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Peptide Detergent A_6K Stabilize Photosystem II from Spinach *in vitro*

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ABSTRACT

Detergents, including a new type of detergent—the designed peptide detergent acetyl-AAAAAAK (A_6K), were used to study their effects on stabilizing photosystem II (PS II). Their effects were studied at different concentrations. Detergents at higher concentration showed greater solubilization effects on PS II. The microenvironments of protein-binding Chls have been observed for a period of 28 days, using absorption and fluorescence spectra. Triton X-100 and SDS caused dissociation of Chls from PS II and the inhibition of excitation energy transfer from Chl *b* to Chl *a*. OG showed little effect on stabilizing PS II. DM partially stabilized the PS II, while the peptide detergent A_6K stabilized PS II more effectively than the other common membrane protein-stabilizing detergents. Our results indicate that designed peptide detergents A_6K could stabilize PS II particle and may be useful for the study of the structure and function of thylakoid membrane proteins.

Keywords: Detergent, Photosystem II, Chlorophyll, Absorption Spectrum, Fluorescence Spectrum

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INTRODUCTION

Although over 30% of currently sequenced genomes encode membrane protein, only 0.2% of solved structure are membrane proteins [1-5]. The many practical problems of working with membrane proteins resulted in the great disparity between our understanding of soluble proteins and membrane proteins. One common problem is their poor stability, which lead to rapid inactivation. In membrane protein research, detergents are ubiquitous and necessary reagents that maintain a target membrane protein in a functional, folded state in the absence of membrane. Detergent is usually composed of a hydrophilic head and a hydrophobic tail group. Depending on the types of their hydrophilic group, detergents are divided into three kinds: ionic, nonionic and zwitterionic. Types of head group have great impact on the character and behavior of detergents. Thus the discovery and design of new detergents are acutely needed to facilitate the study of membrane protein crystallization and structure [4,6].

Photosystem II (PS II) is a membrane-bound pigment-protein complex which contains a great many of chlorophylls (Chls) that play crucial roles in harvesting and transferring light energy and initiating electron-transfer reactions. Detergents are widely used in the isolation and stabilization of pigment-protein complexes of photosynthetic membrane, as well as in the studies on the structures and functions of these complexes. The isolation of protein on photosynthetic membranes usually involves extracting the protein out of the membrane and inserting it into detergent micelles. Although lots of detergents have been used in the membrane protein studies, few have been proved satisfactorily effective in stabilizing membrane proteins, because the membrane proteins tend to denature or aggregate quickly in solution [9]. Therefore, choosing the right detergent and experimental conditions is crucial for the experiment of a membrane protein. An effective detergent should prevent membrane proteins from denaturing or aggregating and retain the structure and function of the proteins. Evidences show that short-chain detergents are more effective in solubilizing membrane proteins than detergents with long chains, which usually induce protein denaturation [10,11].

There is a new type of designed short-chain detergent that is composed of several acid residues, called peptide detergent. It is reported these detergents consisting of 6~10 amino acid residues, are 2~3 nm long, and when dissolved in water, they undergo self-assembly to form micelles, nanovesicles, or nanotubes when they are above their critical aggregation concentration (CAC). The peptide detergents that previously reported include A_6K, A_6D, G_4D_2 G_6D_2, G_8D_2, L_6D_2, V_6D, and V_6K [12-14]. Previous works have shown that the designed peptide detergents stabilize some membrane proteins successfully, such as photosystem I complex [15], glycerol-3-phosphate dehydrogenase [16], and GPCR bovine rhodopsin [17]. A_6K, one of these designed detergents, is a cationic peptide detergent. It has a hydrophilic amino acide-lysine at C terminus and followed by 6 alanines which are hydrophobic amino acids [15].

In our work, we investigated the ability of the peptide detergent A_6K to stabilize the

PS II membrane isolated from spinach, using visible absorption spectra and fluorescence spectra. We used four traditional chemical detergents as comparisons. *n*-dodecyl-β-D-maltoside (DM) and *n*-octyl-β-D-glucopyranoside (OG) are generally used to stabilize membrane protein [18,19], while Triton X-100 is often used in isolation of thylakoid membrane proteins [20-22]. Sodium dodecyl sulfate (SDS), an ionic detergent, is quite effective in solubilizing membrane protein but usually leads to protein denatureation [19,23]. We tested the stability of PS II with detergents in 28 days. Compared with the other detergents, here we reported that the peptide detergent A_6K stabilized PS II more effectively.

MATERIALS AND METHODS

Materials

Spinach **(***Spinacia Oleracea* L.) was bought from supermarket.

Reagents

Analytical grade SDS and the Triton X-100 were purchased from Sigma. Peptides were obtained from the MIT Biopolymers Laboratory and Synpep Corporation. All other reagents used in this study made in home were also of analytical grade.

Isolation and purification of PS II membrane

Thylakoid membranes were prepared according to Bassi and Simpson [24]. Particles of PS II were isolayed according to Kuwaraba and Murata [25]. The purified PS II particles were finally resuspended in the solution of 0.4 mol/ L sucrose, 0.05 mol/L Mes-NaOH, 0.01 mol/L NaCl, 5 mmol/L $MgCl_2$, pH 6.0 and characterized by measuring its absorption spectra, fluorescence emission spectra and SDS- PAGE at room temperature.

Measurement of chlorophyll concentration

The chlorophyll concentration of the preparation was measured by the method of Arnon [26].

Spectroscopy measurement

The fluorescence emission spectrum was measured with Hitachi F-4500 fluorescence Spectrophotometer at room temperature; Excitation was at 436 nm and 475 nm, slits set at 2 nm. The absorption spectra were measured Shimadzu Graphicord UV-240 UV-Visible Recoding Spectrophotometer. The chlorophyll concentration of the samples was 5µg/ ml.

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RESULTS

Addition of peptide detergent A_6K stabilize the absorption spectra of PS II particle at room temperature

In order to determine how concentration of detergent affects the stabilization of PS II particle, different concentrations (0.031%, 0.062%, 0.125%, 0.25% and 0.5%) of detergents were used to stabilize PS II.

The visible absorption spectra of PS II were measured at room temperature. The absorption spectrum of PS II in red region is characterized by a band at around 680 nm and a shoulder at 650nm. The former originates from Chl *a*, while the later corresponds to Chl *b*. There are two peaks in blue region: one at approximately 436 nm is mainly due to Chl *a*, and the other at around 470 nm belongs to Chl *b*.

As shown in figure 1(a), the absorption peaks changed a little when the concentration of DM ranged from 0.031% to 0.25%. There was an apparent increase of PS II absorption peaks in both red and blue region when DM concentration was increased to 0.5%. With the increase of the concentration of OG and Triton X-100, the PS II absorption peaks increased. When Triton X-100 added to PS II, Chl *b* absorption peak in red region and Chl *a* in blue region blue-shifted about 10 nm. Higher concentrations of Triton X-100 led to further blue-shifts of peaks. When Triton X-100 concentration was increased to 0.5%, the Chl *b* absorption peak in red region shifted 2 nm to the blue and the Chl *a* in blue region blue-shifted 3 nm. Blue shifts of absorption bands were generally ascribed to microenvironment changes around Chls [27]. It was reported that pigments departing from proteins might lead to the blue-shifts of peaks [22,28]. Figure 1(c) showed that SDS at concentration of 0.031% could properly stabilize PS II particle. However, after the addition of SDS at higher concentration, the peaks changed obviously. On one hand, in blue region, there was a new peak at around 417 nm arose and increased with concentration, and the peak at about 470 nm decreased and became only a shoulder. On the other hand, in red region, the shoulder at 650 nm disappeared and there was a great blue-shift of Chl *a* absorption peak (from 672 nm to 666 nm). As concentration of A_6K increased, the maximums of PS II absorption peaks increased with little shift of peaks, indicating that A_6K with higher concentration could better solubilize PS II. The results shown in Figure 1 that detergents with higher concentration resulted in higher absorption intensity, suggested that higher concentration of detergents solubilized PS II particle more effectively. However, greater solubilization effect of SDS with higher concentration always accompanied with destruction of Chls arrangement.

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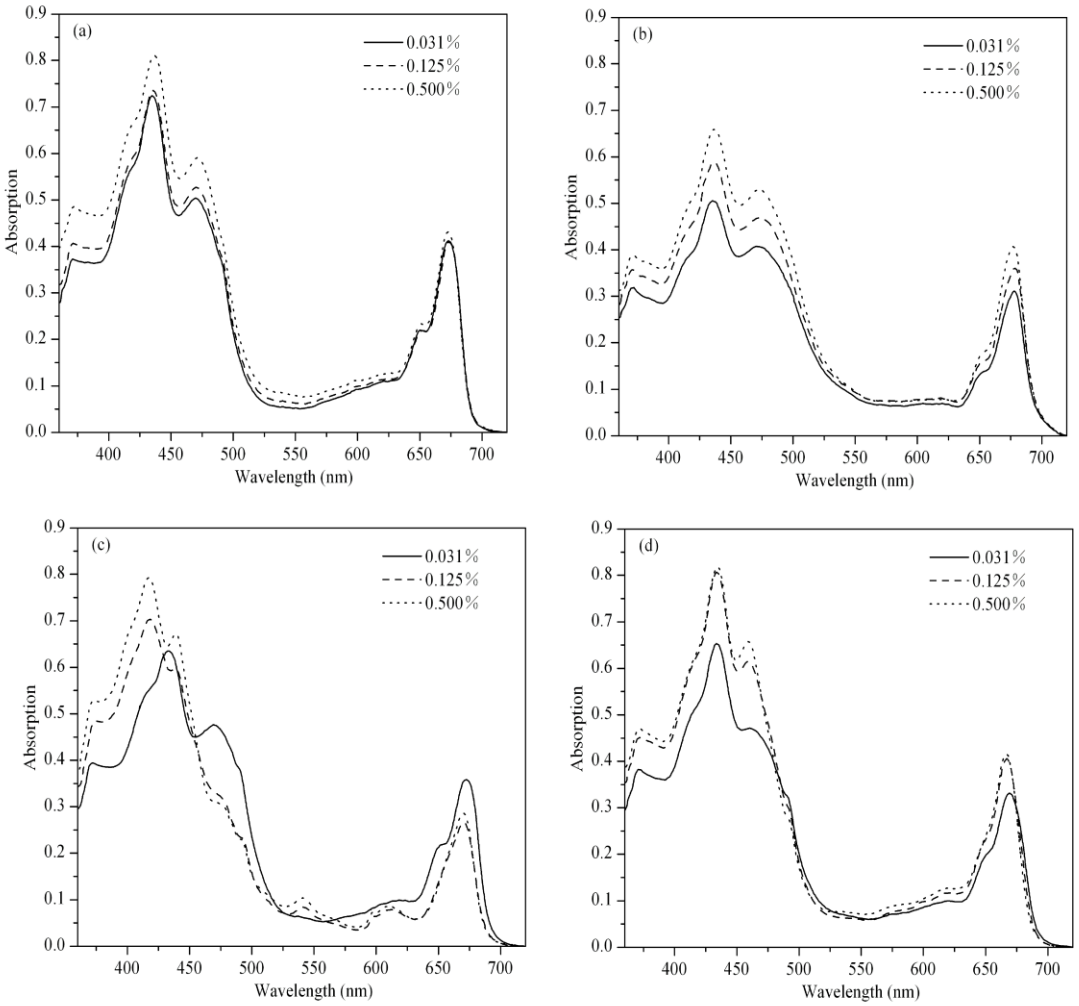

Fig. 1 Absorption spectra at room temperature of PS II dealt with detergents at concentrations of 0.031%, 0.125% and 0.500%.

(a) DM. (b) OG. (c) SDS. (d) Triton X-100.

Therefore, detergents at concentration of 0.5% were added to PS II particle solution to investigate their ability to solubilize and stabilize PS II particle. Absorption spectra were measured after one-day incubation at room temperature (Figure 2). There was almost on shift of peaks compared with control sample, when OG or A_6K added into PS II solution. Treated with A_6K resulted in higher absorption intensity, showing its greater solubilization effect than OG. Although DM showed even greater solubilization effect, it led to 8 nm blue-shift of the peak at around 680 nm, indicating a loss of Chl *a*. Triton shown the greatest solubilizaton effect, nevertheless it severely disturbed the microenvironment of Chls, for the absorption peaks shifted blue more than 10 nm. As a comparison, the absorption peak of PS II treated with SDS exhibited differently. There was a new band peaking at around 417 nm owing to pheophytin *a* [29], which was probably transformed from Chl *a*. SDS also did great damage to Chl *b*, for the Chl *b* absorption peak in red region shifted 10 nm to the blue.

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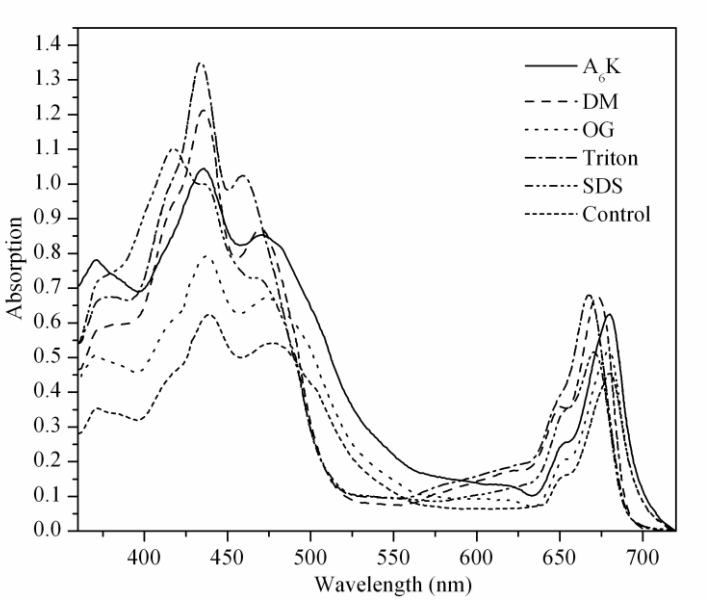

Fig. 2 Comparison of absorption spectra of PS II incubated with A6K, DM, OG, Triton X-100 and SDS at room temperature for 1day.

Control sample was PS II solution without addition of detergent.

Addition of peptide detergent A_6K stabilize the fluorescence spectra of PS II particle at room temperature

The fluorescence spectrum is more sensitive than the absorption on reflecting the changes of Chls microenvironment. The fluorescence emission spectra of the pigment-protein complexes reflect the properties of the contributions of different pigments to the fluorescence and the excitation energy transfer [13]. Upon excitation at 436 nm, all of the samples showed a prominent emission peak at around 680. This peak corresponded to the spectral origin and maximum in the emission spectrum of Chl *a* [27]. Light at 475 nm mainly excited the Chl *b* molecule, and the excitation energy of which either transferred to the Chl *a* molecule that in turn emits fluorescence emission, or is deactivated in form of internal conversion followed by fluorescence emission. Thus, fluorescence emission from both Chl *a* and Chl *b* can be observed [27]. The peak at approximately 680 nm belongs to Chl *a*, and the peak at around 660 nm is attributed by Chl *b*. The fluorescence emission spectra of the pigment-protein complexes reflect the properties of the excitation energy transfer and the contributions of various pigments. As shown in fig. 3, detergent, when applied to the suspensions of PS II, the fluorescence spectra changed evidently. The spectrum of PS II with the continuous increase of the DM concentration had small changes, the main fluorescence emission peak at approximately 680 nm decreased a little gradually. Similarly, with the increased concentration of OG, the main fluorescence yield declined gradually. When PS II was subjected to Triton X-100, under excitation at 436 nm, the change consisted of an increase in the intensity of the fluorescing chlorophyll in the approximately 680-nm region and an associated blue shift of this peak (from 675.2 nm to 673.6 nm). Upon excitation at 475 nm, when the concentration of Triton X-100 was increased to 0.125%, a new emission peak at about 655 nm appeared, and the shoulder at 655 nm tended to

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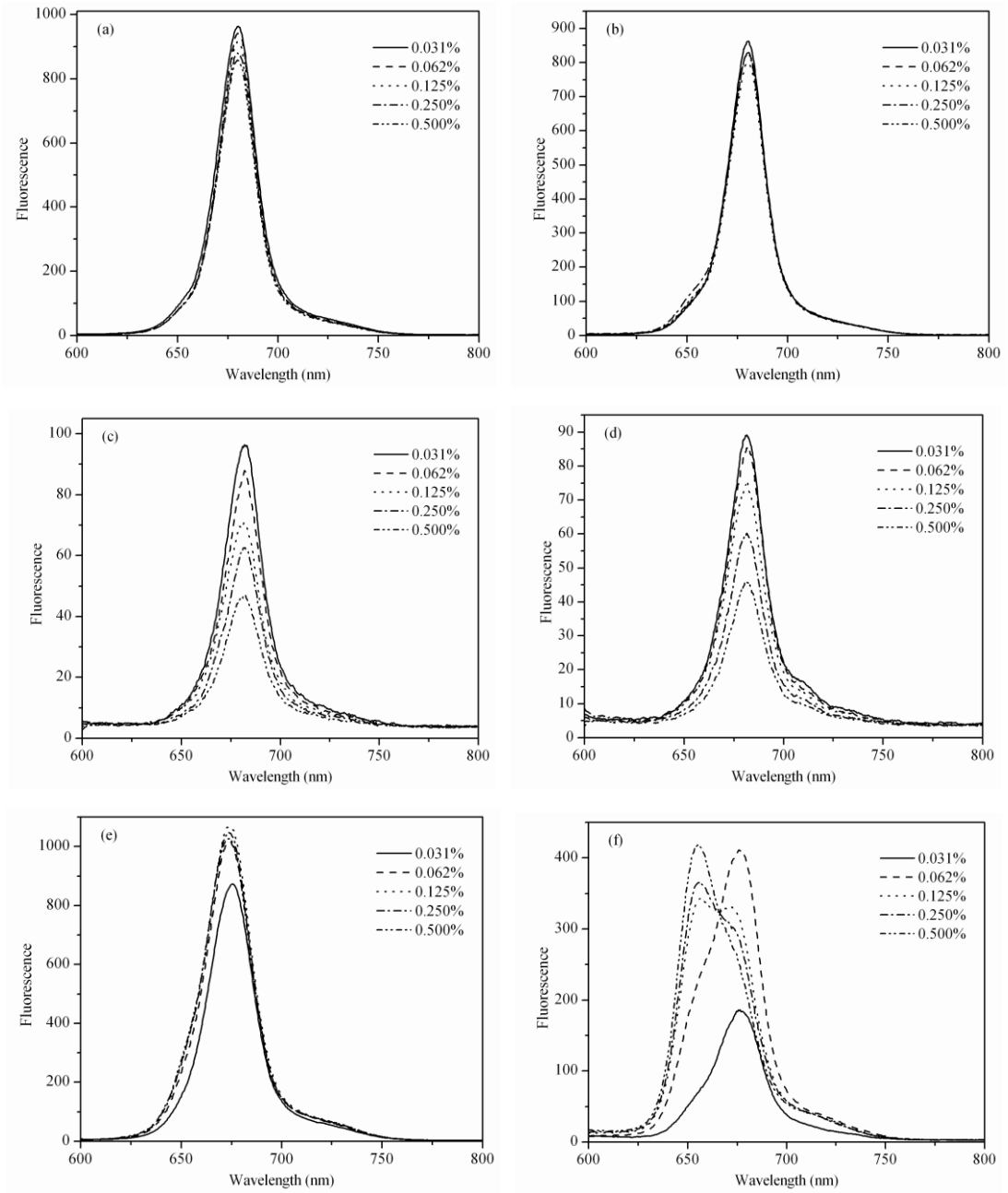
become a prominent peak. When PS II particles were treated with SDS, under excitation at 436 nm, the main fluorescence emission peak increased and accompanied by obvious blue-shift (from 680.2 nm to 662.6 nm). Upon excitation at 475 nm, the main fluorescence emission peak increased at first and then declined coupled with large blue shift (from 679.4 nm to 666.6 nm).When the PS II particles were treated with A_6K, the main fluorescence emission peak at approximately 680 nm increased gradually. These results suggested that lower concentration of DM, OG, SDS, and Triton X-100 and high concentration of A_6K were the optimized concentration to stabilize PS II and maintain its energy transfer ability.



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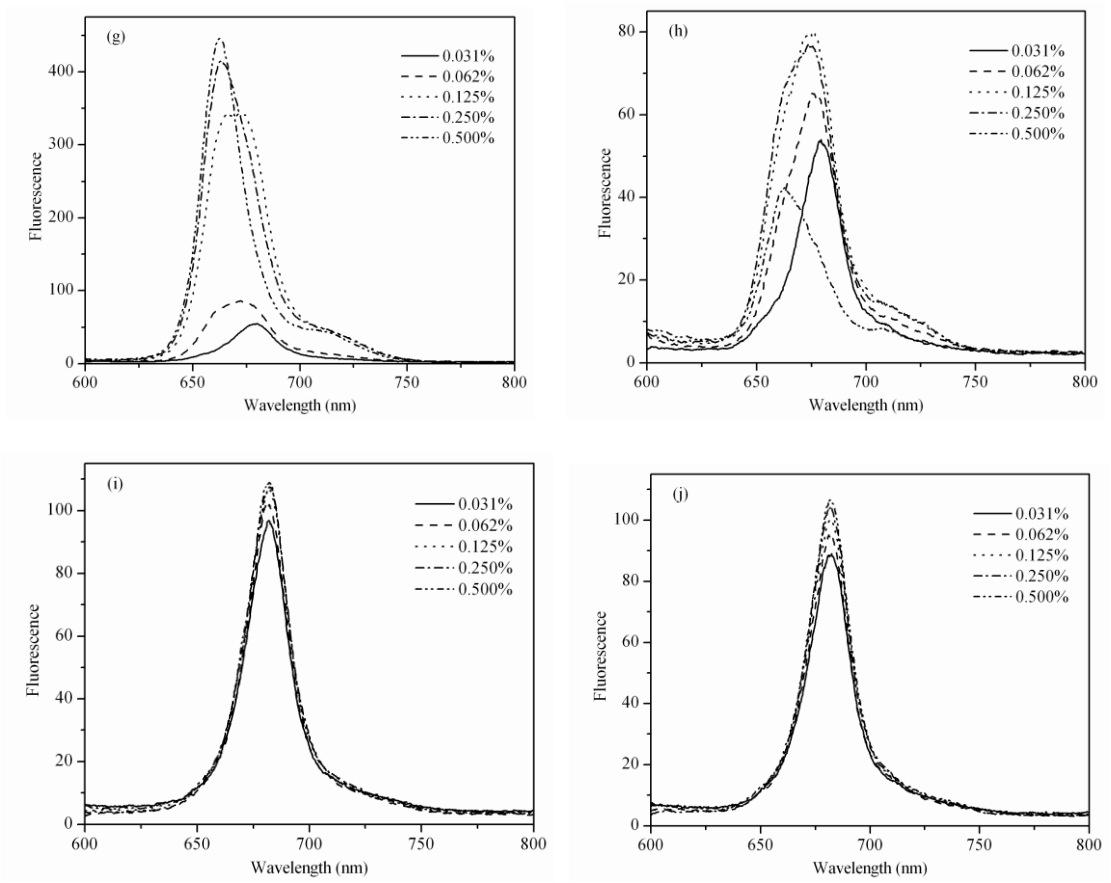

Fig. 3 Emission spectra at room temperature of PS II dealt with detergents at concentrations of 0.031%, 0.062%, 0.125%, 0.250% and 0.500%.

Emission spectra of samples contained A_6K (i), as well as DM (a), OG (c), TX-100 (e) and SDS (g), excited at 436 nm; Emission spectra of samples contained A_6K (j), as well as DM (b), OG (d), TX-100 (f) and SDS (h), excited at 475 nm.

Peptide detergent A_6K stabilizes the PS II complex for an extended time

To investigate whether these detergents could satisfactorily stabilize PS II, they were incubated with PS II individually for a month. Absorption spectra at room temperature were reported in Figure 4 along with their second derivative analysis.

After DM and Triton X-100 were mixed with PS II for 1week, a new peak at approximately 417 nm appeared. When DM was used, this peak decreased with time and the peaks in blue region both shifted 3 nm to the blue and decreased 25% of the maximum after 4 weeks. The 417 nm peak increased with time when PS II was incubated with Triton X-100. After 28 days, the peak shifted to 413 nm and became the main peak in blue region. The Chl *a* and Chl *b* absorption bands in blue region disappeared while the bands in red region reduced to only 50% of the maximum. Figure 4(g) showed that OG could hardly stabilize PS II particle. When incubated for 2 weeks, the absorption peaks in blue region decreased to about 40% and peaks in red region reduced to 20% of the maximum, and the peaks disappeared as time extended. When SDS was used for 1 week, the peak at 670 nm

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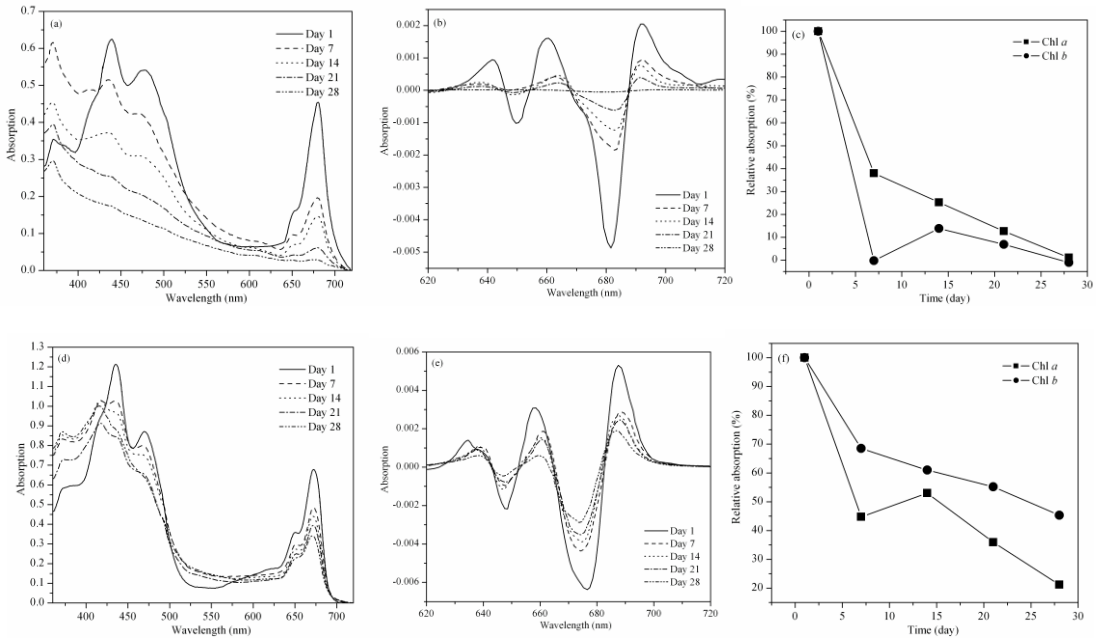
shifted to 660 nm and the shoulder at 469 nm and 650 nm disappeared. After 28 days, the absorption peaks of PS II without detergent decreased to only 10~30% of the maximum and all the peaks flatted. The absorption peaks decreased gradually when PS II was incubated with A_6K. After 28 days about 60% of the absorption maximum remained. The peaks shifted little even after 28 days.

Similar changes were detected in the second derivative analysis of absorption spectra. Previous studies has demonstrated that the main negative signal at around 680 nm corresponds to Chl *a*, and the other at 650 is due to Chl *b* [31,32]. In the presence of OG (Figure 4h, 4i), the Chl *a* signal sharply decreased to 10%, and signal of Chl *b* 40% after 1 week. Four weeks later, all the signals flatted out. This was similar with control sample, proving that OG has little effect on stabilizating PS II. Almost 10 nm blue-shift of Chl *a* signal was observed after incubated with Triton, and the signal of Chl *b* disappeared after 1 week, indicating bad damage had been made to Chls binding state. SDS showed greater destructive effect on the Chls, for the Chl *b* signal vanished and Chl *a* signal shifted 20 nm to the blue just 1 week later. DM led to a 5 nm blue-shift of Chl *a* signal and after 4 week there was half intensity of Chl *a* signal left, however, there was only 20% for Chl b. In the case of peptide detergent A_6K, both signals of Chl *a* and Chl *b* were well preserved after 4 weeks: the signals scarcely shifted and about 50% of the intensity was maintained.

These results indicated that A_6K could better stabilize PS II particle than the other common used detergents.



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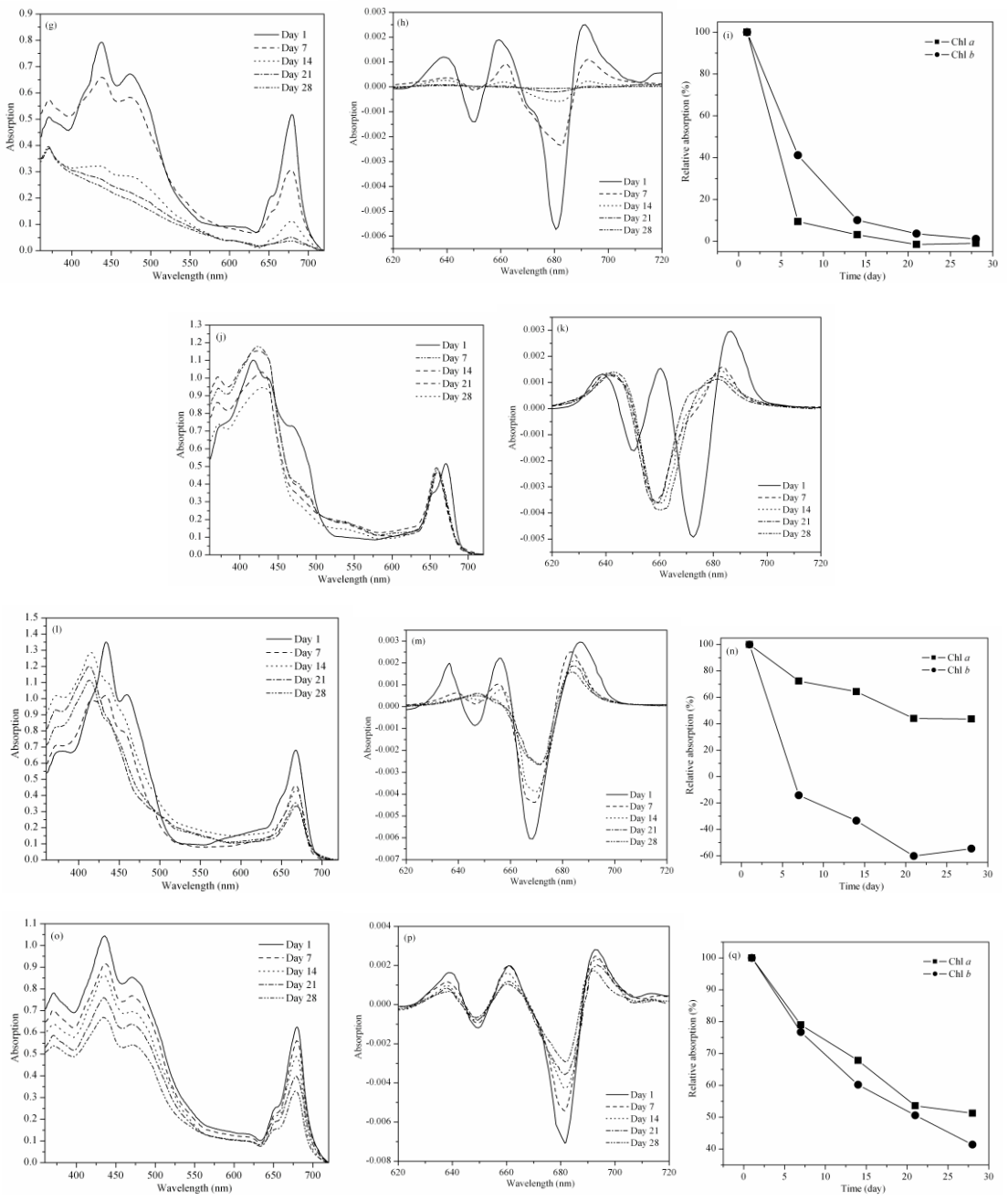

Fig.4 Absorption spectra of PS II particle incubated with detergents at room temperature for an extend time.

Absorption spectra of PS II particle treated with peptide detergent A6K (o), as well as DM (d), OG (g), SDS (j) and Triton (l) for a period of 4 weeks. Control sample was PS II solution incubated without addition of detergent (a). (e), (h), (k), (m) and (p) were second derivative analysis of the RT absorption spectra of PS II incubated with DM, OG, SDS Triton and A6K, with (b) as a control. (c), (f), (i), (l), (n) and (q) were Chls analysis of second derivative absorption spectra. Intensity of Chls absorptions in first day was regarded as 100%.

As shown in fig. 5, dealt with DM, OG, SDS and Triton X-100, PS II not only showed a significantly reduced fluorescent emission, but also showed a blue shift. When sample was

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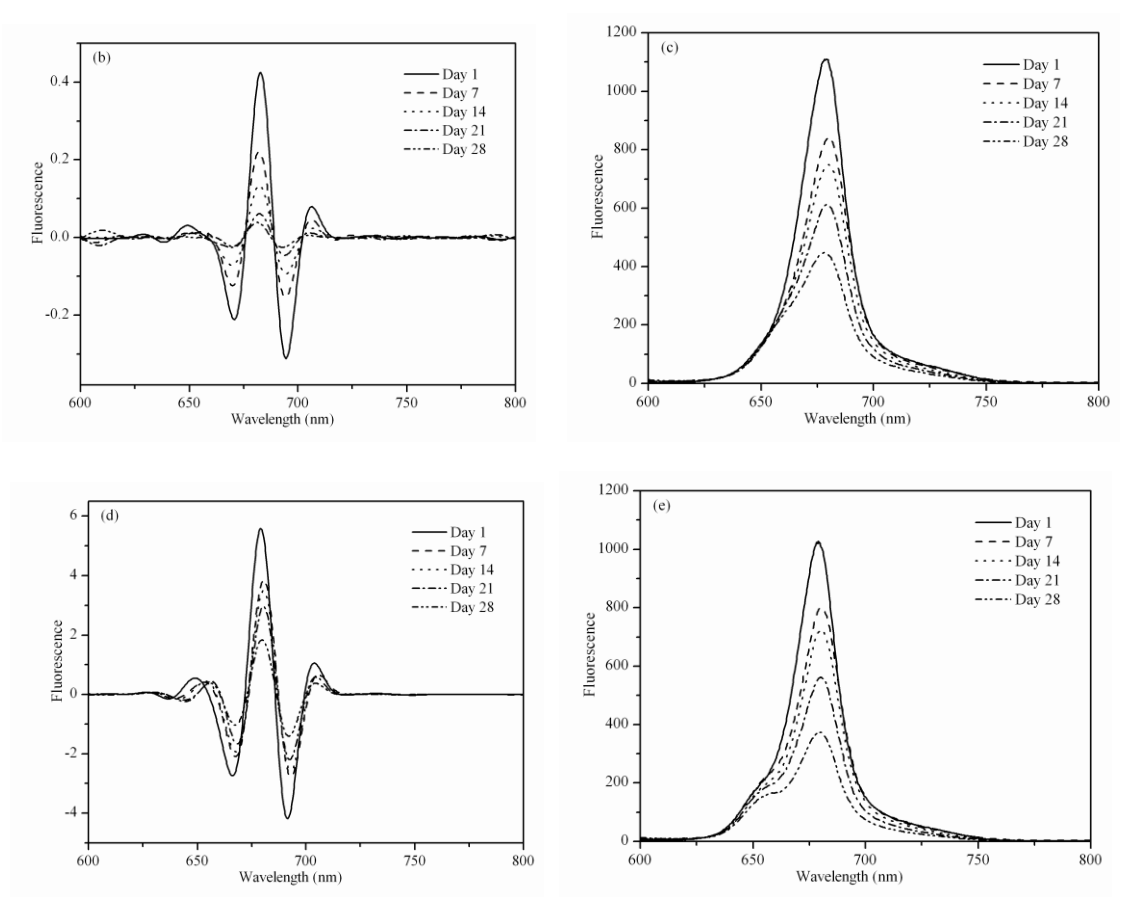
subjected to DM, the intensity in the approximately 680 nm region gradually decreased coupled with slight blue shift, and a little shoulder appeared at approximately 650 nm when excited at 475 nm. Fluorescence intensity declined to 50% of the maximum after 3 weeks. When the sample was treated with OG, similar to the sample dealt without detergents, the main fluorescence yield declined dramatically, and the fluorescence yield reduced to 50% of the maximum when time extended to 2 weeks. Furthermore, the peak vanished completely when time extended to 4 weeks. When the PS II particles were treated with Triton X-100, under excited at 436 nm, the fluorescence yield in the approximately 680 nm region increased at first and then decreased along with a noticeable blue shift. Upon excitation at 475 nm, the peak blue shifted to 655 nm when time extended to 1 week and the fluorescence intensity declined gradually. When the PS II particles were subjected to SDS, similar to the samples added with Triton X-100, under excitation at 436 nm, the intensity of the fluorescing chlorophyll in the approximately 680 nm region increased at first and then decreased coupled with a large blue shift. Upon excitation at 475 nm, the main fluorescence emission peak decreased with an associated blue shift (from 676.8 nm to 661.4 nm). On the other hand, When the PS II particles were treated with A_6K, the intensity of fluorescence in the approximately 680 nm region decreased without a change in the shape of the spectrum, and fluorescence yield still preserved 60% of the maximum after 4 weeks. Based on the fluorescence emission spectra of PS II, DM demonstrated modest effect on PS II. SDS, OG and Triton X-100 did not show a stabilizing effect on PS II. On the other hand, PS II appeared to be stabilized by A_6K.



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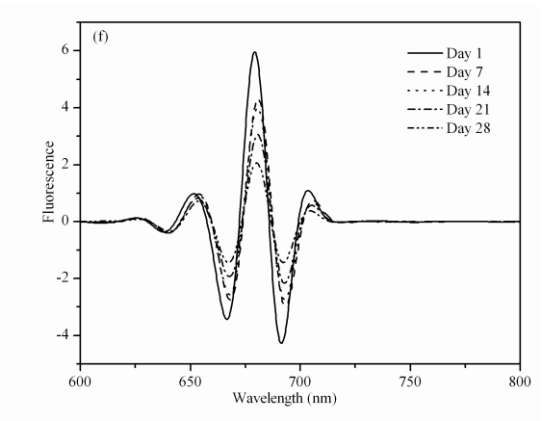

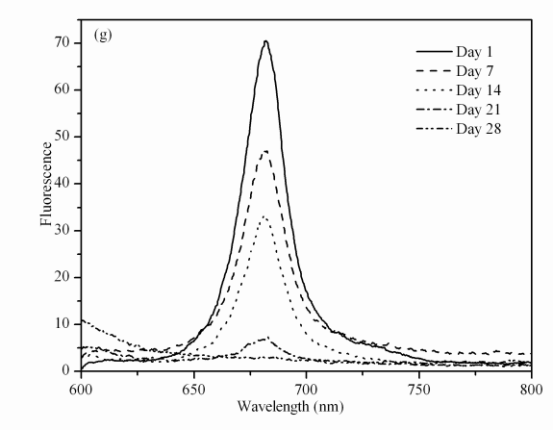

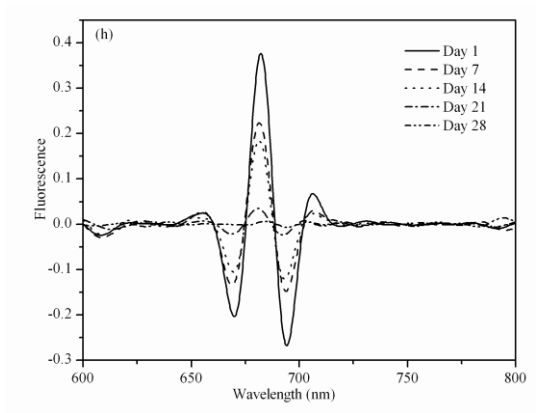

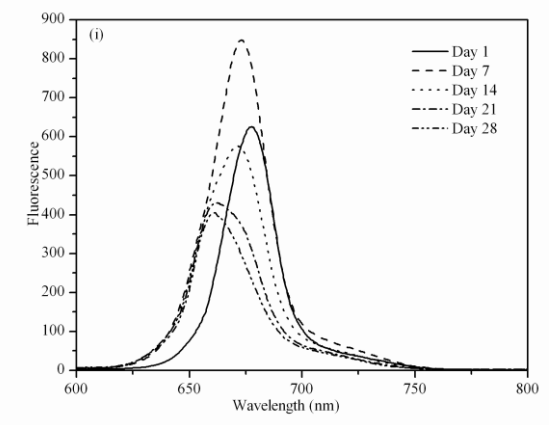

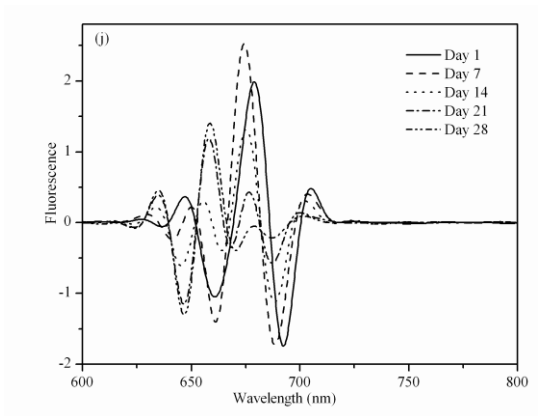

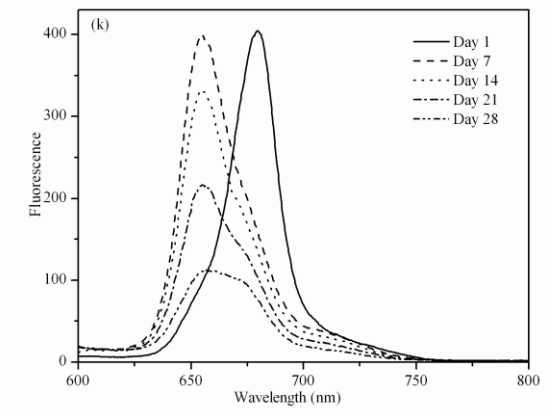

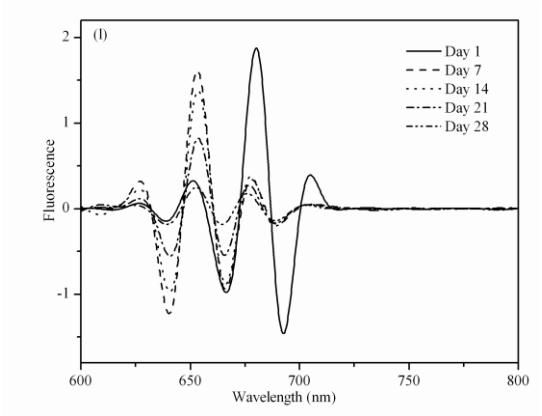

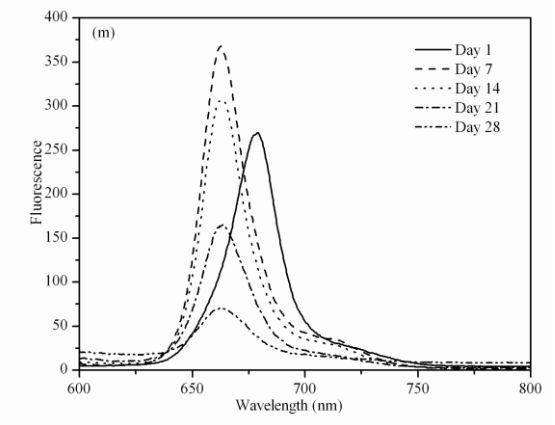

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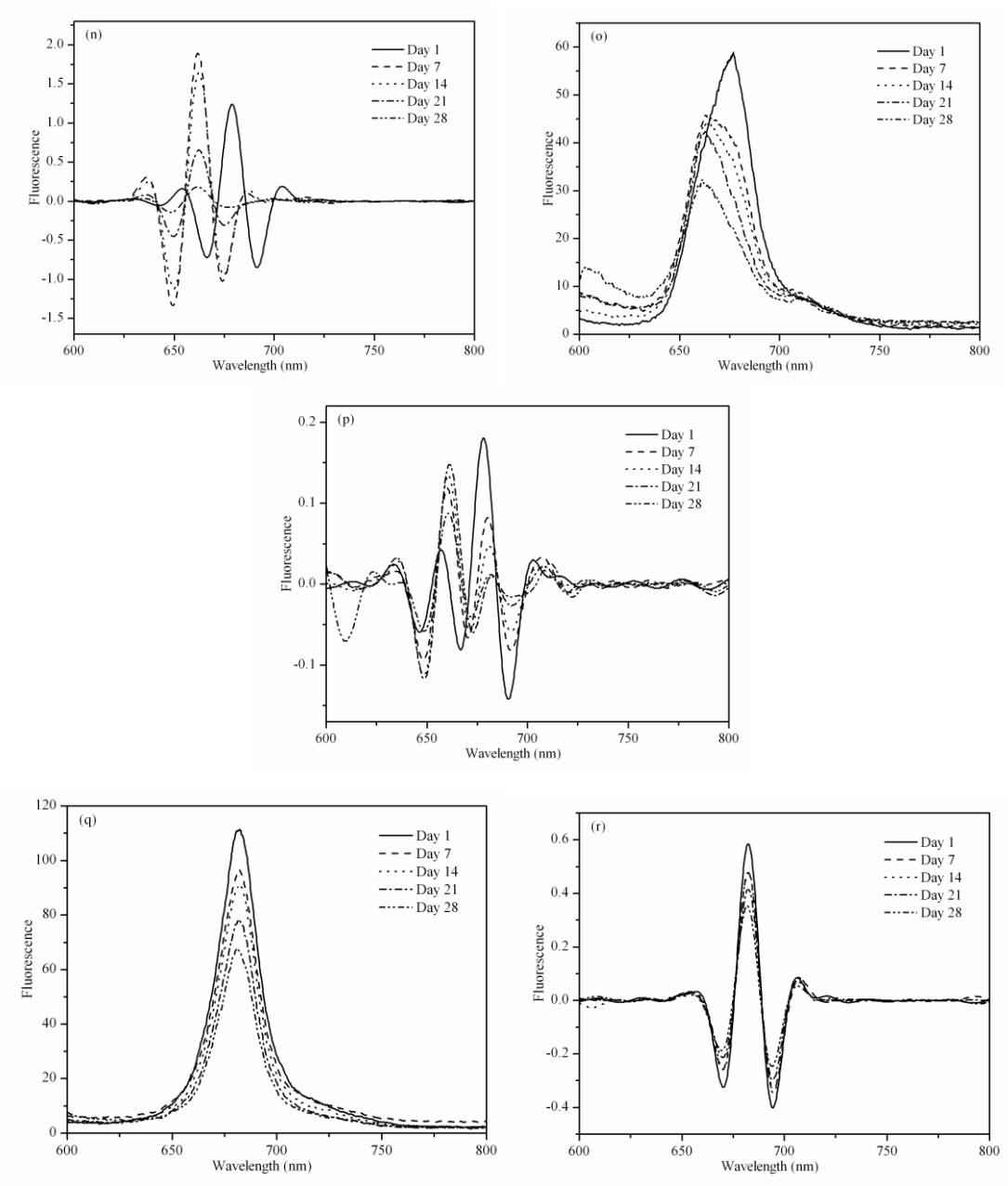

Fig. 5 Emission spectra at room temperature of PS II incubated with detergents for an extended time.

It is proved by the second derivative analysis of the room temperature emission spectra of PS II. Components at 650 nm, 680 nm and 705 nm can be observed. When sample was subjected to DM, the main positive signals at 650 nm, 680 nm and 705 nm all decreased gradually coupled with a slight red shift. When the sample was treated with OG, similar to the samples dealt without detergents, the intensity in the approximately 650 nm, 680 nm and 705 nm region all decreased declined accompanied by a slight blue shift. When the PS II particles were treated with Triton X-100, the main positive signals at 650 nm shifted to 635 nm, when time extended to the 7th day. The main positive signals at 680 nm shifted to 655 nm gradually. The main positive signals at 705 nm shifted to 701 nm and declined gradually. When the PS II particles were subjected to SDS, similar to the samples added with Triton X-100, the main positive signals at 650 nm shifted to 636 nm, when time extended to the 7th

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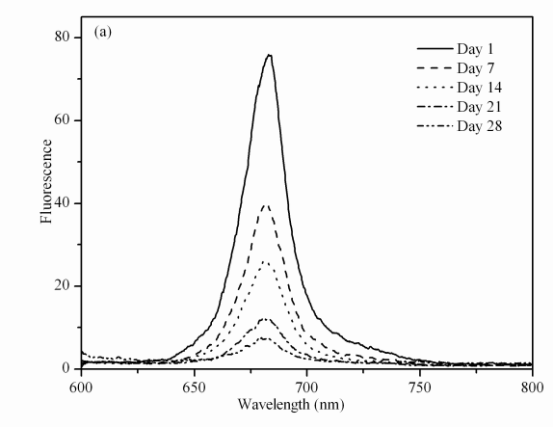
day. The main positive signals at 680 nm shifted to 661.5 nm gradually. The main positive signals at 705 nm shifted to 686 nm and declined gradually. On the other hand, When the PS II particles were treated with A_6K, the main positive signals at 650 nm, 680 nm and 705 nm decreased without a change in the shape of the spectrum.



Emission spectra of samples contained A_6K (q), as well as DM (c), OG (g), TX-100 (i) and SDS (m), excited at 436 nm; Control sample was PS II particles incubated without addition of detergents, excited at 436 nm. Emission spectra of samples contained DM (e), as well as TX-100 (k) and SDS (o), excited at 475 nm; (d), (h), (j), (n) and (r) were second derivative analysis of the RT emission spectra of PS II incubated with DM, OG, TX-100, SDS and A_6K respectively, excited at 436 nm; (f), (l) and (p) were second derivative analysis of the RT emission spectra of PS II incubated with DM, TX-100, and SDS respectively, excited at 475 nm; (b) was second derivative analysis of the RT emission spectra of PS II incubated without detergents, excited at 436.

DISCUSSION

Detergents are widely used as a tool in the study of membrane proteins. They are with great importance in the isolation, purification and stabilization of the membrane proteins. The selection of a proper detergent is usually vital in the study of membrane protein. The nonionic detergents, OG, DM and Triton X-100 are frequently used, especially in the study of thylakoid membrane proteins and complexes. They are generally considered to be mild detergents and lead to no denaturing of proteins, probably because they break interactions of lipid-lipid and lipid-protein without making damage to protein-protein interactions [18]. Long chain nonionic detergents, like DM, are proved to be more efficient in stabilizing proteins, for the short chain nonionic detergents, such as OG, often lead to deactivation of proteins [33]. On the other hand, the ionic detergents, such as SDS, generally cause protein denaturation to some extent, although they're excellent at solubilizing membrane proteins [18].

It is known detergent molecule is characterized by a hydrophilic "head" region and hydrophobic "tail" region. As a result of this characteristic, detergents form thermodynamically stable micelles with hydrophobic cores in aqueous media. When they interact with proteins, the core would provide a hydrophobic environment for the

hydrophobic domains of proteins. Addition of detergents to PS II solution will more or less change the microenvironment of chlorophylls attached to PS II. PS II complex was surrounded by detergents which maintain its hydrophobic structure when PS II was treated with detergents. We inferred that this hydrophobic environment would affect the arrangement of Chls, as well as the interactions between Chls and their ligands. The insertion of detergents into the PS II particles disturbed the microenvironment of chlorophyll molecules [34], and affected the interaction in protein-pigment complex, hence changed the spectral properties of PS II. When environments of protein are destroyed by external factors, such as excessive detergent, the intrinsic twist configurations are lost, which results in lower excitation energy transfer efficiency and higher quenching efficiency occurring [35].

Stability of membrane proteins in vitro depends on the detergents used for solubilization and storage. Solubilization of a membrane protein must be accomplished without disrupting its native structure; hence the solubilizing medium should simulate the microenvironment of the protein in the membrane. We often use detergents to meet this requirement. In our condition, we used four widely known membrane protein-solubilizing detergents (Triton X-100, SDS, DM and OG) and one peptide detergent (A_6K) for PS II. They were previously shown to have diverse effects on PS II.

Obvious changes occurred in the microenvironment of Chls following the addition of Triton X-100 or SDS. Upon excitation at 436 nm, the increase in Chl *a* fluorescence intensity coupled with the blue shift indicated that the Chl *a* in PS II dissolved from the protein in solution [22]. Upon excitation at 475 nm, the intensity (680 nm) in the fluorescence emission spectra dramatically decreased, while a new emission peak at around 660 nm appeared. Obviously, both detergents inhibited the Chl *b*-to-Chl *a* excitation energy transfer, and they had much stronger inhibition effect than the others. Triton X-100 was previously reported to disrupted the excitation energy transfer from Chl *b* to Chl *a* in algae [36]. In addition, the emission fluorescence spectra excited at 475 nm changed more obviously than that excited at 436 nm, indicated that Chl *b* microenvironment was more ready than that of Chl *a*. Although more than half of the absorption ability was preserved by Triton X-100 and SDS respectively after 4 weeks, they also led to great blue-shifts of absorption peaks, which proved the disassociation of some Chls originally bound to PS II. Incubated with Triton X-100 or SDS both resulted in a new peak at around 417 nm which originated from pheophytin *a* [29], and the disappearance of Chl *b* absorption peak at blue region, suggesting that great changes of Chls surroundings took place. A large number of Chls *a* were destroyed and transformed into pheophytin *a*, and Chls *b* disassociated from binding proteins in PS II. These may be the major reason why the Chl *b*-Chl *a* energy transfer was greatly disrupted.

When treated with OG, the peak vanished completely in fluorescence emission spectra, and there was only 20% left in absorption spectra when time extended to 4 weeks, which was coincident with the control sample with no detergent in it, suggesting that OG had little effect on stabilization of PS II.

DM resulted in a decreasing fluorescence emission at 680 nm with a slight blue shift, and showed a small shoulder when excited at 475 nm, indicating that DM also disrupted the Chl *b*-to-Chl *a* excitation energy transfer a little. It demonstrated that it has modest stabilizing effect on PS II. Absorption spectra showed that a new peak at around 417 nm arising from pheophytin *a* appeared after incubated with DM for 1 week, indicating a transform from Chl *a* to pheophytin *a* happened. In addition, the Chl *b* absorption peak shifted to the blue as time extended, suggesting that there was a lost of Chl *b*. Observed from the second derivative analysis of absorption spectra, signals of Chl *b* decreased more dramatically than Chl *a*. The results demonstrated the stabilization effect of DM was greater than Triton X-100 and OG, but was still limited.

OG, SDS and Triton X-100 did not show a proper stabilizing effect based on the emission and absorption spectra of PS II. Thus, the common used membrane protein-stabilizing detergents were not ideal stabilizing detergents for PS II under our conditions. However, A_6K shown a better effect on stabilizing PS II than the four detergents mentioned above. The apex of fluorescence peak did not shift after A_6K treatment, and the fluorescence intensity still preserved 60% of the maximum after 4 weeks. Absorption spectrum and its second derivative analysis of PS II in A_6K solution also showed little peak-shift and preserved approximately 50% of the PS II absorption ability after 28 days, indicating A_6K could maintain the binding state of Chls to the proteins in effect, especially the arrangement of Chl *b* compared with other detergent.

CONCLUSION

The application of peptide detergents on the study of membrane proteins is still in its early stage. It has been used in stabilizing several proteins, including photosynthetic apparatus PS I. PS I was successfully stabilized by peptide detergent A_6K on a dry surface for at least 3 weeks [15]. It is known that PS I particles are more stable than PS II. In our work, PS II particles were stabilized for 4 weeks in solution, and A_6K showed better efficiency compared to other detergents used.

The mechanism by which peptide detergent stabilize membrane proteins is still unknown. It is assumed that peptide detergents, similar to other common detergents, surround the hydrophobic domains of membrane proteins and protect them from denaturation or aggregation. [15, 17]

The prospect of application of peptide detergent on solubilizing and stabilizing membrane protein looks bright. If the stabilizing mechanism of peptide detergent is revealed, it becomes possible that we design a specific peptide detergent to stabilize a certain membrane protein or complex. It is more convenient in the usage of detergent.

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