Paper

Dependence of Fluorescence Spectra of Susabi-nori (*Porphyra yezoensis*) on the Excitation Wavelength

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ABSTRACT

This research paper aims to investigate dependence of fluorescence spectra of Susabi-nori (*Porphyra yezoensis*) on the excitation wavelength by comparing with absorption spectra of photosynthetic pigments. The intensities of the fluorescent peaks located at approximately 685 nm and 720 nm relatively increased by using the excitation light with the wavelength of approximately 430 nm, because the excitation light was mostly absorbed by chlorophyll-a. On the other hand, the intensity of the 580 nm peak relatively increased by using that of approximately 500 nm, because the excitation light was mostly absorbed by phycoerythrin. As a result, the intensity ratio of the 580 nm peak to the 685 nm peak was maximal at the excitation wavelength of approximately 500 nm. Therefore, it was concluded that the optimum excitation wavelength was approximately 500 nm for diagnosis of diseases and stresses of Susabi-nori.

KEYWORDS: Susabi-nori, *Porphyra yezoensis*, laser-induced fluorescence, red rot disease

1. Introduction

In the conventional nori (laver; *Porphyra*) cultivation, fishermen diagnose the influences of diseases and stresses by means of the visual inspection or the optical-microscope observation. However, a quantitative evaluation technique is required for more stable nori production. In addition, diagnosis technique is also required in the early stage when diagnosis of nori is impossible by the visual inspection or the optical-microscope observation.

Up until now, we have proposed the laser-induced fluorescence (LIF) method for diagnosis of Susabi-nori (*Porphyra yezoensis*), and revealed that fluorescence spectra of Susabi-nori were affected by the diseases and the stresses of fresh water and that the diseases and the stresses of Susabi-nori could be diagnosed by the fluorescence method¹⁾. However, the fluorescence mechanisms are not clear yet.

In this work, we investigated the dependence of fluorescence spectra of Susabi-nori on the excitation wavelength by comparing with absorption spectra of photosynthetic pigments.

2. Materials and methods

The samples of Susabi-nori were cultivated from October to December, 2007 by Kaneda fisheries cooperative and Shin-Futtsu fisheries cooperative, Chiba prefecture, Japan. The fluorescence spectra were measured immediately after gathering these samples.

In order to investigate the dependence of fluorescence spectra on the excitation wavelength, a Xe lamp (L6759, Hamamatsu Photonics, Japan) was used as an excitation light. The Xe-lamp light was monochromated by a monochromator (H20 IR, Horiba Jobin Yvon, France). The intensity of the monochromated Xe-lamp light ranged from 15 to 100 $\mu\text{W}/\text{cm}^2$. The laser lights with wavelengths of 325 nm (cw He-Cd laser, IK3301R-G-S, Kimmon, Japan, 10 mW), 375 nm (cw laser diode, LDM375.10CW, Omicron, Germany, 190 μW), 405 nm (cw laser diode, 405-50-COL-003, Oxxius, France, 10 mW) and 488 nm (cw Ar⁺ laser, GLG3020, NEC, Japan, 6 mW) were also used as excitation lights for comparison. The fluorescence spectra were measured in sea water at approximately 20°C, and analyzed using a multichannel monochromator (PMA-12, Hamamatsu Photonics, Japan). The quantity of the sea water was so limited that it had little influence on the fluorescence measurement of Susabi-nori. The fluorescence was guided to the monochromator using the optical fiber. The short-wavelength cut-off filter under 530 nm was attached to the optical fiber in order to prevent the influence of the excitation light.

3. Results and discussion

Figure 1 and 2 show the dependences of fluorescence spectrum of Susabi-nori on the excitation wavelength by using the monochromated Xe-lamp lights and the lasers, respectively. As reported in the previous paper, the

Figure 1 Dependence of fluorescence spectrum on the excitation wavelength by using the monochromated Xe-lamp lights

Figure 2 Dependence of fluorescence spectrum on the excitation wavelength by using the lasers

fluorescent peaks located at approximately 580, 660, 685 and 720 nm were observed in the spectra¹⁾. The emissions at approximately 580 nm and 660 nm probably originate in phycoerythrin and allophycocyanin, respectively²⁾, and those at approximately 685 nm and 720 nm are probably due to chlorophyll-a³⁾. Distinctive individual differences were not observed in terms of intensity ratio of each peak. As shown in Figure 1, the fluorescence spectra were clearly observed even by using the monochromated Xe-lamp lights. From these figures, it was found that the fluorescence intensity ratios such as the ratio of the 580 nm peak to the 685 nm peak were affected by the excitation wavelength.

Figure 3 shows the dependence of the fluorescence intensity ratio of the 580 nm peak to the 685 nm peak (F_{580}/F_{685} ratio) on the excitation wavelength by using the monochromated Xe-lamp lights and the lasers, and the reported absorption spectra of photosynthetic pigments⁴⁾⁻⁷⁾. The vertical dashed lines denote the

wavelengths of 430 and 500 nm, at which the absorbances were maximal in chlorophyll-a and R-phycoerythrin, respectively. It was found that the F_{580}/F_{685} ratio was minimal at the excitation wavelength of approximately 430 nm. The 685 nm peak probably originates in chlorophyll-a, and the absorbance of the chlorophyll-a is maximal at the wavelength of approximately 430 nm. Therefore, this result suggests that the 685 nm peak intensity relatively increases by using the excitation light with the wavelength of approximately 430 nm, because the excitation light is mostly absorbed by chlorophyll-a. On the other hand, the F_{580}/F_{685} ratio increased at the excitation wavelength of approximately 500 nm. The 580 nm peak probably originates in phycoerythrin, and the absorbance of the phycoerythrin is high at the wavelength of approximately 500 nm. Therefore, this result indicates that the 580 nm peak intensity relatively increases by using the excitation light with the wavelength of approximately 500 nm, because the excitation light is mostly absorbed by phycoerythrin.

Figure 3 Dependence of the intensity ratio of the 580 nm peak to the 685 nm peak on the excitation wavelength and absorption spectra of the photosynthetic pigments⁴⁾⁷⁾

Figure 4 shows the dependence of the intensities of the peaks located at approximately 580 nm (F_{580}), 660 nm (F_{660}), 685 nm (F_{685}) and 720 nm (F_{720}), and the integrated intensity between 530 to 800 nm on the excitation wavelength by using the monochromated Xe-lamp lights. The integrated intensities were the integral of the fluorescence intensity from 530 to 800 nm. In the measurement system, the intensities of the monochromated Xe-lamp lights varied with the wavelength. Therefore, the intensities of the fluorescent peaks were confirmed to be proportional to the excitation-light intensity in preliminary experiment, and the fluorescence intensity at each wavelength was revised after divided by the excitation-light intensity in order to remove the variance influences in the excitation-light intensity. At the excitation wavelength of approximately 430 nm, the intensities of the 580 and 660 nm peaks were

Figure 4 Dependence of the intensities of the 580, 660, 685 and 720 nm peaks and the integrated intensity between 530 to 800 nm on the excitation wavelength

Figure 5 Fluorescence spectra of the control sample and the sample infected with the red rot disease by using the excitation lights with wavelengths of 430 and 500 nm

very weak, because the excitation light with the wavelength of approximately 430 nm was mostly absorbed by chlorophyll-a. On the other hand, the intensities of any peaks were maximal at the excitation wavelength of approximately 500 nm. The excitation light with the wavelength of approximately 500 nm was mostly absorbed by phycoerythrin, but the energy absorbed by phycoerythrin was transferred to chlorophyll-a as a photosynthetic reaction center. Therefore, the intensities of the 685 and 720 nm peaks, which probably originated in chlorophyll-a, increased by using the excitation light with the wavelength of approximately 500 nm. Moreover, the integrated intensity between 530 to 800 nm was the minimum at the excitation wavelength of approximately 430 nm, and was greatly increased near the excitation wavelength of 500 nm. This result indicates that the fluorescence yield is low at the excitation wavelength of 430 nm, at which most photons are absorbed by chlorophyll-a as a photosynthetic reaction center.

Furthermore, we investigated the dependence of the fluorescence spectrum of Susabi-nori infected with the red rot disease on the excitation wavelength. Figure 5 shows fluorescence spectra of the control sample and the sample infected with the red rot disease by using the excitation lights with wavelengths of 430 and 500 nm. At the excitation wavelength of 500 nm, the F580/F685 intensity ratio distinctly increased by the effect of the red rot disease as reported in the previous paper¹⁾. On the other hand, the difference between the fluorescence spectrum of the control sample and that of the sample infected with the red rot disease was not clear at the excitation wavelength of 430 nm, because the intensities of the 580 nm peak were very weak. We reported that the F580/F685 intensity ratio was useful to the evaluation of diseases and stresses in Susabi-nori¹⁾. Therefore, we concluded that the optimum excitation wavelength was approximately 500 nm for diagnosis of diseases and stresses of Susabi-nori.

4. Conclusions

We investigated dependence of fluorescence spectra of Susabi-nori (*Porphyra yezoensis*) on the excitation wavelength by comparing with absorption spectra of photosynthetic pigments. It was found that the intensity ratio of the 580 nm peak to the 685 nm peak was maximal at the excitation wavelength of approximately 500 nm. Furthermore, we investigated the dependence of the fluorescence spectrum of Susabi-nori infected with the red rot disease on the excitation wavelength, and it was found that the intensity ratio of the 580 nm peak to the 685 nm peak distinctly increased by the effect of the red rot disease at the excitation wavelength of 500 nm. Therefore, we concluded that the optimum excitation wavelength was approximately 500 nm for diagnosis of diseases and stresses of Susabi-nori.

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