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The Stabilization Effect of Glutaraldehyde on the *Spirulina platensis* Phycobilisomes

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Abstract: The spectral properties of the glutaraldehyde-treated phycobilisomes were studied. The results showed that glutaraldehyde was effective in preventing phycobilisomes from dilution-induced dissociation and preserving the intra-phycobilisomes energy transfer.

Keywords: Phycobilisomes, *Spirulina platensis,* energy transfer, fluorescence, glutaraldehyde.

Phycobilisomes are supramolecular complexes of phycobiliproteins and linker polypeptides locked together mainly by hydrophobic forces in blue-green and red alga. The phycobiliproteins and the linker polypeptides assemble in specific configurations for optimized light harvesting and energy transfer to photosynthetic complexes[1]. Some of the purified phycobiliproteins (*i.e.* phycoerythrin and allophycocyanin), the component fluorescent proteins of phycobilisomes, have been widely used as labels in immunoassay. Compared to those phycobiliproteins, the phycobilisomes have extremely high molecular weights (5-20×10^6 daltons), large molecular sizes (30-80 nm), efficient energy transfer (>90%) among constituent phycobiliproteins, large Stokes shifts (>80 nm), and high quantum yields (>0.5) of constituent phycobiliproteins, so the phycobilisomes can constitute brighter probes for fluorescent detection and they are ideal nano-labels of high sensitivity[2].

The phycobilisomes are preferably stabilized against dissociation before they are used as detectable markers because they readily dissociate into phycobiliproteins especially under the conditions of low ionic strength. The criterion for the functional integrity of phycobilisomes is the demonstration that they exhibit highly efficient energy transfer between component phycobiliproteins. In *Spirulina platensis* phycobilisomes composed of only two kinds of phycobiliproteins, the energy transfer sequence is from phycocyanin to allophycocyanin[3]. Therefore, the intact phycobilisomes of *Spirulina platensis* emit allophycocyanin fluorescence (approx. F660) when phycocyanin is excited. On the contrary, the dissociation of phycobilisomes interrupts intra-phycobilisomes energy transfer, so that phycocyanin (approx. F650) or allophycocyanin fluoresces individually upon excitation.

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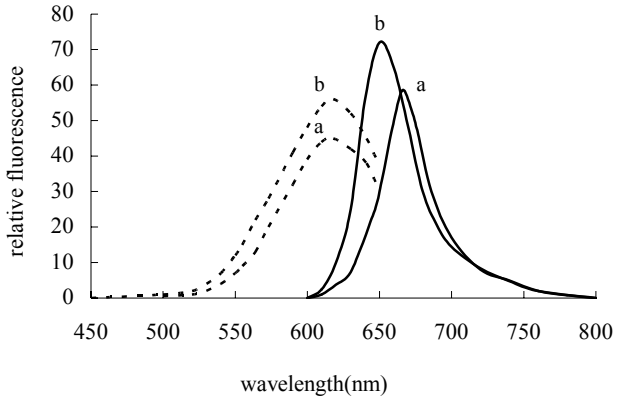
Figure 1 Fluorescence spectra of (a) the intact phycobilisomes and (b) the dissociated phycobilisomes in *Spirulina platensis* at room temperature



Emission curve (—) were measured at 580 nm excitation. Excitation curve (---) were measured at 670 nm emission for a and at 650 nm emission for b, respectively.

In this paper, glutaraldehyde, the medium chain-length homobifunctional protein crosslinking reagent, was used to stabilize the phycobilisomes of *Spirulina platensis.* The glutaraldehyde could respectively react withε-amino groups of lysine residues of two different component proteins in the form of Schiff's base, so connect the component proteins through covalent bonds instead of hydrophobic forces[4].

In this work, the phycobilisomes of *Spirulina platensis* isolated by sucrose density gradients ultracentrifugation were intact in 1 mol/L Na_2HPO_4-KH_2PO_4 buffer ,which was indicated by the fluorescence emission maximum at 665 nm; and the emission maximum was blue-shifted to 650 nm in 1 minute when diluted to 0.1 mol/L with deionized water, which suggested that the phycobilisomes had dissociated into multimeric phycocyanin and allophycocyanin, as shown in **Figure 1**. In the same way, the phycobilisomes (1.8 mg/mL of phycobiliproteins) treated at different glutaraldehyde concentrations were diluted to 0.1 mol/L and incubated for 72 hours at 22℃, only the phycobilisomes treated by 0.02% glutaraldehyde could keep the emission maximum at 665 nm. The emission maximum was gradually blue-shifted to 645 nm with the decrease in the concentration of glutaraldehyde, as shown in **Figure 2**. The strong shoulders at 645 nm of curve c and 665 nm of curve b in **Figure 2** suggested that there had been dissociation in phycobilisomes treated respectively by 0.01% and 0.005% glutaraldehyde during the incubation.

The 0.02% glutaraldehyde-treated phycobilisomes were purified again by sucrose density gradients ultracentrifugation to remove the free glutaraldehyde. The absorptionspectrum and the fluorescence spectra of the purified phycobilisomes were shown in **Figure 3** and **Figure 4**, respectively.

In **Figure 3**, although the absorption maximum of the 0.02% glutaraldehyde-treated phycobilisomes was red-shifted by 3 nm to 618 nm, the shape of their absorption spectrum was similar to that of the native phycobilisomes absorption spectrum.

Figure 2 Fluorescence emission spectra of phycobilisomes treated by different concentrations of glutaraldehyde. (The excitation wavelength was 620 nm)

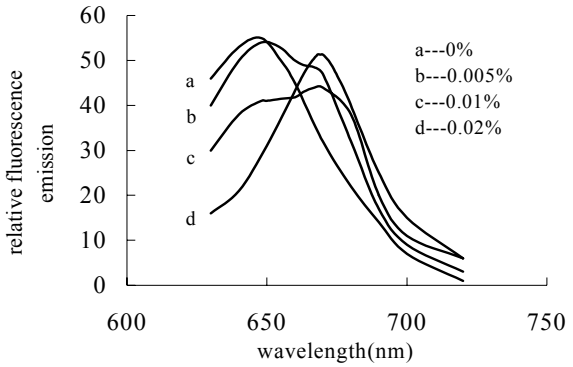


Figure 3 The absorption spectra of 0.02% glutaraldehyde-treated phycobilisomes (—) and the native phycobilisomes (---). (The spectra were normalized at the maximum)

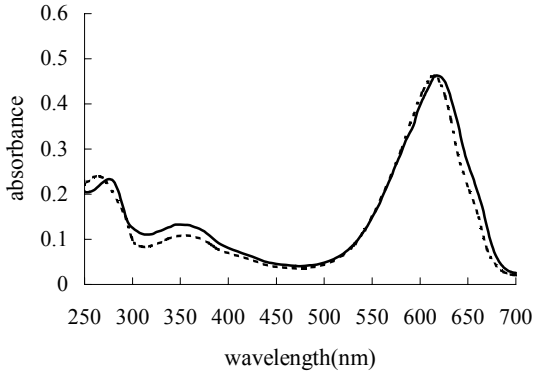
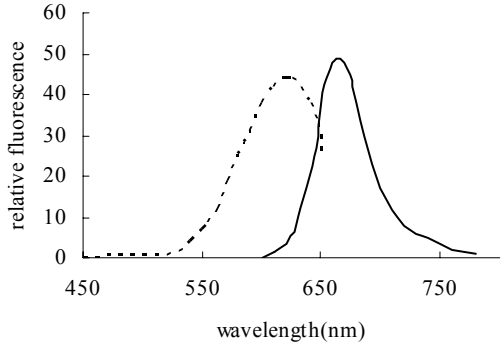


Figure 4 Fluorescence spectra of 0.02% glutaraldehyde-treated phycobilisomes at room temperature



Emission curve (—) was measured at 580 nm excitation.
Excitation curve (---) was measured at 670 nm emission.

The emission and the excitation maximum of 0.02% glutaraldehyde-treated phycobilisomes were, respectively, at 662 nm and at 620 nm in **Figure 4**, which demonstrated the efficient energy transfer within the glutaraldehyde-treated phycobilisomes.

The results of the spectral analysis showed that the glutaraldehyde was effective in protecting phycobilisomes from dilution-induced uncoupling of energy transfer, and preserving the structural integrity of phycobilisomes without much alteration in spectral properties. This experiment provided fundamental work for further using the stabilized phycobilisomes as highly sensitive labels.

Acknowledgments

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