN-PAGE contained 0.05% DOC and 0.02% α-DDM. No amphipol A8-35 was added to the cathode buffer for amphipol-based CN-PAGE.

Fig. 6 *Physcomitrella* thylakoid protein complexes resolved by amphipol-based and DOC-based CN-PAGE. *Physcomitrella* thylakoid protein complexes were solubilized by 1% DDM and resolved by and amphipol-based (A) and DOC-based (B) CN-PAGE. After solubilization, amphipol A8-35 was added to the supernatants at a final concentration of 1% (A). The samples were loaded and separated by 4-13% acrylamide gels. The cathode buffer of the DOC-based CN-PAGE contained 0.05% DOC and 0.02% α-DDM. No amphipol A8-35 was added to the cathode buffer for amphipol-based CN-PAGE.

Fig. 1

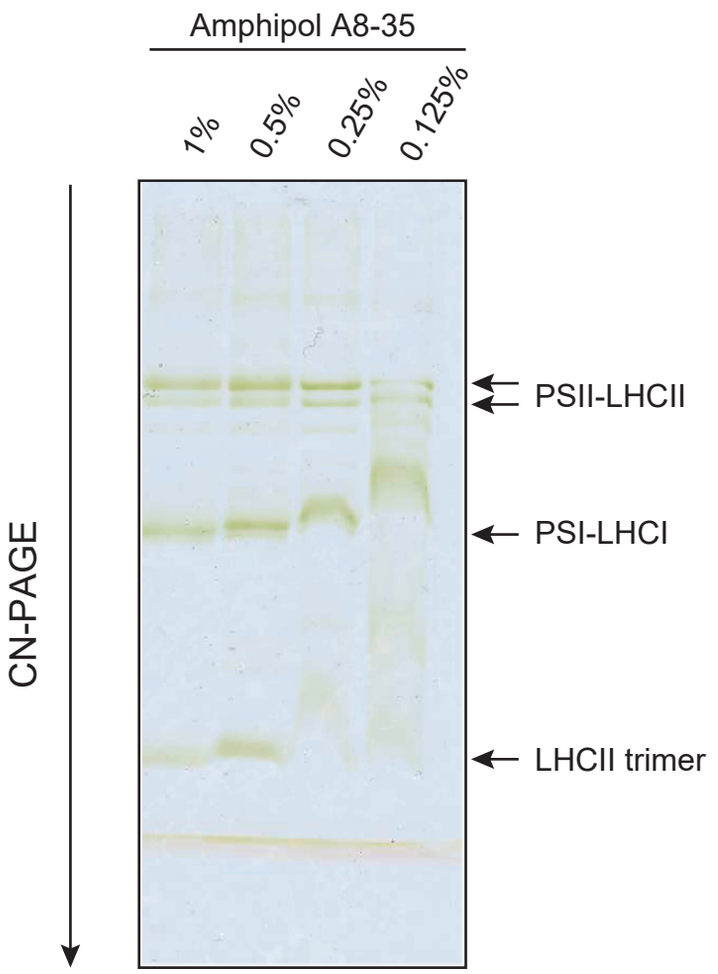
Amphipol A8-35
1%
0.5%
0.25%
0.125%
CN-PAGE
PSII-LHCII
PSI-LHCI
LHCII trimer

Fig. 2

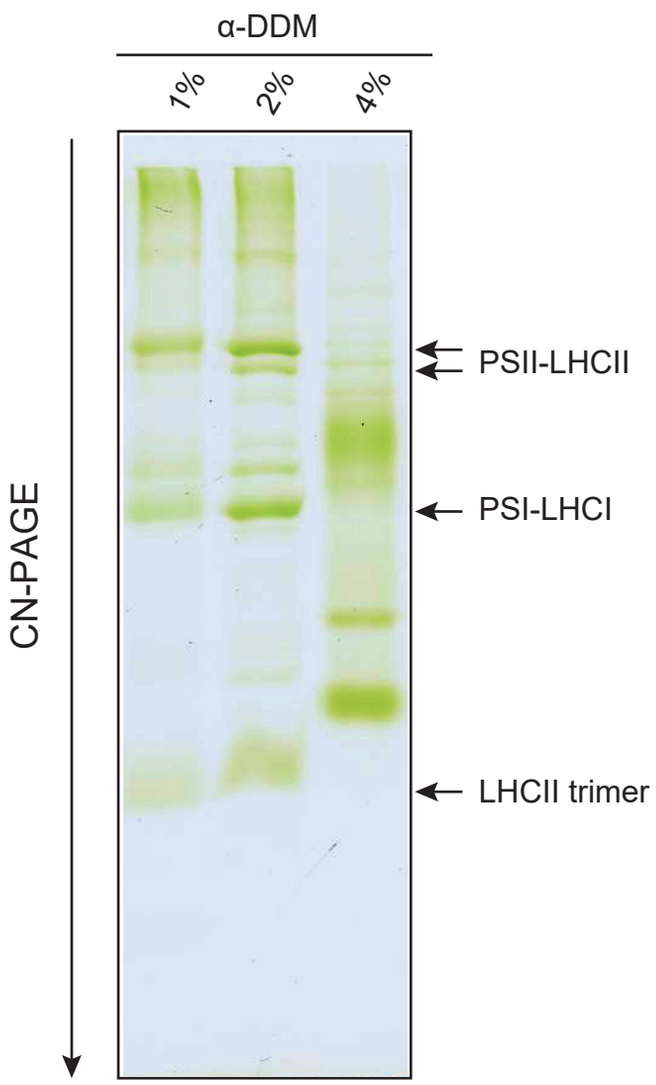

Fig. 3

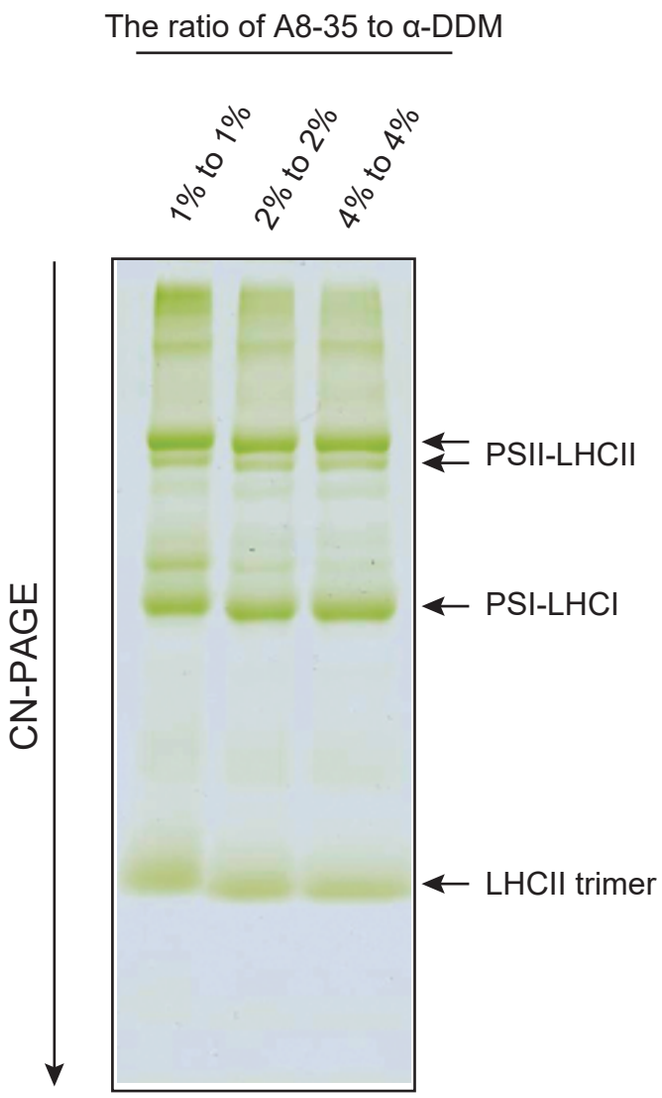
The ratio of A8-35 to α-DDM
1% to 1%
2% to 2%
4% to 4%
CN-PAGE
PSII-LHCII
PSI-LHCI
LHCII trimer

Fig. 4

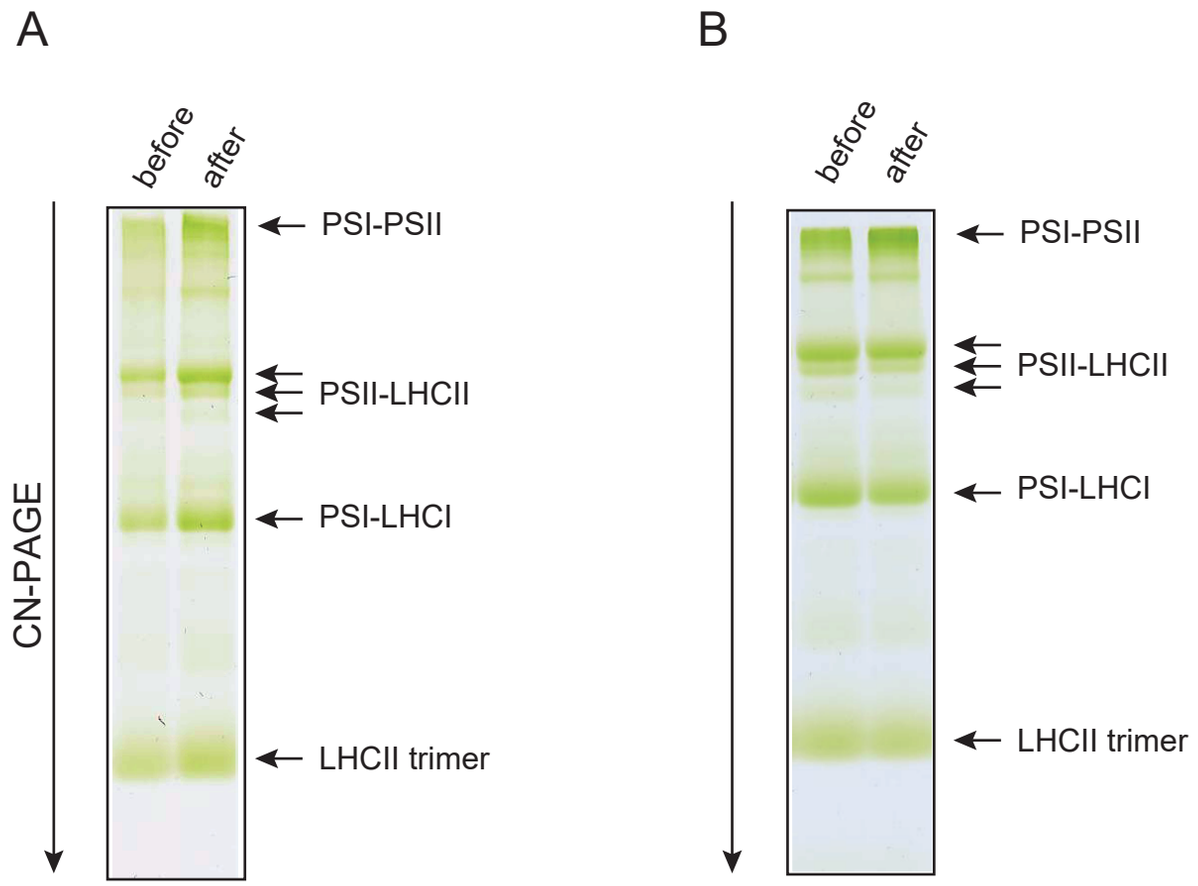
A
before
after
CN-PAGE
PSI-PSII
PSII-LHCII
PSI-LHCI
LHCII trimer
B
before
after
PSI-PSII
PSII-LHCII
PSI-LHCI
LHCII trimer

Fig. 5

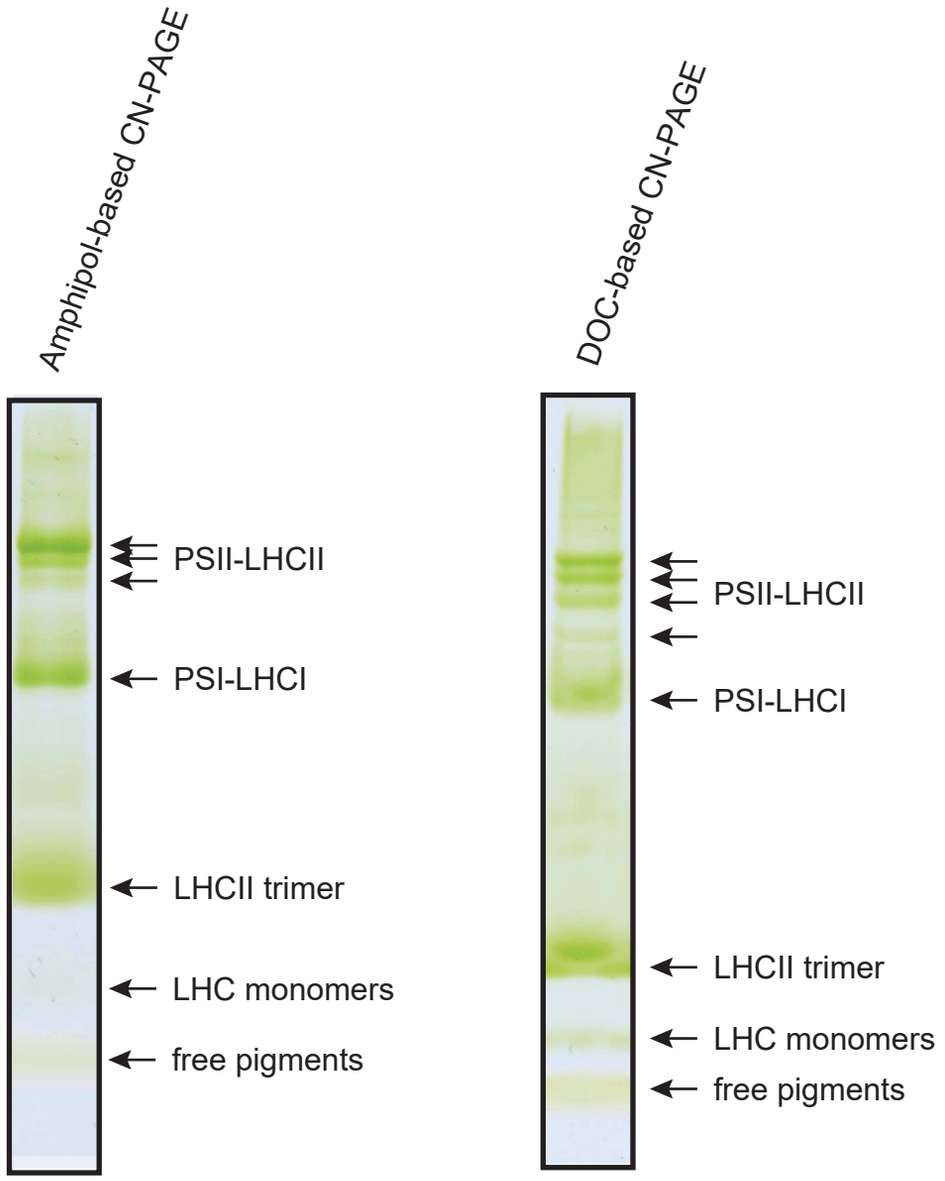
Amphipol-based CN-PAGE
PSII-LHCII
PSI-LHCI
LHCII trimer
LHC monomers
free pigments
DOC-based CN-PAGE
PSII-LHCII
PSI-LHCI
LHCII trimer
LHC monomers
free pigments

Fig. 6

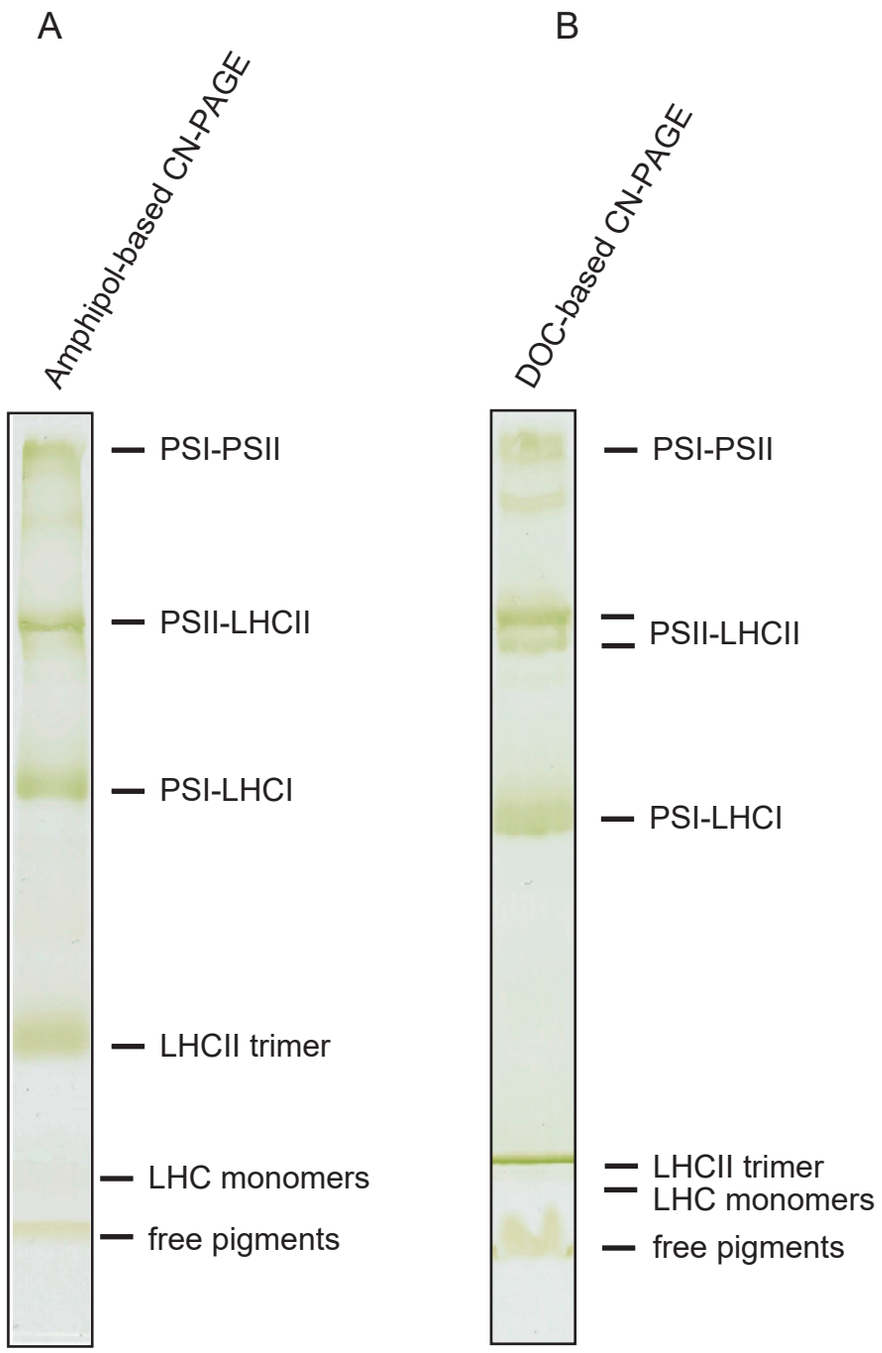

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Fig. S1

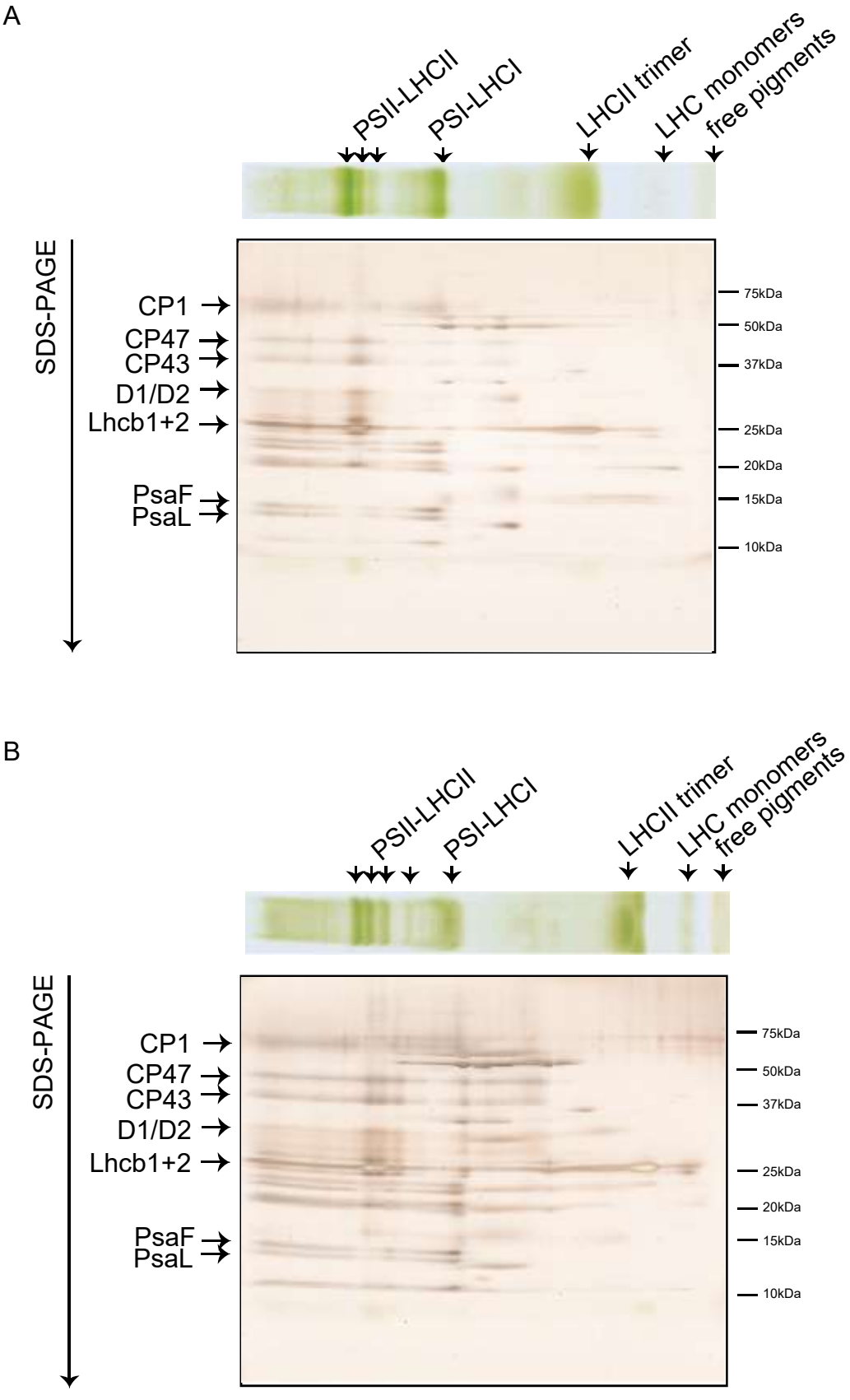

Fig. S1 2D-CN/SDS-PAGE of *Arabidopsis* protein complexes in thylakoid membranes followed by silver staining. The amphipol-based (a) and DOC-based (b) CN-PAGE gel strips (Fig. 5) were soaked in 1% SDS and 50 mM DTT solution for 30 min. Proteins in the gel strips were resolved by SDS-PAGE using a 14% acrylamide gel containing 6 M urea and visualized by silver staining.

Fig. S2

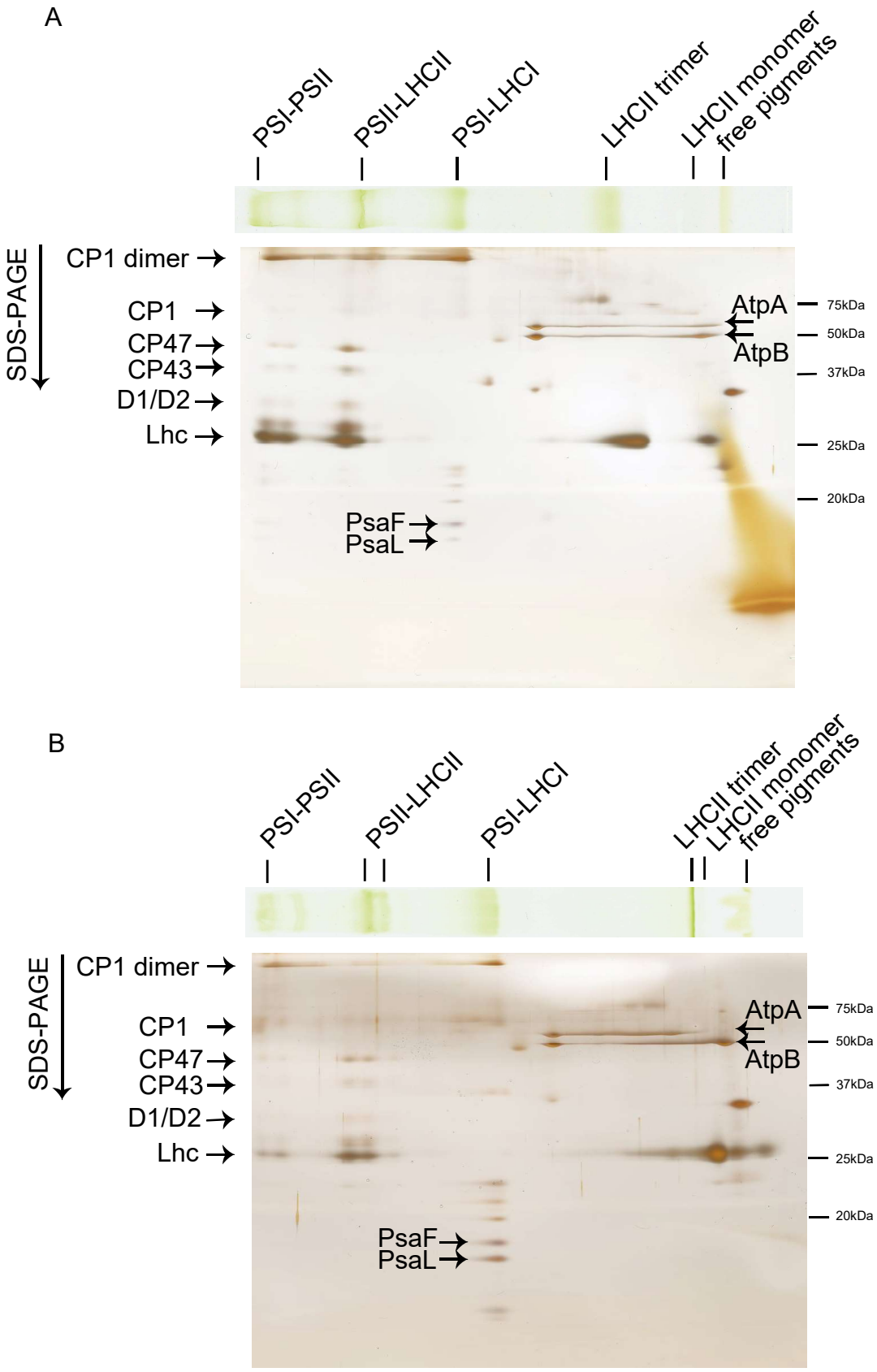

Fig. S2 2D-CN/SDS-PAGE followed by silver staining using the amphipol-based and DOC-based CN-PAGE gel strips for separation of *Physcomitrella* protein complexes in thylakoid membranes. The amphipol-based (A) and DOC-based (B) CN-PAGE gel strips (Fig. 6; upper) were soaked in 1% SDS and 50 mM DTT solution for 30 min. Proteins in the gel strips were resolved by SDS-PAGE using a 14% acrylamide gel containing 4 M urea and were visualized by silver staining.

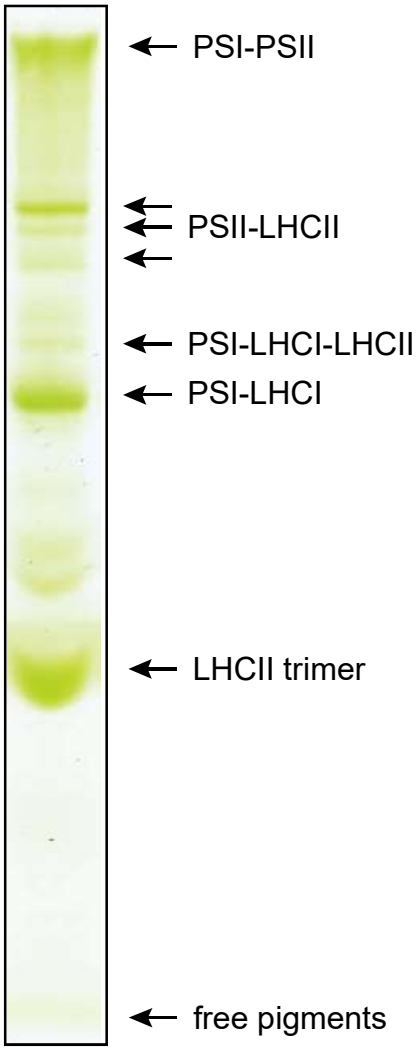

Fig. S3 Amphipol-based CN-PAGE of *Arabidopsis* protein complexes in thylakoid membranes solubilized by digitonin. *Arabidopsis* protein complexes in thylakoid membranes were solubilized by 1% digitonin and resolved by amphipol-based CN-PAGE using 4-13% acrylamide gels.