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Spectrophotometric Determination of Pigments in Full-mature and Vegetative Parts of *Ulva pertusa*

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The fertile thalli of *Ulva pertusa* were collected from intertidal and subtidal zones in Notojima, Noto Peninsula, Ishikawa Prefecture. These thalli were divided into three groups as the male, the female gametophytes and the sporophytes. The absorption spectra of pigments in the intact thalli and in the methanol extracts were analyzed by spectrophotometric measurement.

The absorption spectra indicated that the full-mature parts were characterized by higher content of carotenoids and lower that of chlorophylls than the vegetative parts.

Absorption difference spectra of the full-mature parts versus the vegetative parts of the intact thalli showed a distinct absorption band around 505 nm. The formation of this red pigment seemed to be an important characteristic change in the course of the fertilization of this algae.

Absorption difference spectrum of pigments in the methanol extracts of the full-mature parts versus the vegetative ones showed a maximum at 453 nm and a shoulder at about 473 nm. The absorption maximum at 505 nm in the intact thalli sifted toward shorter wavelength as a result of extract into methanol.

Key words: spectrophotometric determination, *Ulva pertusa*, carotenoids, chlorophylls, red pigment

In general, the fruiting of *U. pertusa* usually occurs at biweekly intervals during the spring neap tides of a lunar month. When they become fertile the full-mature parts of the thalli differ in color from the vegetative interior ones. The full-mature parts of thalli always turn rather yellowish green than the vegetative ones. The color change in the full-mature parts of three plants, i.e. the male, the female gametophytes and the sporophytes, is somewhat different from each other.[1-5]

Such color difference suggests that some modification of the pigments or the change in the pigment composition might have taken place between the vegetative parts and the full-mature ones.[6-9]

In the present study the absorption and the absorption difference spectra of pigments in the intact thalli and the methanol extracts of the vegetative and the full-mature parts of the three plants of *U. pertusa* were measured by spectrophotometric determination. The difference of pigment composition in the respective materials was investigated by comparing the spectra obtained.

Materials and Methods

Materials

The fertile thalli of *U. pertusa* used in this study were collected in Notojima, Noto Peninsula during march, 1985. After return to the laboratory of Ishikawa prefecture marine culture station in Notojima, they were divided into groups as the male, the female gametophytes and the sporophytes. Because color change was quite remarkable in fertile fronds, it was easy to cut off the full-mature parts from the thalli with scissors.

Preparation of Pigment Extracts

The vegetative and the full-mature parts of *U. pertusa* were carefully wiped with filter papers. These materials (5-10 g wet weight) which were weighed according to color tone were cut up into small pieces and were put into Erlenmeyer flasks of 100 m*l* volumes. Pigments in them were extracted with absolute methanol containing 0.5 mg basic $MgCO_3$ in the dim light at room temperature until they were colorless. The residue in these pigment extracts was removed by being filtered out through the 3g-2 glass filters.

Spectrophotometric Determination

Absorption spectra of the intact thalli were measured with a Shimazsu multipurpose spectrophotometer MPS 50L. Opal glass method was used for this analysis. The vacancy opal glass slide and the opal glass slide with a small piece of thallus which was spread smoothly were set to the cell holder of reference side and sample side of spectrophotometer, respectively. The absorption spectra were examined as usual.

The absorption spectra of the methanol solutions of pigments were determined with a Shimadzu spectrophotometer UV-200. This analysis was also carried out in 1 cm-cells.

The absorption difference spectra were examined by way of setting the vegetative parts to reference side and the full-mature ones to the sample side of the spectrophotometer.

Results

Pigments in the Intact Thalli

Fig. 1 shows the spectra of the pigments in the male (A), the female gametophyte (B) and the sporophyte (C) examined by the opal glass method. The absorption spectra of the vegetative (a) and the full-mature part (b) and the absorption deference spectrum (c) between the each are in this figure.

The six absorption spectra were essentially similar in shape. The main absorption bands in those spectra were commonly located at 425 nm and 670 nm and the shoulder at about 575 nm and 645 nm, respectively.

The 650-675 nm band was higher in the absorption spectrum of the vegetative parts than in that of the full-mature one of the male gametophyte, but the 450-570 nm band conversely was lower. The height of the 650-675 nm band in the vegetative spectrum of the female gametophyte was almost identical with that in the full-mature one, but the 450-570 nm band is low in the vegetative.

The two absorption spectra of the sporophyte extremely resembled each other in shape, though the ratio of 500-550 nm to 665 nm was somewhat lower in the vegetative spectrum than in the full-mature one.

The spectral difference of the full-mature parts and the vegetative ones of three plants shows that the pigments in them were different in composition each other.

The main maxima of the absorption difference spectra (c) of pigments in the vegetative part versus in the full-mature one of the male, the female gametophyte and the sporophyte were located at 505 nm. Shapes of absorption maxima were sharply in the male spectrum, somewhat sharply in the female one and shortly in the sporophyte one.

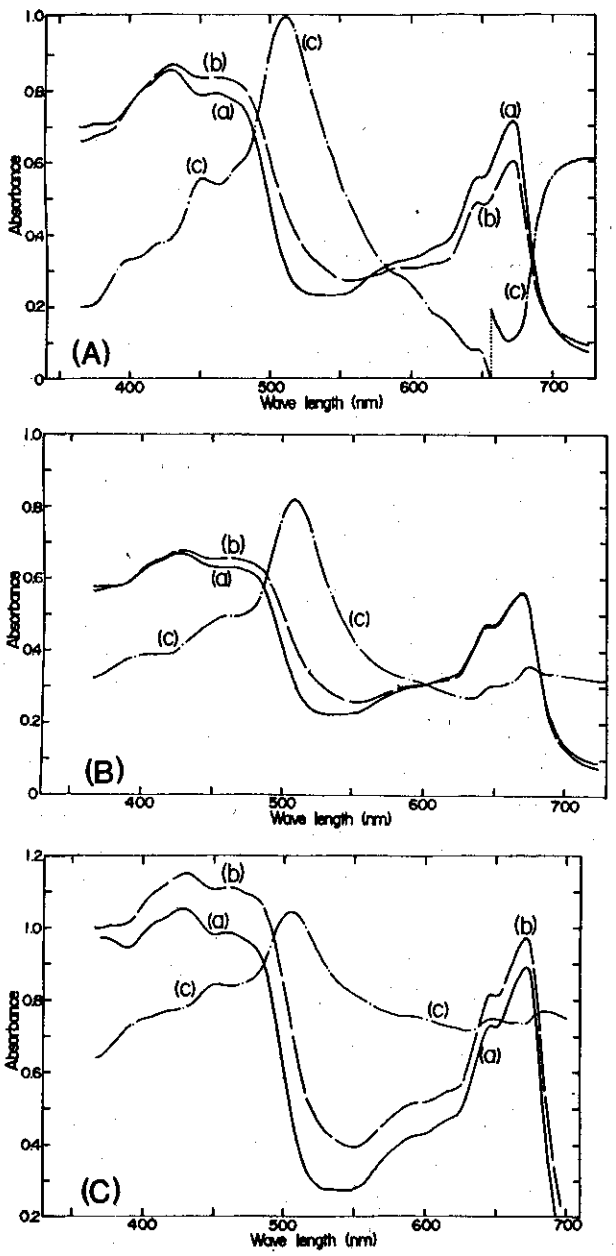

Fig. 1. Absorption and absorption difference spectra of the vegetative and the full-mature part of the male, the female gametophyte and the sporophyte of *Ulva pertusa*. (A), male gametophyte; (B), female gametophyte; (C), sporophyte; (a), vegetative part; (b), full-mature part; (c), full-mature part versus vegetative one.

Pigments in the Extracts

Fig. 2 shows the absorption spectra ((a) and (b)) and the absorption difference spectrum (c) of pigments extracted by methanol from the vegetative and the full-mature parts of the male thalli.

The absorption spectra of extracts from the vegetative parts had the major absorption maxima at 436 nm, 468 nm and 665 nm. The main absorption maxima of the full-mature parts was located at 438 nm, 468 nm and 665 nm. The absorption maximum at 436 nm of the vegetative spectrum sifted 2 nm toward longer wavelength as a result of maturity. The

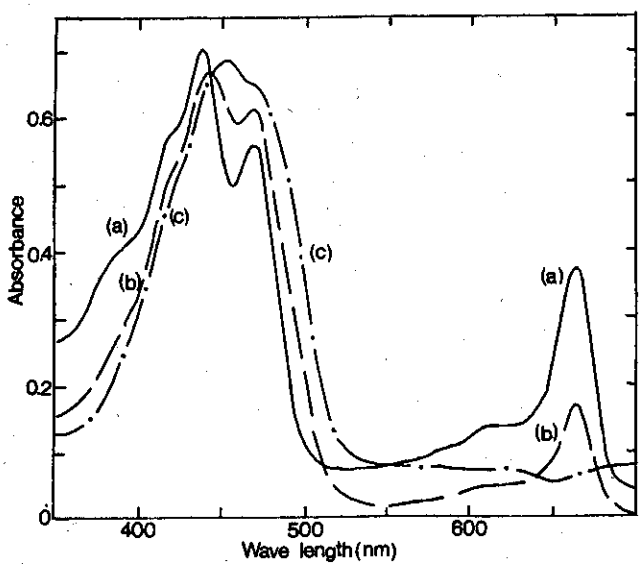

Fig. 2. Absorption and absorption difference spectra of the pigments extracted into 100% methanol from the vegetative and the full-mature parts of *U. pertusa*. (a), vegetative parts; (b), full-mature parts; (c), full-mature parts versus vegetative ones.

absorption maximum of the vegetative at 665 nm was higher than that of the full-mature parts, but the absorbance at 440-525 nm was lower. Those results could be interpreted that there was some differences in the pigments composition between the two parts.

The absorption difference spectrum of the full-mature parts versus the vegetative ones showed that the absorbance curve at 650-750 nm (red region) was low and the absorbance at 450-520 nm (green from blue region) was high. Moreover there was the maxima at 453 nm and the shoulder at about 473 nm attributed to carotenoids. It is seemed that the ratio of the carotenoids / chlorophylls content in the full-mature parts is higher than that in the vegetative ones.

Discussion

The absorption spectra of pigments in the intact thallus of the vegetative and the full-mature part of the male, the female gametophyte and the sporophyte were comparatively similar in shape, but the ratio of absorbance of 400-500 nm to 665 nm was lower in the vegetative spectra than in the full-mature ones. On the absorption spectra in the methanol extracts from the male gametophytes, the absorption maxima in the vegetative spectrum and in the full-mature one were at 430 nm and at 428 nm, respectively. Moreover the ratio of absorbance at 400-500 nm to 665 nm in the vegetative ones was lower than that in the full-mature ones. The spectral difference between the vegetative and the full-mature parts shows that the pigment component changed and the ratio of chlorophylls / carotenoids contained was higher as a result of full-maturity.

In the intact thalli of three plants, main maxima of the absorption difference spectra of the full-mature part versus the vegetative one were located at 505 nm. The absorption maximum around this wavelength is assumed to be primarily due to a red pigment which has absorption activity in green light.

The band at 505 nm in difference spectra of the male, the female gametophyte and the sporophyte differed in the height. This indicates that there was the difference in the content of the red pigment among the full-mature part of each plants.

In vivo absorption spectra of deep-water green algae, *U. japonica*, showed a distinct band around 540 nm (green region).[10] This pigment seemed to be siphonaxanthin. Siphonaxanthin was presumed to be an efficient photosynthetic pigment which was important for the green algae living in deep coastal water.[10]

The maximum of the absorption difference spectrum of the full-mature part versus the vegetative one was at 505 nm, therefore the red pigment in the full-mature part of *U. pertusa* is not siphonaxanthin. It is suggested that this red pigment was relevant to fruiting process or the formation of spores and their germination.

In methanol extracts, the absorption difference spectrum of the full-mature parts versus the vegetative ones had the maxima at 453 nm and the shoulder at about 473 nm attributed to carotenoids. Previously, the author have carried out the analysis of pigments in the full-mature parts of *U. pertusa* and *Enteromorpha linza*, and detected a characteristic pigment with the absorption maxima bands at 443, 472 and 502 nm in diethylether.[11] In the present study, the measurement of the absorption difference spectrum of the intact thallus cleared that this pigment had the absorption maxima bands at 505 nm. It is presumed that this change is due to a disruption of a carotenoid protein link, rather than to a destruction of the pigment itself.

The color change to yellowish green of the edge due to maturing of *U. pertusa* is probably caused by the change of the carotenoids / chlorophylls ratio and by the formation of a red pigment absorbing at 505 nm. The difference of color tone of the full-mature parts among the male, the female gametophytes and sporophytes is due to the difference of the red pigment content.

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