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Photoabsorption Study of Pigments in Mosses: *Scopelophila ligulata* Has an Abnormally High Formation Rate of Pheophytin

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To understand the characteristics of a Fe-accumulating moss *Scopelophila ligulata*, we compared the photoabsorption spectra of pigments from this and several other mosses and found that some of the absorption bands observed only for *S. ligulata* can be assigned to pheophytin *a*. The conversion rate from chlorophyll to pheophytin in *S. ligulata* was calculated to be 25%. This striking characteristic of *S. ligulata* could be explained by considering its habitat conditions.

Pigments are essential for all green plants. Their activities and stresses can be evaluated and assessed by absorption and fluorescence spectroscopy of their pigments.[1] Hence, the spectral properties of pigments of a green plant are fundamental for the understanding of its activity and stress. For mosses, however, spectral data of their pigments are still lacking. Moreover, there are no studies on pigments in metal-accumulating mosses except our study.[2] The colors of these mosses are expected to reflect metal accumulated in them. *Scopelophila ligulata*, which is known as a Fe-accumulating moss, lives on the metal ion-rich surface of rock walls under low pH conditions; its color is yellowish-green above and blackish below.[3] However, the constitution and concentrations of pigments of this moss are unknown; the effect of Fe on color of *S. ligulata* remains unclear. This is a similar situation to *S. cataractae*, which is known as a Cu-accumulating moss.

In this study, we measured photoabsorption spectra of pigments extracted from *S. ligulata*, *S. cataractae*, and other common mosses and found a significant difference between the spectra of *S. ligulata* and the other mosses. Some of the absorption bands were observed only for *S. ligulata* and can be assigned to pheophytin (Phe) *a*, which is formed by replacing the central Mg^{2+} in chlorophyll (Chl) *a* with two hydrogen ions.[4] The conversion rate from Chl to Phe in *S. ligulata* was calculated to be 25%, which is three times the average rate in the other common mosses. Furthermore, this rate is more than 25 times that of higher plants.[5,6] Such an abnormally high rate in *S. ligulata* could be explained by considering its habitat conditions.

The moss samples used in this study were collected from their natural habitats in Japan: *S. ligulata* in Chichibu, Saitama; *S. cataractae* in Nikko, Tochigi, in Taito-ku, Tokyo, and in Kawasaki, Kanagawa; six other common mosses in each place (*Polytrichum commune* in Tsubame, Niigata; *Didymodon constrictus* in Taito-ku, Tokyo; *Pogonatum inflexum* in Kamakura, Kanagawa; *Brachythecium buchananii* in Yokohama, Kanagawa; *Atrichum undulatum* in Yokohama, Kanagawa; *Myuroclada maximoviczii* in Chichibu, Saitama). All the samples were dried in paper envelopes under normal laboratory conditions for about two weeks.

The pigments of the dried moss samples were extracted as follows. A dried sample cut at about 5 mm from the tip of the body was homogenized using a mortar and pestle in buffered aqueous acetone, which is 80% aqueous acetone containing 2.5 mM sodium phosphate buffer of pH 7.8 to minimize conversion of Chl to Phe during the extraction.[7] Pigments were extracted from the homogenized sample in buffered aqueous acetone. The extract was centrifuged at 3000 rpm for 5 min, and then its absorption spectrum was measured using a spectrophotometer (Jasco V-550). From the spectral data, the Chl concentrations and the conversion rate from Chl to Phe ((Phe *a* and *b*)/[(Chl *a* and *b*) + (Phe *a* and *b*)]) were calculated using Porra's and Dietrich's equations.[7,8] Reflectance spectra of the dried samples were also measured using the spectrophotometer with an integrating sphere attachment.

Figure 1 shows the photoabsorption spectra of pigments extracted from the dried moss samples. We found a significant difference between the spectra of *S. ligulata* and the other mosses. In particular, the band around 410 nm for *S. ligulata* is

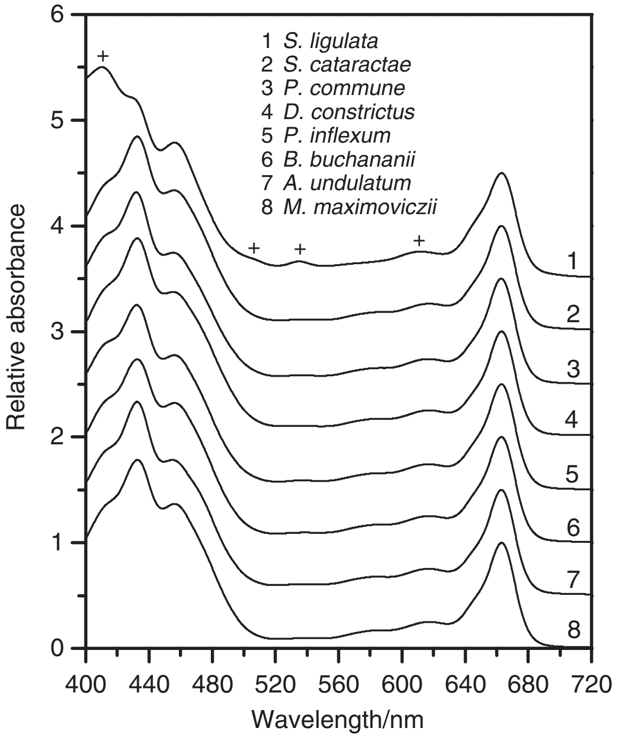

Figure 1. Photoabsorption spectra of pigments from the dried moss samples. The spectra are normalized to unity at 663 nm and arranged at regular intervals of 0.5. Plus marks at 410, 505, 535, and 610 nm represent the characteristic bands of *S. ligulata*.

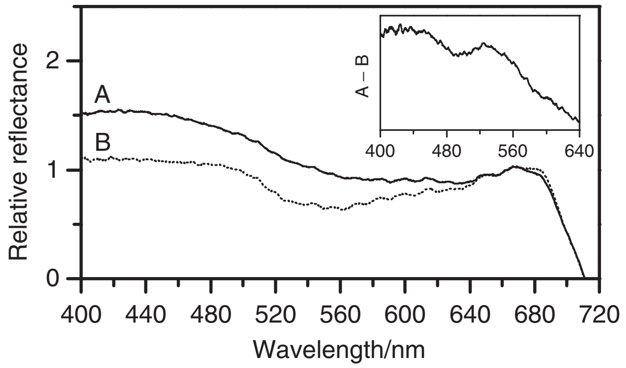

Figure 2. Reflectance spectra of the dried samples of *S. ligulata* (A) and *M. maximoviczii* (B). The spectra of (100 minus reflectance [%]) are normalized to unity at 663 nm. The inset represents the difference spectrum (A − B) between the two spectra.

Table 1. The conversion rates from chlorophyll to pheophytin, the concentrations of chlorophyll *a*, and the chlorophyll *a*/*b* ratios for *S. ligulata*, *S. cataractae*, and other six mosses[a]

Sample	Conv. rate /%	Chl *a* /mg g^{-1}	Chl *a*/*b* ratio
S. ligulata	24.6 ± 12.9	3.5 ± 0.7	2.0 ± 0.4
S. cataractae	5.8 ± 2.1	6.5 ± 0.7	2.2 ± 0.1
Six mosses	7.7 ± 1.7	4.8 ± 1.1	2.4 ± 0.2

[a] Values are given as average ± standard deviation ($n = 3$ for *S. ligulata* and *S. cataractae*, $n = 6$ for other six mosses).

the strongest in its spectrum, whereas the band around 430 nm is for the other mosses. Bands around 410, 505, 535, and 610 nm were observed only for *S. ligulata*. On the other hand, the bands which are common to all the moss samples can be assigned to Chl *a* and *b*.[9] To be specific, the bands around 430 and 663 nm can be assigned to Chl *a*, whereas the bands around 460 and 645 nm to Chl *b*.[9] The photoabsorption spectrum for *S. cataractae* agrees with those for the other six mosses.

Moreover, a band around 530 nm was observed for the dried sample of *S. ligulata* by reflectance spectroscopy. Figure 2 shows the reflectance spectra of *S. ligulata* and *M. maximoviczii*; the inset represents the difference spectrum between the two spectra. The absorption band around 530 nm was observed even in the difference reflectance spectrum.

The characteristic bands of *S. ligulata* can be assigned to Phe *a*,[10] which means that *S. ligulata* has a much higher formation rate of Phe *a* than the other mosses have. In fact, the conversion rate from Chl to Phe in *S. ligulata* was calculated to be 25%, which is three times the average rate in the other common mosses (Table 1). Furthermore, it is noted that the conversion rate in *S. ligulata* is more than 25 times that of higher plants.[5]

This striking characteristic of *S. ligulata* could be explained by considering its habitat as follows. This moss lives on metal ion-rich surfaces of rock walls under low pH conditions,[3] and consequently the pH in the dried sample is likely to be lower than those for the other mosses. Acidic conditions promote the displacement of the central Mg^{2+} in Chl *a* by two hydrogen ions,[4] and thus so lower pH in *S. ligulata*.

Table 2. The concentrations of Fe, Cu, and Zn in the dried moss samples of *S. ligulata* and *S. cataractae*

Sample	Metal concentrations/wt %		
	Fe	Cu	Zn
S. ligulata	1.25	0.01	0.00
S. cataractae	0.85	2.06	0.05

The Chl concentrations in the dried moss samples and the Chl *a*/*b* ratios were calculated from the spectral data (Table 1). All the ratios agree with that (2.4 ± 0.5) for thirty-nine species of mosses.[11] Moreover, the ratio for the other six mosses agrees well with the published ratio.[11] These suggest that the Chl *a*/*b* ratios in Table 1 are reasonable for mosses and that the other six mosses are suitable for reference. Table 1 shows that the Chl *a*/*b* ratio for *S. ligulata* is lower by 17% than the average ratio for the other six mosses. We found that this value is in accordance with the difference (17%) between the conversion rate from Chl to Phe in *S. ligulata* (25%) and the average conversion rate in the other six mosses (8%). It is reasonable to consider that the decrease in Chl *a* due to the conversion reducing the Chl *a*/*b* ratio.

In preliminary experiments, the concentrations of Fe, Cu, and Zn in the dried moss samples of *S. ligulata* and *S. cataractae* were determined by ICP analysis (Table 2). Before this analysis, a dried sample cut at about 10 mm from the tip of the body was ultrasonicated in ultrapure water, and the attached soil and sand were removed. The cleaned sample was dried at 358 K for 24 h, heated at 773 K for 2 h, and dissolved in concd nitric acid at high temperature under high pressure. Then the concentrations of Fe, Cu, and Zn in the acid solution were determined by ICP analysis. Table 2 indicates that the samples of *S. ligulata* and *S. cataractae* are Fe- and Cu-accumulating mosses. The relation between Phe *a* and Fe in *S. ligulata* remains unclear. The Fe concentration in *S. cataractae* is the same level as that in *S. ligulata* (Table 2), and hence the presence of high concentration of Fe is not a sufficient condition for the high conversion rate from Chl to Phe in this moss.

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