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Hydrostatic Pressure Effects on the Fluorescence and FRET Behavior of Cy3-Labeled Phycocyanin System

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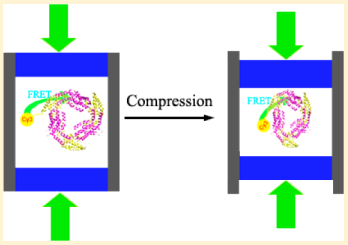
Supporting Information

ABSTRACT: FRET has been used as a powerful tool in biological fields as biosensors, bioimaging, protein folding/unfolding monitoring, biomolecular interactions, and so on. It is also important to applying FRET to high hydrostatic pressure studies on biosystems or biorelated systems. Herein, we construct a FRET system by labeling Cy3 on C-phycocyanin (C-PC) to investigate the effect of hydrostatic pressure on the fluorescence and FRET behavior between them. The fluorescence spectra of individual Cy3, C-PC, and integrated Cy3/C-PC system are measured separately under compression. An enhanced FRET efficiency under compression is concluded based on fluorescence behavior differences between them. To further reveal the origination of the enhanced FRET efficiency with pressure, the overlap integral between Cy3 emission and C-PC absorption is also calculated, and several possible explanations are proposed.



INTRODUCTION

Hydrostatic pressure is an important tool to modulate various biochemical processes. By combining Fourier transform infrared (FT-IR), UV−vis absorption, fluorescence spectroscopy, and light scattering, the effects of pressure on protein structure, stability, function, folding, and dynamics have been investigated.[1−6] Förster resonance energy transfer (FRET) is the nonradiative transfer of energy from a donor to an acceptor chromophore, which can take place when the emission spectrum of the donor overlaps with the absorption spectrum of the acceptor. The FRET efficiency also varies as the sixth power of the distance between the donor and acceptor from ∼10 to ∼100 Å and, hence, can be used as a molecular ruler.[7] Therefore, FRET has been used as a powerful tool in many biological fields such as biosensors, bioimaging, protein folding and unfolding, biomolecular interactions, and so on.[8−12] It is also potential to applying FRET to high hydrostatic pressure studies on biomacromolecules. In some articles, the RNA tertiary structure changes, protein−DNA binding affinity, and protein denaturation and aggregation have been explored through FRET.[13−15] Herein, we construct a FRET system between Cy3 label and C-phycocyanin and try to explore how pressure affects the fluorescence of the donor chromophore and acceptor protein, and especially on the FRET behavior between them in a Cy3-labeled C-phycocyanin (Cy3/C-PC) system. In addition, understanding the pressure effect on FRET behavior will highlight the investigation on the pressure-induced protein conformational changes, stability, function, and weak interaction between proteins, and so on.

Phycobilisomes (PBS) are light-harvesting complex present in cyanobacteria and eukaryotic red algae, which absorb light and transfer the energy step by step to the photosynthetic reaction centers.[16−18] A typical phycobilisome (in *Gracilaria chilensis*) contains three phycobiliproteins (PBP): phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC), which consist of a core of face-to-back cylinders formed by stacked discs of PBP and several rods that radiate from the core composed of stacked back-to-back discs of PBP.[19−21] The absorption and emission spectra of these proteins are overlapping, which allows a nonradiative, direct, and efficient transfer of the excitation energy among them.[22] This transfer is channeled along an energy gradient from the rods to the core and finally transferred to chlorophyll *a* in the thylakoid membrane through linker polypeptides.[23] Schreiber et al. once investigated the effects of hydrostatic pressure on photosynthetic energy transfer processes, which showed that pressure reversibly inhibited energy transfer between phycobilins and chlorophyll *a*.[24] But the pressure range they employed was low (0−0.12 GPa), and such a system contained several proteins and FRET processes in which intermolecular interactions were too complex to study the pressure-induced FRET variation and protein conformation changes. Therefore, we label C-PC by using Cy3 to construct a relatively simple and easily controlled FRET model and further study the pressure effect on it.

Cy3 is one of the most widely used fluorescent sulfoindocyanine for covalently labeling proteins and nucleic acids, due to its remarkable stability against photobleaching, compatibility with commonly available laser lines and detectors, and commercial availability.[25] It has been applied to study the intracellular imaging, the protein−protein and protein−DNA interactions, protein and DNA structures, biosensors, and so on.[26−28] Through combining with the structurally related red-

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absorbing fluorophore Cy5, Cy3 has also been used in many FRET experiments. Besides its widespread popularity, the photophysics of Cy3 are also investigated. There have been reports on the changes in the fluorescence properties of Cy3 when covalent or noncovalent binding to biomolecules occurs[29] and in the Cy3−nucleobase interactions.[30] The interactions between biomolecules can also induce changes in the fluorescence properties of Cy3 analogues.[31] As the fluorescence of chromophore can be affected by pressure,[32] the understanding of pressure-induced Cy3 fluorescence behavior in a protein-contained system is also necessary for using Cy3 to investigating pressure-induced FRET changing in the Cy3/C-PC system.

In this article, we investigate the difference of Cy3 fluorescence changes, as a FRET donor, under compression before and after labeling on protein. The results show that the pressure-induced fluorescence changes of Cy3 can be affected by the contiguous protein, which leading the FRET behavior between them more complicated. We also study the FRET behavior between Cy3 and C-PC and the overlap integral between the Cy3 emission and C-PC absorption at elevated pressure. A reduction in distance between donor- and acceptor-induced FRET enhancing is concluded.

MATERIAL AND METHODS

Materials. Cy3 succinimidyl ester (Cy3 SE) is purchased from Mycome Co. C-PC, supplied in a 100 mM sodium phosphate buffer with 60% ammonium sulfate, is purchased from Express Biotechnology Co. BSA is purchased from Beijing DingGuo Changsheng Biotechnology Co. Ltd. Other reagents are purchased from Aldrich and used without further purification.

Preparation of C-PC and Cy3-Labeled C-PC in Solution. The C-PC as received from the manufacturer is first centrifugated at 3000 rpm for 3 min. Then the precipitate is redissolved in 5 mM phosphate buffer solution (PBS, pH 7.5) and dialyzed with the same buffer solution to gain purified C-PC in buffer solution. To label C-PC with Cy3, Cy3 SE is dissolved in DMF first and then is mixed with the purified C-PC and incubated at 4 °C for 4 h to connect Cy3 with amino residues in C-PC. After that a dialysis in 5 mM PBS (pH 7.5) is performed to purify the Cy3-labeled C-PC (Cy3/C-PC) by removing the nonreacted Cy3 and other remnant. The concentration of C-PC in solution is determined by UV−vis spectrum using an absorption coefficient of ε = 281000 M^{-1} cm^{-1} at 620 nm.[33] And the number of Cy3 labeled on C-PC is determined by the difference amounts of the added Cy3 and those left in the dialysis solution. For an initial ratio of 3:1 for Cy3 to C-PC, the determined number of Cy3 labeled on C-PC is 2.2.

Photoluminescence and UV−Vis Absorption Measurements under Normal Pressure. The measurements of UV−vis absorption spectra are carried out with a Lambda 800 spectrophotometer, while steady static fluorescence emission spectra are recorded on a Shimadzu (Japan) RF-5301PC fluorescence spectrophotometer.

High-Pressure Generation and Measurement. The high-pressure experiments are performed using a symmetric diamond anvil cell (DAC) with 1 mm diamond culets. A 0.4 mm diameter hole is drilled in a T301 stainless steel gasket, which is preindented to 0.24 mm thick, as the sample chamber in the photoluminescence experiment. While in the UV−vis absorption experiment, to get the better signal, the thickness of the gasket is 0.45 mm. The solution is introduced into the chamber, and a small ruby chip is also placed in the chamber. Using the well-known ruby fluorescence technique, by observing the separation and widths of both R1 and R2 lines of ruby fluorescence, the hydrostatic pressure is evaluated.

Photoluminescence and UV−Vis Absorption Spectral Measurements under High Pressure. The in situ photoluminescence measurements under high pressure are performed on the QuantaMaster 40 spectrometer (produced by Photon Technology Inc.) in the reflection mode. A xenon lamp is used as the excitation source. The DAC with the sample is put on a Nikon fluorescence microscope in order to focus the light on the sample. The emission spectra are recorded by a monochromator equipped with a photomultiplier at room temperature. The typical resolution in spectrum of the present study is set as 0.25 nm. The in situ UV−vis absorption measurements under high pressure are performed on an Ocean Optics QE65000 Scientific-grade spectrometer under identical conditions.

Analysis on Overlap Integral between Cy3 and C-PC. The overlap integral, I, is defined as

$$I = \int_0^\infty \mathrm{PL}_{\mathrm{D\text{-}corr}}(\lambda)\varepsilon_{\mathrm{A}}\lambda^4 \mathrm{d}\lambda \quad (1)$$

It is a quantitative measure of the donor−acceptor spectral overlap over all wavelengths λ; it is a function of the normalized donor emission spectrum (dimensionless property), $\mathrm{PL}_{\mathrm{D\text{-}corr}}$, and the acceptor absorption spectrum (expressed as an extinction coefficient), ε_{A}. Because it is difficult to get emission spectra of donor and absorption spectra of acceptor at exactly the same pressure, we choose both only at very closed pressures to calculate the integral of the spectral overlap between them.

RESULTS AND DISCUSSION

First, we measure the optical properties of Cy3 and C-PC. Figure 1 shows the normalized UV−vis absorption and

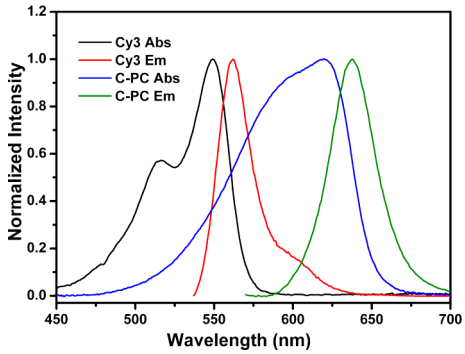

Figure 1. Normalized UV−vis absorption and fluorescence spectra of Cy3 and C-PC, respectively.

emission spectra of Cy3 and C-PC, respectively. Cy3 displays a main absorption band at 548 nm, companied with a shoulder at 515 nm and a main fluorescence band at 562 nm with a small shoulder at ~600 nm. While the purified dilute solution of C-PC displays a broad absorption band from 500 to 650 nm and a fluorescence emission band at 638 nm. Figure 1 also illustrates that the fluorescence of Cy3 and the absorption of C-PC have a remarkable spectral overlap. Therefore, there is potential to produce FRET if the distance between Cy3 and C-PC fluorogen is shorter than 10 nm.

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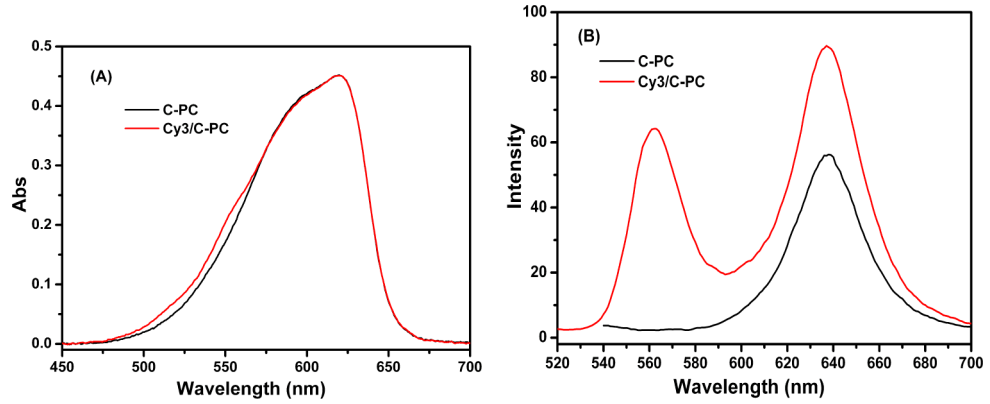

Figure 2. (A) UV−vis absorption spectra and (B) fluorescence spectra of C-PC and Cy3/C-PC (λ_{ex} = 490 nm), respectively. The fluorescence spectra of C-PC before and after Cy3 labeling have been corrected by the concentration of C-PC.

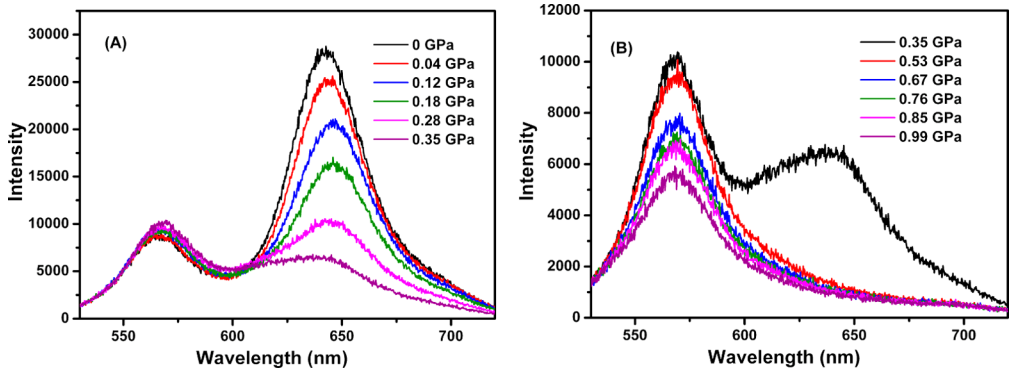

Figure 3. Fluorescence spectra of Cy3/C-PC at different pressures (λ_{ex} = 490 nm): (A) from 0 to 0.35 GPa and (B) from 0.35 to 0.99 GPa.

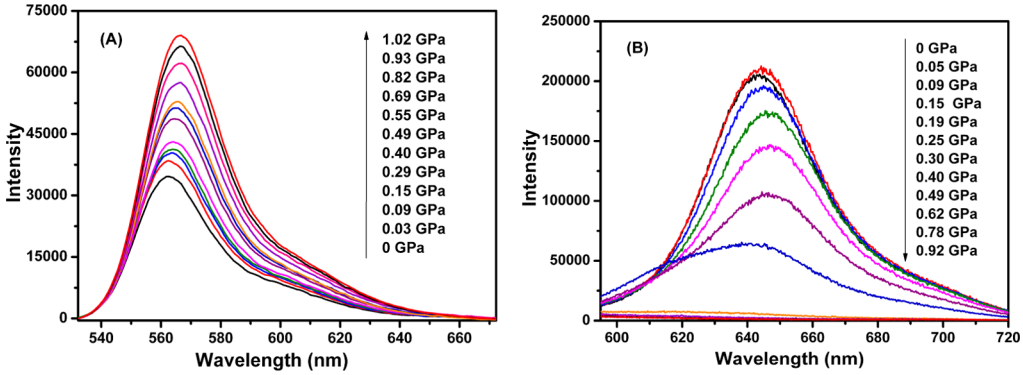

Figure 4. Fluorescence spectra of (A) Cy3 (λ_{ex} = 490 nm) and (B) C-PC (λ_{ex} = 560 nm) measured at different pressures.

After the C-PC is labeled by Cy3, we use UV−vis absorption spectrum and fluorescence spectrum to confirm the induced FRET between them. Figure 2 shows the differences of UV−vis absorption spectra as well as the fluorescence spectra of C-PC before and after Cy3 labeling. It indicates that, after labeled by Cy3, the absorption bands at 548 and 515 nm increase a little, which is definitely ascribed to Cy3 (Figure 1).[25] In the fluorescence spectra, a band at 637 nm is attributed to C-PC and a new band appeared at 562 nm after Cy3-labeling should be assigned to Cy3 (Figure 1).[34] Of note is that the fluorescence spectra of C-PC before and after Cy3 labeling have been corrected by the concentration of C-PC, and therefore, the fluorescence enhancement of C-PC at 637 nm after Cy3 labeling should be attributed to the energy gained from Cy3. The existence of the fluorescence at ~562 nm suggests that the FRET efficiency from Cy3 to C-PC may not be very high.

Once we have confirmed the existence of FRET between the Cy3 and C-PC, further we compress such Cy3/C-PC system and investigate the effect of hydrostatic pressure on the fluorescence spectra of it, especially on the monitoring of FRET behavior between them. Figure 3A,B shows the fluorescence emission spectra of Cy3/C-PC in response to the elevated pressure. The spectra are divided into two groups, the pressures below 0.35 GPa and those above 0.35 GPa, to demonstrate more explicitly where dramatic changes are clearly observed: at the pressure lower than 0.35 GPa, the C-PC emission at 637 nm is reduced drastically, while that for Cy3 at 562 nm increases only slightly with pressure; however, as the pressure is increased higher than 0.53 GPa, the former band almost

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disappears, while the later one starts to decrease obviously with compression. That is, two-step processes are observed for the intensity changes of Cy3 on C-PC in response to compression, which will be illustrated more clearly later. These observations give a complicated appearance about the changes of FRET from Cy3 to C-PC with pressure, which make it difficult to give conclusion before considering the pressure effects on solo Cy3 and C-PC. Therefore, fluorescence spectra of separated Cy3 and C-PC under compression are recorded (Figure 4) and compared with those obtained from Cy3/C-PC system in following.

Figure 4A shows the photoluminescence spectra of free Cy3 under compression. The results illustrate that, under the compression, the emission band of Cy3 shifts to red a little, while its intensities increase remarkably (Figure 4A). The reason for the red-shift may be due to the increased interaction with the environment lowering the energy gaps of the $\pi-\pi^*$ and the $n-\pi^*$ excitation of Cy3.[32] Schuster et al. studied the spectroscopy of a cyanine dyes in fluid solution at atmospheric and high pressure, and the results showed that the fluorescence efficiency of such cyanine dye increased with viscosity in a certain range, ascribing to the increase of the barrier to bond rotation in the excited cyanine.[35] The low barrier to bond rotation that induces *trans* → *cis* isomerization of Cy3 is responsible for the low fluorescence quantum yield of free Cy3.[29] As the pressure can induce the viscosity increase of solution at room temperature,[36] we suggest a similar conclusion here that the fluorescence increase results from the pressure-induced increase of viscosity, which increases the barrier to bond rotation in the excited Cy3 and reduces the efficiency of *trans* → *cis* isomerization from the first excited state.[30] After releasing pressure, the Cy3 fluorescence spectrum coincides almost with its initial state, showing pretty reversible property of Cy3 under such compression (Figure S1).

To illustrate more vividly the behavior differences between individual Cy3, C-PC, and the Cy3/C-PC system, curve fitting is used to extract the separated emission band for Cy3 and C-PC from the spectrum of Cy3/C-PC system first. Then we integrate the peak area of Cy3 and C-PC, respectively, at each pressure, and divide the area by A_0, the peak area measured at ambient pressure. Figure 5 shows the pressure-dependent area ratios (A/A_0) of Cy3 alone and those of it in the Cy3/C-PC system. Under compression, a monotonous increase is clearly indicated for separated Cy3. However, after labeled on C-PC, at pressure lower than 0.35 GPa the A/A_0 increase yet with pressure but the degree it increased is lower than that of separated Cy3; while at a pressure higher than 0.35 GPa the A/A_0 changes oppositely to that of separated Cy3, it decreases with pressure. The large behavior differences of Cy3 in two occasions should be related to the connection of it with C-PC. That is, the covalent binding of C-PC to Cy3 may affect its configuration and consequently the emission behavior to pressure. To further investigate the protein effect on the labeling dye, we construct another Cy3-protein system as control, labeling Cy3 on an often used protein, bovine serum albumin (BSA). Pressure-dependent fluorescence spectra are also measured for the Cy3/BSA system where there is no FRET effect between them (Figure S2). As the experiment conditions are close to each other for two systems, it will provide bases for evaluating the fluorescence behavior of Cy3 under compression after labeling on proteins.

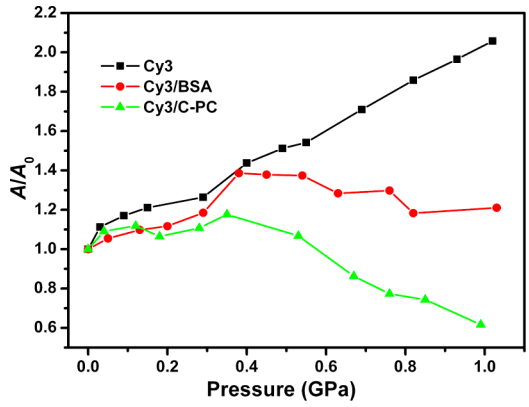

Figure 5. Integrated area ratio changes of the fluorescence bands for separated Cy3 and those of it in Cy3/C-PC and Cy3/BSA systems under compression.

Figure S2 shows that the maximum positions of Cy3 emission in Cy3/BSA shift to red a little under compression, while after curve fitting the integrated area ratios (A/A_0) exhibit large difference from those of solo Cy3 (Figure 5): increase first and then decrease with pressure (Figure 5). At the pressure lower than 0.4 GPa, being similar as solo Cy3, the increasing of the integrated area ratios in Cy3/BSA system can be attributed to the pressure-induced viscosity increase;[35] but as shown in Figure 5, such an increasing rate is lower than that in separated Cy3, which should be ascribed to the connection with BSA. It was reported that the interaction with amino acids and proteins could induce fluorophores quenching[37,38] by both static and dynamic mechanisms, which generally was attributed to the dye−dye and dye−protein interactions.[31] At the pressure higher than 0.4 GPa, the decrease of the integrated area should be attributed to the further enhancement of the dye−protein interactions induced by conformation and hydration changes of BSA. The pressure-induced increase in β-sheet structure and decrease in α-helical structure of BSA was reported,[39] which might enhance the dye−protein interactions and, therefore, induce a decrease in the integrated area of Cy3 at higher pressure.

In comparing the Cy3 emission behaviors in Cy3/C-PC and Cy3/BSA systems (Figure 5), parallel results are illustrated between them: increase at low pressure first and then decrease at high pressure. Therefore, similar mechanisms as Cy3/BSA are proposed for the fluorescence changing processes observed for Cy3 in Cy3/C-PC (Figure 5). However, for Cy3 in Cy3/C-PC, the integrated area ratios (A/A_0) exhibit also an obvious difference from those of Cy3/BSA (Figure 5); the changing scope to pressure are clearly extensive than that of Cy3/BSA. As no FRET exists between Cy3 and BSA, the differences observed between Cy3/C-PC and Cy3/BSA should be ascribed to the FRET response to pressure. Because the Cy3 emission behavior in Cy3/C-PC system has a more lower increasing rate at low pressure and a more notable decreasing process at high pressure, we conclude that with elevated pressure more energy is transferred from Cy3 to C-PC, that is, FRET efficiency is enhanced under compression.

From the Cy3 fluorescence behavior under compression, we suggest that three issues at least have contributed to the fluorescence changes of Cy3 in the Cy3/C-PC system: response of solo Cy3 itself, dye−protein interaction changes and the FRET efficiency changes. The emission of separated Cy3 increases with elevated pressure. As compared to the behavior of Cy3 alone, the dye−protein interactions in Cy3/

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BSA depreciate the increasing rate of Cy3, which is enhanced by the pressure-induced conformational changes of BSA at higher pressure (Figure 5). Due to the Cy3 fluorescence having a lower increasing rate and a more notable decrease in Cy3/C-PC system in comparison to that of Cy3/BSA, the pressure-induced FRET efficiency enhancement is concluded here. More evidence will be supplied to further validate this conclusion based on the analysis focusing on the FRET receptor, C-PC, in the system.

The fluorescence spectra of C-PC alone under compression are illustrated in Figure 4B, which show that under compression the emission bands of C-PC shift to red a little with remarkable intensity decrease. Such results agree well with the previously reported fluorescence measurements for monomer and trimer C-PC under various hydrostatic pressures, where a stepwise reduction in the intensity and an expansion in emission bandwidth were observed for C-PC trimer.[40] It was also reported there that, under high pressure, the displacement coordinates of chromophores was changed and reached to lower equilibrium state in which the nonradiation processes was increased. Therefore, we conclude that the pressure-induced conformational changes of C-PC are the main cause of its fluorescence quenching. The gradual quenching process for C-PC at the pressure lower than 0.4 GPa indicates that some conformational changes do occur at this pressure range. Further extended compression on it may destroy the general conformations of it. Following recycle pressure treatments confirm well this point: once the pressure is released from 1.0 GPa to ambient, the fluorescence spectrum of C-PC can not be recovered to its original state (Figure S3), which shows irreversibility of the pressure-induced conformational changes of C-PC under the present situation.

Then we compare the fluorescence behavior of C-PC before and after being labeled by Cy3 with elevated pressure. Figure 6

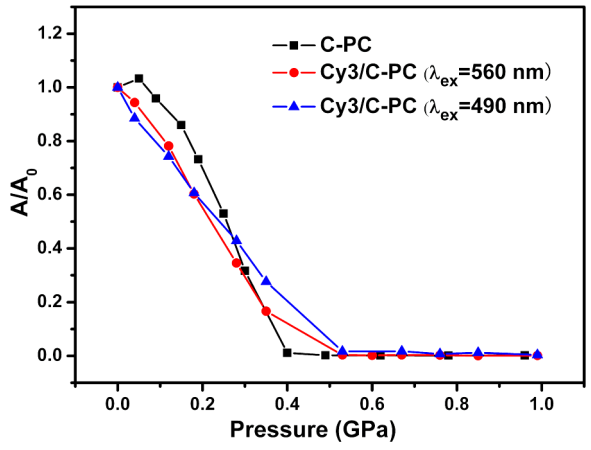

Figure 6. Changes of fluorescence area ratio of separated C-PC and in the Cy3/C-PC system under compression. Comparison of the spectra under two excitation wavelengths is also performed.

shows the integrated area ratio of separated C-PC and the Cy3/C-PC system with pressure. As the reducing rates of C-PC along pressure are independent of the excitation wavelengths (Figure S4), then we compare the fluorescence responsing plots of C-PC and Cy3/C-PC against pressure under different excitation wavelengths. The results shown in Figure 6 illustrate that after being labeled by Cy3, the reducing rate of area ratio for C-PC is slightly lower than that of separated C-PC under compression. In addition, it needs to be noted that in the Cy3/C-PC system, the reducing rate of the area ratio plot for C-PC under the excitation of 490 nm is relatively lower than that excited at 560 nm, as the former wavelength can be used for the excitations of Cy3 and C-PC while the later one is special for C-PC. Both differences come from the dye−protein interaction and the FRET variations from Cy3 to C-PC. In considering that C-PC is much larger in size than Cy3, the dye−protein interaction effect on C-PC should be minor and can be neglected. Therefore, the fluorescence changing behavior for C-PC under pressure should be attributed to the variation of FRET efficiency. That is, the FRET efficiency is strengthened with elevated pressure, which induces the C-PC gained more energy from the Cy3 donor. As a result, the decreasing rate of C-PC in Cy3-labeled C-PC is relatively lower than that of C-PC alone.

Furthermore, the decompression effects on the Cy3/C-PC system are also investigated. After the release of pressure, the fluorescence of Cy3 in Cy3/C-PC system recovers to its initial state, while that of C-PC can not (Figure S5), agreeing well with the pressure response of individual Cy3 and C-PC (Figures S1 and S2). Such results show that, albeit Cy3 and C-PC are connected in one system, the response of each to pressure is independent of each other in the present study. In addition, as the FRET is based on the states of both donor and acceptor, it can not be reversible between Cy3 and C-PC under pressure.

As the integral of the spectral overlap between donor emission and acceptor absorption is one of the important factors to affect the FRET efficiency,[41] we calculate the integral of the spectral overlap to analyze how it changes with pressure. The UV−vis absorption spectra of separated C-PC at different pressures are shown in Figure 7, which reduce remarkably and

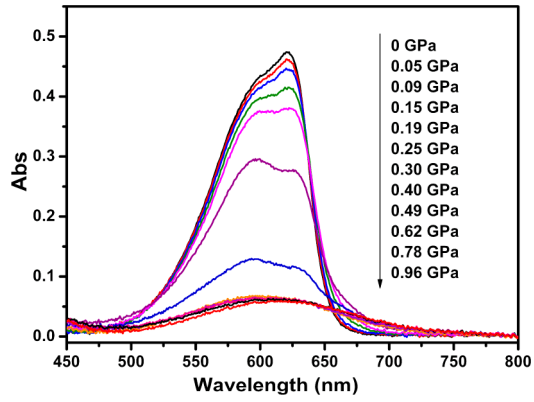

Figure 7. UV−vis absorption spectra of C-PC at different pressures.

have a minor red-shift with pressure increase. The results coincide well with the fluorescence changing of C-PC, which may be induced by the conformational change of C-PC. Because it is difficult to get emission spectra of donor and absorption spectra of acceptor at exact the same pressure, we choose both only at very closed pressures to calculate the integral of the spectral overlap between them. The results shown in Table 1 indicate that the integral of the spectral overlap reduce obviously with increasing of pressure. Generally, a decreasing of overlap between donor emission and acceptor absorption induces weakening of FRET efficiency, however, as aforementioned, an enhanced FRET efficiency is observed for the Cy3/C-PC system under pressure. Therefore, we attribute

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Table 1. Overlap Integrals between C-PC Absorbance and Cy3 Emission Spectra at Several Representative Pressures

pressure (GPa)	I ($\times 10^{-12}$ cm^3/M)
0	2.03
0.09	1.98
0.15	1.90
0.4	0.38
0.49	0.37
0.96	0.32

such an enhancement to the shortening of the distance between donor and acceptor, which could enhance their interaction and might be the major reason for the reduction of the Cy3 fluorescence. Such a result is in accord with that concluded from Cy3 emission behavior.

In addition, we evaluate the pressure-induced unfolding of Cy3/C-PC in the presence of urea by in situ tracking its fluorescence spectra. Figure 8A shows the pressure-dependent area ratios (A/A_0) of Cy3 in the Cy3/C-PC system in the absence and presence of 2 and 4 M urea, respectively. At a pressure lower than 0.4 GPa, in the presence of urea, the A/A_0 increases with pressure; its increasing rate is more rapid than that without urea. Such results show that the increasing extent of FRET efficiency between Cy3 and C-PC is weakening after the addition of urea. In addition, as shown in Figure 8B for the pressure-dependent area ratios (A/A_0) of C-PC in Cy3/C-PC system in the presence of urea, the A/A_0 decrease more faster than that without urea, which illustrates again the weakening FRET efficiency between Cy3 and C-PC in the former case. As urea-induced unfolding of Cy3/C-PC will change the secondary and tertiary structure of C-PC,[42,43] which leads C-PC adopting a relative loose structure and hence changes its fluorescence response to pressure. Consequently, the FRET efficiency is affected directly by the urea-induced unfolding process of Cy3/C-PC. Therefore, we suggest that the presence of urea leads C-PC in Cy3/C-PC more sensitive to pressure, inducing a faster rate of losing initial structure for C-PC and the increasing extent of FRET efficiency between Cy3 and C-PC is weakening after addition of urea. At a pressure higher than 0.4 GPa, the increasing rates of A/A_0 for Cy3 in Cy3/C-PC slow down (Figure 8A), while those for C-PC in Cy3/C-PC nearly disappeared, no matter in the presence or absence of urea (Figure 8B). A rational conjecture is that the C-PC structure is destroyed at a pressure higher than 0.4 GPa; the interaction and FRET effect between Cy3 and C-PC are nearly broken, which makes the increasing rate of A/A_0 for Cy3 in Cy3/C-PC not increase either (Figure 8A), especially in the case of 4 M urea. Furthermore, in the presence of 2 M urea the plot of the Cy3/C-PC system under decompression (Figure S6) shows that the fluorescence of C-PC can recover slightly, while that of Cy3 fluorescence is a little higher than that in the initial state. Such results illustrate that the addition of urea indeed change the C-PC structure, which decrease the reversibility of C-PC and break the FRET effect between Cy3 and C-PC more seriously.

CONCLUSIONS

In the present study, we construct and confirm a FRET system through labeling Cy3 on C-PC and the further spectral affirmance. The fluorescence spectra of such a FRET system under elevated pressure are measured and compared with those of solo Cy3 and C-PC under identical conditions. Three possible issues induced by pressure in the Cy3/C-PC system, which can affect the fluorescence of Cy3/C-PC system, are proposed: responses of solo Cy3 and C-PC itself, dye−protein interaction, and FRET efficiency between them. The enhanced FRET efficiency under compression is concluded for the Cy3/C-PC system, based on fluorescence differences between the individual and interacted Cy3 and C-PC under pressure. In addition, the overlap integral between the Cy3 emission and C-PC absorption is calculated. In combining it with the FRET enhancement, it is assumed that the distance between Cy3 and C-PC chromophore is shortened with pressure, which is a crucial factor inducing a Cy3 fluorescence decrease. Such a study will highlight the investigation on the pressure-induced protein folding/unfolding, weak interactions, biological functions, and so on.

ASSOCIATED CONTENT

Supporting Information

The fluorescence spectra of Cy3, C-PC, and Cy3/C-PC at ambient pressure, high pressure, and released to ambient pressure, in the absence and presence of urea. The fluorescence spectra of Cy3/BSA at elevated pressure. The changes of fluorescence area ratio of C-PC under two excitation wavelengths with elevated pressure. This material is available free of charge via the Internet at http://pubs.acs.org.

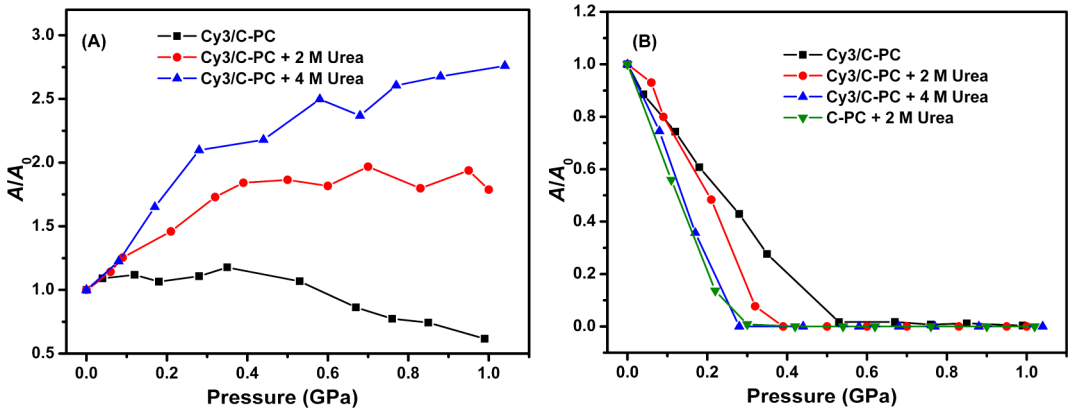

Figure 8. Changes of fluorescence area ratio (A/A_0) of (A) Cy3 in Cy3/C-PC and (B) C-PC in Cy3/C-PC systems under compression, which are measured in the absence and presence of urea, respectively.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Scire, A.; Pedone, E.; Ausili, A.; Saviano, M.; Baldassarre, M.; Bertoli, E.; Bartolucci, S.; Tanfani, F. *Mol. BioSyst.* **2010**, *6*, 2015−2022.

(2) Wang, J.-M.; Yang, X.-Q.; Yin, S.-W.; Zhang, Y.; Tang, C.-H.; Li, B.-S.; Yuan, D.-B.; Guo, J. *J. Agric. Food Chem.* **2011**, *59*, 7324−7332.

(3) Gebhardt, R.; Takeda, N.; Kulozik, U.; Doster, W. *J. Phys. Chem. B* **2011**, *115*, 2349−2359.

(4) Davydov, D. R.; Davydova, N. Y.; Halpert, J. R. *Biochemistry* **2008**, *47*, 11348−11359.

(5) Staiano, M.; Saviano, M.; Herman, P.; Grycznyski, Z.; Fini, C.; Varriale, A.; Parracino, A.; Kold, A. B.; Rossi, M.; D'Auria, S. *Biopolymers* **2008**, *89*, 284−291.

(6) Okuno, A.; Kato, M.; Taniguchi, Y. *Biochim. Biophys. Acta, Proteins Proteomics* **2006**, *1764*, 1407−1412.

(7) Morgner, F.; Geissler, D.; Stufler, S.; Butlin, N. G.; Lohmannsroben, H. G.; Hildebrandt, N. *Angew. Chem., Int. Ed.* **2010**, *49*, 7570−7574.

(8) Tomasz, H. *Curr. Opin. Biotechnol.* **2002**, *13*, 292−296.

(9) Roy, R.; Hohng, S.; Ha, T *Nat. Methods* **2008**, *5*, 507−516.

(10) Piston, D. W.; Kremers, G.-J. *Trends Biochem. Sci.* **2007**, *32*, 407−414.

(11) Schuler, B.; Eaton, W. A. *Curr. Opin. Struct. Biol.* **2008**, *18*, 16−26.

(12) Gambin, Y.; Deniz, A. A. *Mol. BioSyst.* **2010**, *6*, 1540−1547.

(13) Downey, C. D.; Crisman, R. L.; Randolph, T. W.; Pardi, A. *J. Am. Chem. Soc.* **2007**, *129*, 9290−9291.

(14) Senear, D. F.; Tretyachenko-Ladokhina, V.; Opel, M. L.; Aeling, K. A.; Wesley Hatfield, G.; Franklin, L. M.; Darlington, R. C.; Ross, J. B. A. *Nucleic Acids Res.* **2007**, *35*, 1761−1772.

(15) Stapelfeldt, H.; Skibsted, L. H. *J. Dairy Res.* **1999**, *66*, 545−558.

(16) Grossman, A. R.; Schaefer, M. R.; Chiang, G. G.; Collier, J. L. *Microbiol. Mol. Biol. Rev.* **1993**, *57*, 725−749.

(17) Tandeau de Marsac, N. *Photosynth. Res.* **2003**, *76*, 193−202.

(18) Guan, X.; Qin, S.; Zhao, F.; Zhang, X.; Tang, X. *Int. J. Biol. Sci.* **2007**, *3*, 434−445.

(19) Stec, B.; Troxler, R. F.; Teeter, M. M. *Biophys. J.* **1999**, *76*, 2912−2921.

(20) Liu, L.-N.; Chen, X.-L.; Zhang, Y.-Z.; Zhou, B.-C. *Biochim. Biophys. Acta, Bioenerg.* **2005**, *1708*, 133−142.

(21) Wu, M.; Goodwin, P. M.; Ambrose, W. P.; Keller, R. A. *J. Phys. Chem.* **1996**, *100*, 17406−17409.

(22) Melkozernov, A. N.; Barber, J.; Blankenship, R. E. *Biochemistry* **2006**, *45*, 331−345.

(23) Glazer, A. N. *J. Biol. Chem.* **1989**, *264*, 1−4.

(24) Schreiber, U.; Vidaver, W. *Photochem. Photobiol.* **1973**, *18*, 205−208.

(25) Mujumdar, R. B.; Ernst, L. A.; Mujumdar, S. R.; Lewis, C. J.; Waggoner, A. S. *Bioconjugate Chem.* **1993**, *4*, 105−111.

(26) Sapsford, K. E.; Blanco-Canosa, J. B.; Dawson, P. E.; Medintz, I. L. *Bioconjugate Chem.* **2010**, *21*, 393−398.

(27) Richards, C. I.; Hsiang, J.-C.; Khalil, A. M.; Hull, N. P.; Dickson, R. M. *J. Am. Chem. Soc.* **2010**, *132*, 6318−6323.

(28) Teramoto, T.; Kobayashi, T. *Phys. Chem. Chem. Phys.* **2010**, *12*, 13515−13518.

(29) Sanborn, M. E.; Connolly, B. K.; Gurunathan, K.; Levitus, M. *J. Phys. Chem. B* **2007**, *111*, 11064−11074.

(30) Harvey, B.; Levitus, M. *J. Fluoresc.* **2009**, *19*, 443−448.

(31) Luschtinetz, F.; Dosche, C.; Kumke, M. U. *Bioconjugate Chem.* **2009**, *20*, 576−582.

(32) Drickamer, H. G. *Annu. Rev. Phys. Chem.* **1982**, *33*, 25−47.

(33) Glazer, A. N.; Fang, S.; Brown, D. M. *J. Biol. Chem.* **1973**, *248*, 5679−5685.

(34) Stryer, L.; Glazer, A. N.; Oi, V. T. U.S. Patent 5055556, 1991.

(35) Murphy, S.; Sauerwein, B.; Drickamer, H. G.; Schuster, G. B. *J. Phys. Chem.* **1994**, *98*, 13476−13480.

(36) Abramson, E. H. *Phys. Rev. E* **2007**, *76*, 051203.

(37) Marmé, N.; Knemeyer, J.-P.; Sauer, M.; Wolfrum, J. *Bioconjugate Chem.* **2003**, *14*, 1133−1139.

(38) Levitus, M.; Ranjit, S. Q. *Rev. Biophys.* **2011**, *44*, 123−151.

(39) Hosseini-nia, T.; Ismail, A. A.; Kubow, S. *J. Food Sci.* **2002**, *67*, 1341−1347.

(40) Xia, A.-D.; Zhu, J.-C.; Jiang, L.-J.; Zhou, Y.-X.; Liu, S.-X.; Zhang, X.-Y. *Chin. Sci. Bull.* **1992**, *37*, 1671−1672.

(41) Milos, M.; Pal, P.; Hauser, A. *ChemPhysChem* **2010**, *11*, 3161−3166.

(42) Thomas, B. A.; McMahon, L. P.; Klotz, A. V. *Biochemistry* **1995**, *34*, 3758−3770.

(43) Kupka, M.; Scheer, H. *Biochim. Biophys. Acta* **2008**, *1777*, 94−103.