THE EFFECT OF pH ON BOTH SPECTRAL TYPES OF R-PHYCOERYTHRIN*

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Abstract

There are two spectral types of R-phycoerythrin (RPE) in red algae. One is the triple peak type RPE with absorption maxima at 498,540 and 565 nm. The other is the double peak type RPE with absorption maxima at 498 and 565 nm, and an absorption shoulder at 540 nm. The former is present only in higher classes of the more advanced Florideophyceae, the latter occurs chiefly in the more primitive Bangiophyceae. When these two types of RPE are treated with buffer solutions with a broad pH range from 2.0 to 11.0, their absorption and fluorescence spectra remain unchanged within the range adjacent to neutral pH. At pH values higher than 9.6, the absorption and fluorescence spectra of triple peak type RPE still remain stable, but the absorption spectra of double peak type RPE produce gradually a protrusion similar to an absorption peak at about 540 nm. This phennomenon shows that there are certain differences in structure between these two types of RPE. Both types of RPE are naturally occurring products showing systematic developmental meaning in red algae.

Phycoerythrin (R-PE) is a light-harvesting pigment protein present in large quantities in red algae. According to a model of the energy transfer in phycobilisome (PBS)[4], RPE first absorbs light energy, then excitation energy transfer occurs in the sequence: phycoerythrin (PE)—phycocyanin (PC)—allopycyanin (APC). Eventually, the light energy absorbed by PBS is transferred through APC to chlorophyll in the lamellar system to start the process of photosynthesis. There are two RPE types in the absorption spectrum. One is the triple peak RPE with absorption maxima at 498, 540, and 565nm. The other is the double peak RPE with absorption maxima at 498 and 565 nm, and a shoulder at 540nm. The absorption spectrum characteristic is significant for judging biliproteins. As early as 1887 Sorby[8] had noted some relationships between the spectral properties of biliproteins and the systematic position of algae containing these pigments. Basing their conclusion on the measured absorption spectra of extracts of 56 species of red algae from Japan, Hirose[9] considered that double peak type RPE (designated as type IV PE in Hirose's paper) occurs in red algae below Nemaliales of Florideophyceae, including the majority of lower red algae in Bangiophyceae and a part in Nemaliales, and that triple peak type (designated as type V PE in Hirose's paper) is only present in higher red algae above Nemaliales, including a higher species in Nemaliales. According to absorption spectra measured by the authors for biliproteins from 62 species of red algae collected from Qingdao, China, the Atlantic coast of Canada, and the Pacific coast of America, RPE from 17 species of red algae in Bangiophyceae were all double peak type, RPE from 7 of 47 species of red algae in Florideophyceae were double peak type, the remaining 40

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species were all triple peak type. Although our results do not coincide with Hirose's, we believe that the distribution of these two spectral types of RPE in red algae is correlated to the systematic position of these algae.

O'hEocha considered that the wavelength at absorption maxima and relative extinction are related not only to isolated species, but also to the methods of extraction and isolation. Many researchers had noted that the shape and intensity of the absorption spectra of biliproteins depend on the aggregate state of the biliproteins[1,3,6]. Furthermore, there is a close relationship between the aggregate state of biliproteins and the physico-chemical environment, such as biliproteins concentration, pH value and ionic strength of the medium[3,11]. To prove whether these two types of RPE are normally occurring natural products with systematic developmental meaning or are formed under different physico-chemical environments in isolation, we investigated the effect of the pH value on two spectral types of RPE. RPE from *Heterosiphonia japonica* (Florideophyceae) and *Porphyra katadai* var. *hemiphylla* (Bangiophyceae) were used to investigate the effect of different pH values on both types of RPE under constant protein concentration and ionic strength of the medium.

MATERIALS AND METHODS

Biliproteins isolation. Triple peak type and double peak type RPE were respectively isolated from *H.* japonica in Florideophyceae and *P. katadai* var. *hemiphylla* in Bangiophyceae. The seaweeds were common red algae growing in intertidal water near Qingdao. The fresh algae collected were washed with distilled water, and then were immersed in 0.005 M potassium phosphorate buffer (pH6.7), or distilled water. Debris in the crude extracts was removed by filtration or centrifugation. RPEs were isolated and purified by chromatography on a hydroxylapatite column. Biliprotein purity was assayed spectrophotometrically with an acceptable A565/A280 ratio greater than 3.0.

Solution preparation. Two sets of biliprotein solutions, concentration 50 μg/ml, were prepared with a potassium phosphate buffer of 0.05 M ionic strength. The pH values of the solutions were 2.0;3.1;4.0;5.0;6.7;7.0;7.5;8.5;9.6;10.0 and 11.0 respectively. The RPE of one set of solutions, triple peak type, was prepared from *H. japonica*, the RPE of the other, double peak type, from *P. katadai* var. *hemiphylla*. The pH values of the solutions were measured with a Backman Φ-7 pH meter.

Absorption and fluorescence. All absorption spectra were measured with a Shimatzu UV-240 recording spectrophotometer. The measurements were done at room temperature using a standard 1.0 cm pathlength cell. All fluorescence emission and excitation spectra were measured at room temperature with a Hitachi-850 recording spectrofluorophotometer.

RESULTS

The changes of absorption spectra with pH values for both spectral types of RPE are shown in Fig.1 and Fig.2. At pH values 2.0 and 3.1, the absorption spectra of both types of

RPE were basically similiar, and were spectra of denatured RPE. The absorption peaks of the RPE at the long wavelengths changed into one wide absorption band. At a pH value of 3.1, two absorption peaks at the long wavelength can be indistinctly seen, but were not typical. These spectrum characteristics denote a partial denaturation of the biliproteins. In the pH region from 4.0 to 11.0, the shape of absorption curves of triple peak type RPE were all similiar (Fig. 1) and displayed spectral characteristic at neutral pH. The double peak type RPE shows normal absorption spectrum with two absorption peaks in the pH region from 4.0 to 8.5. In the higher pH range from 9.6 to 11.0, the absorption spectra show gradually distinct changes in the ratio of absorption peak. The absorption shoulder at about 540 nm wavelength gradually curves up to evolve into a protrusion similiar to an absorption peak. Fig.2, Fig. 3 (A), (B) and Fig.4(A), (B) respectively show fluorescence emission and excitation spectra of both types of RPE at different pH values. In the pH region from 4.0 to 11.0, all the spectra were not affected by pH change and all displayed the spectral form at neutral pH. The fluorescence emission peak of both types of RPE were at 576 and 577 nm respectively. At a pH value of 3.1, their emission peak shifted to 597.5 and 580 nm respectively. Simultaneously, the shapes of their fluorescence emission spectra were changed.

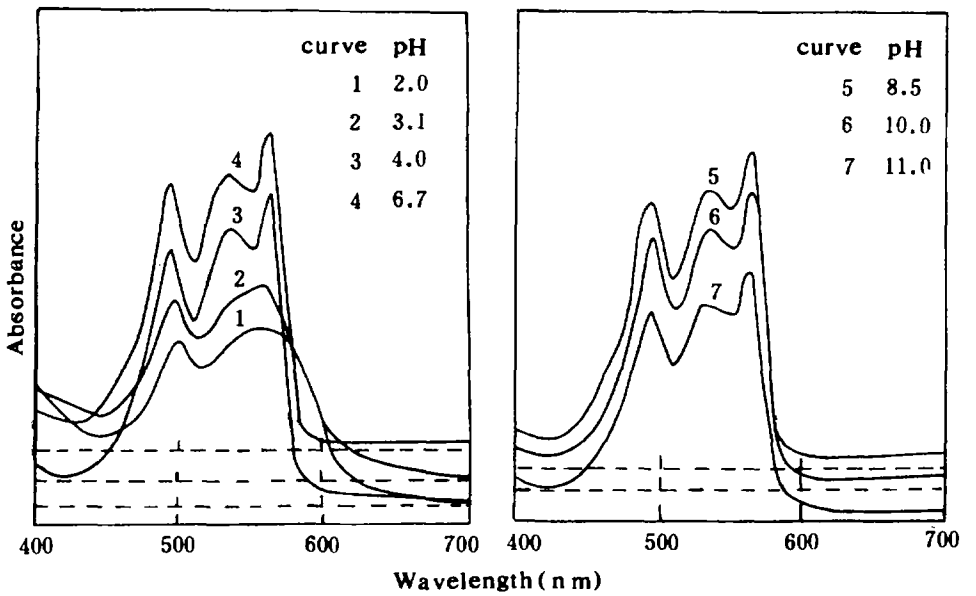

Fig. 1 Absorption spectrum of triple peak type RPE changes with pH.

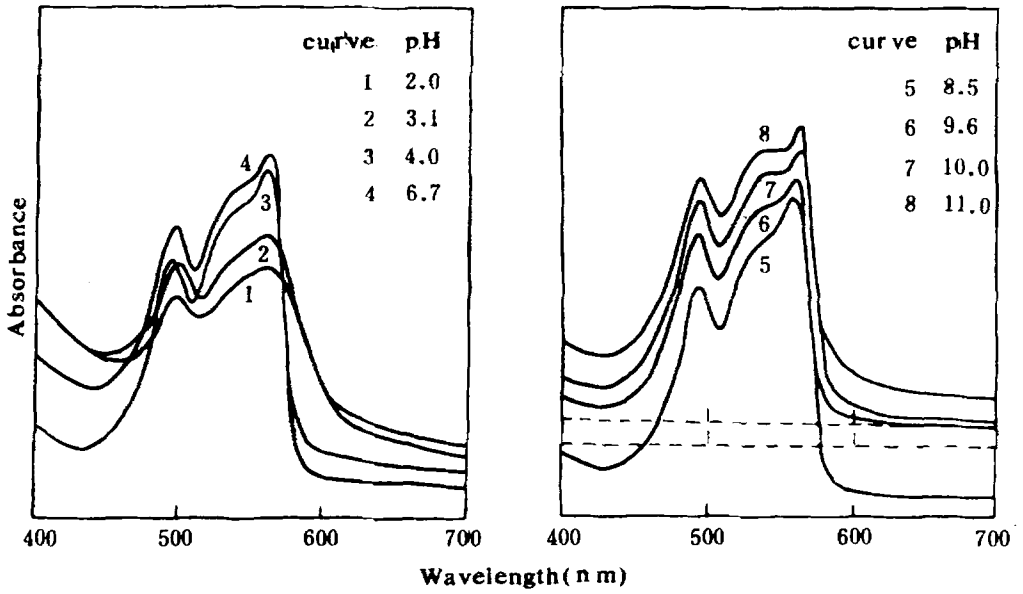

Fig. 2 Absorption spectrum of double peak type RPE changes with pH.

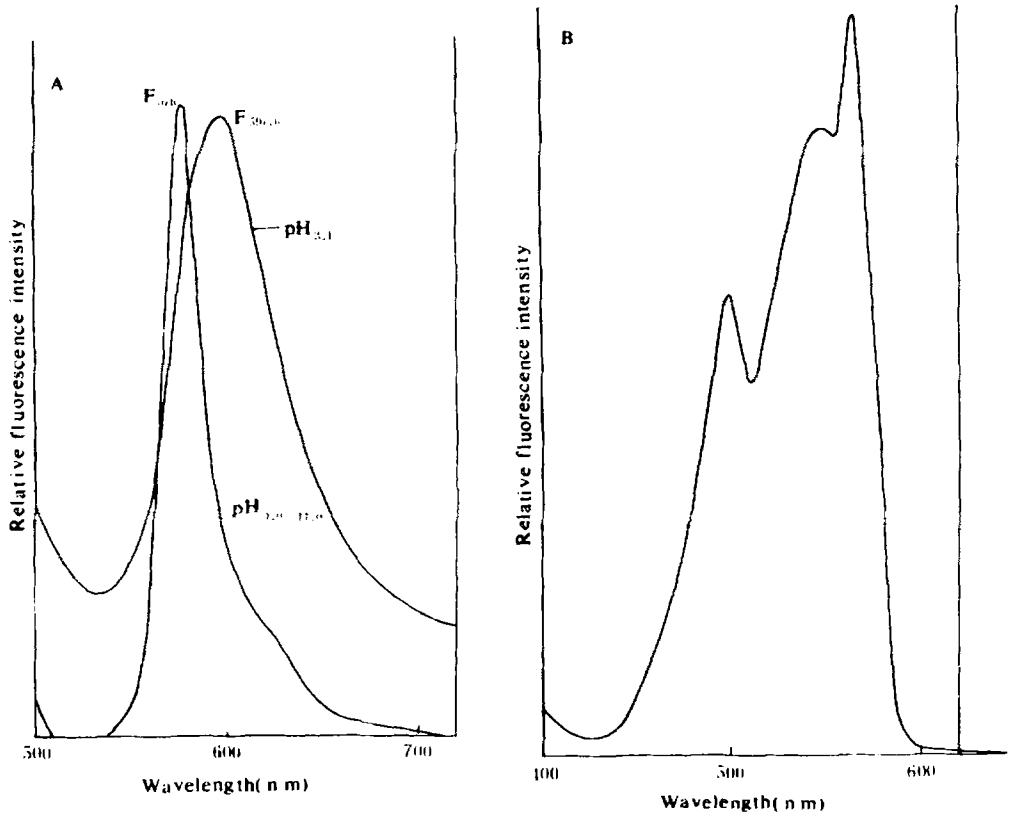

Fig. 3 Fluorescence spectra of triple peak type RPE

(A) Fluorescence emission spectra

(B) fluorescence excitation spectrum

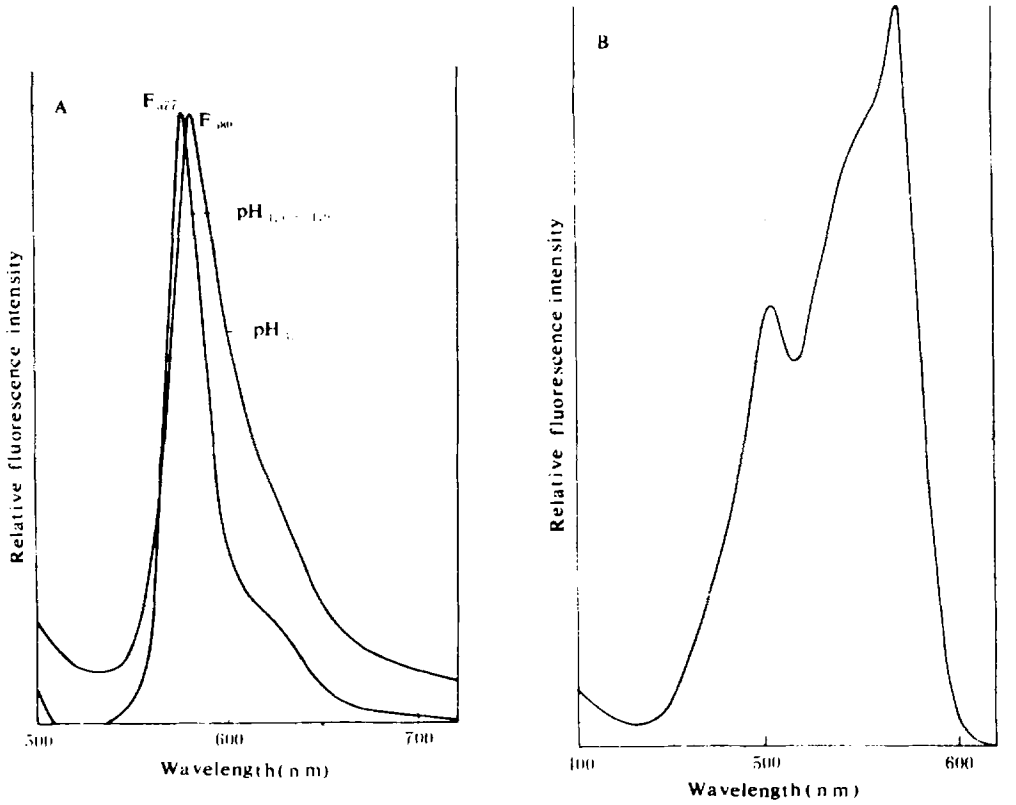

Fig. 4 Fluorescence spectra of double peak type RPE
(A) fluorescence emission spectra
(B) fluorescence excitation spectrum

DISCUSSION

Data on the absorption maxima and fluorescence emission maxima of biliproteins in the visible region from literature on spectral properties of biliproteins were usually the results measured in dilute salt or buffer solutions at nearly neutral pH. In the solutions, RPE from red algae were differentiated into two spectral types, triple peak type and double peak type, depending on whether the absorption at about 540 nm was either a peak or a shoulder. According to Fraskowiak[3] and Murakami et al[11], however, the spectral properties of biliproteins in the visible region will be changed if the measuring conditions described above were changed. They demonstrated that the properties of absorption spectra of biliproteins depend on the physico-chemical environments of the solution measured, such as the pH, protein concentration, ionic strength of medium, etc. Thus, when biliproteins are characterized spectroscopically, it is extremely important to specify the physico-chemical parameters of the biliproteins in question. According to a study by Frackowiak[3] and the authors, both types of RPE display normal absorption spectra with three peaks and two peaks at neutral pH when the biliprotein concentration was 50 μg/ml and the ionic strength of the medium was 0.05 M. In the present study, the biliprotein

concentration and the ionic strength of the medium used were as stated above.

In our results, the shape of the absorption spectral curve for triple peak type RPE from *H. japonica* basically had no change but the absorbance altered somewhat in the region for pH values from 4.0 to 11.0 (Fig.1). The results were identical with that reported by Krasnovskii[10] and Fraskowiak[3]. They respectively prepared RPE from *Callithamnion rubosum* and *Ceramium rubru* and measured their absorption spectra in the pH region from 3.6 to 10.0. The results showed that the absorption spectrum of triple peak type RPE was not affected, or was weakly affected, by the pH values within a wider pH region. Again, it was reported by Erission-Ouense[2] and Vanghan[13] that the triple peak type RPE occurs in the aggregate state $(\alpha\beta)_6\gamma$ in a relatively wider pH range. Fraskowiak[3] also pointed out that the molecular weight of RPE with three peaks varies very little with pH. Thus, it can be considered that the triple peak type RPE can have a remarkably stable structure $(\alpha\beta)_6\gamma$ and is not affected by the pH value.

Unlike the triple peak type RPE from higher red algae, *H. japonica*, the double peak type RPE from *P. katadai* var *hemiphylla* does not always show double peaks at the pH region from 4.0 to 11.0. At pH values higher than 9.0, the absorption spectra emerge as a curve like an absorption peak at about 540nm wavelength (Fig.2). The occurrence of this phenomenon in the double peak type RPE at higher pH values has not been reported yet. Is it possible that the denaturing of the protein is the cause for this phenomenon? To find out, we carefully examined the colour and fluorescence emission and excitation spectra of the biliprotein solution at the pH region from 9.6 to 11.0. Denatured protein is purple and denaturation produces sediments, but the colours of the above solutions were normal and not changed, and sediments were not produced. At the same time, the fluorescence emission and excitation spectra (Fig.4, A, B) were all normal, and identical with that at neutral pH. Thus, it is considered that the phenomenon is not likely the result caused by protein denaturation. As the shape of the absorption curve of biliproteins is very sensitive to the protein aggregate state[1,5,7], the pH value is also an important factor affecting the aggregate state of the biliprotein[6,11], then is the change of the double peak type RPE at higher pH values caused by a change of the aggregate state of the protein? This question still needs further investigation.

It is seen from the results of the measurements that within the physiological pH value both spectral types of RPE displayed absorption properties of triple and double absorption peaks in the visible region. Moreover, the RPE with three absorption peaks is more stable than the RPE with two absorption peaks at higher pH values.

This shows that there are certain structural differences between both types of RPE. For this reason, we think that both these types of RPE are undoubtedly normally occurring natural products found mainly in primitive Bangiophyceae and more advanced Florideophyceae. The significance of their systematic development in red algae is quite clear.

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