Spectral Absorption Property of Pigments in Living Leaves and Its Contribution to Photosynthesis*

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Spectral properties of the absorption of light by leaves have been reported for various plant species^{17,20)}. However, contribution of individual pigments to the absorption spectrum of leaves is still obscure, though the attempts have been described with the pigment extracts from leaves^{22,24)}.

Curves of both absorption and photosynthetic action spectra were compared for various algae^{5,10)} and leaves^{2,3,7,12,19,23,25)}. The thin thallus and cell layers of green algae represented very close correspondence between the absorption and photosynthetic rate, except for the deviation at the region of 700 nm, confirming the red drop of Emerson⁶⁾, and at about 480 nm caused by some inactive absorption⁵⁾. The leaves of higher plants, however, generally showed a considerable divergence between the absorption and the photosynthetic action especially in the blue and ultraviolet regions of the spectrum^{2,7,12,19,23)}.

On the other hand, the use of mutant strains of plants which lacking certain pigments has been attempted to analyze the contribution of pigments to photosynthetic processes^{15,18,21)} and plant growth^{11,21)}. The present study was made to find out the spectral participation of individual pigments in the absorption by leaves using a normal and some mutant strains of rice plant and the contribution of the pigments to the shape of the action and enhancement spectra for photosynthesis.

Materials and Methods

Plant materials

For determining the participation of pig-

ments in the spectral property of leaves, normal and chlorophyll mutant strains of rice (*Oryza sativa* L.) were used. The normal strain was a cv. Norin No. 8 (N 8). The mutant strains were CMV 44, a "viridis" lacking chlorophyll b; CM 213, a "xantha" lacking chlorophylls; and CM 53, an "albina" lacking chlorophylls and most of carotenoids²¹⁾. These mutant strains were derived artificially from N 8¹⁶⁾. Plants of these strains were grown in plastic pots [10×10×9 (h) cm] with paddy soil in a growth cabinet installed "power line, sun fluorescent lamps" (Toshiba, FLR-110-sun/a), under the following conditions: light intensity, about 20 klx; daylength, 16 h; air temperature, 20–30°C; respectively. These plants were subjected to the experiment in 15–19 days after planting.

For determining the photosynthetic action spectrum, rice (N 8 and CMV 44), finger millet (*Eleusine coracana* cv. Yukijirushi-kei), soybean (*Glycine max* cv. Norin No. 2) and cucumber (*Cucumis sativus* cv. Kaga-higan-fushinari) were used. They were grown in a natural light-growth chamber at 20–30°C and subjected to the measurement in 3–10 weeks after planting.

Determination of individual pigments in living leaf

Spectral characters of living leaves were measured with a spectrophotometer, MPS-50L (Shimadzu). The reflectance (R), transmittance (T) and absorbance (log 1/T) spectra were obtained in the wavelengths 340–800 nm for each sample leaf. A smooth surface of calcium carbonate powder was used as the reference of the reflectance. The transmittance and absorbance of the leaf were directly recorded against the reference without sample. For all the measurements, monochromatic lights were

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irradiated to the adaxial surface of a leaf. Absorbance (A) was calculated by means of an equation, $A=1-(R+T)$ for each wavelength.

Spectral absorption of individual pigments in a single leaf was determined by means of difference spectrum between the leaves of different two strains. Difference absorbance was measured directly, while difference absorbance was calculated from the absorbances between the two leaves obtained separately. Individual pigments participated in the absorbance and absorbance spectra of a single leaf was analyzed by the difference between the following pairs of strains: chlorophyll a=CMV 44—CM 213; chlorophyll b=N 8—CMV 44; carotenoids=CM 213—CM 53; and materials other than pigments=CM 53—air.

Analysis of chlorophyll content

Leaf sections with 2.5 cm² in the total area were homogenized with a small amount of 80% aqueous acetone (v/v) in a mortar. Acetone extract of chlorophylls was obtained by filtration from the homogenate and made up to 10 ml with the 80% acetone. Chlorophylls a and b in the acetone solution were determined by spectrophotometry according to Mackinny-Arnon method¹¹.

Measurement of photosynthetic action and enhancement spectra

Photosynthetic action spectrum was measured with the attached leaves using the method similar to that described in the separate paper¹². Data on the enhancement spectrum for photosystem II were quoted from the previous work¹³.

Results

Participation of pigments in the absorbance spectrum of leaf

Spectral absorbance due to each pigment of chlorophylls a and b and carotenoids in a single leaf was obtained from the difference absorbance using the pairs of different two leaves (Fig. 1). For the measurement of chlorophyll b, N 8(2) leaves having a chlorophyll a content (1.39 mg dm⁻²) approaching that of CMV 44 (1.09 mg dm⁻²) were provided by the application of fertilizers without nitrogen. The absorbance curve

due to chlorophyll a has two distinct peaks at 437 and 678 nm, while that of chlorophyll b at 473 and 649 nm. A shoulder found at about 490 nm on the curve of chlorophyll a can not be attributable to chlorophyll a itself, because no peak or shoulder has been found in the corresponding region in the pure chlorophyll a solutions⁹. The ratio of chlorophyll a/b at the maximal absorbance was estimated at about 3.5 either in the red or in the blue region. The absorbance curve due to carotenoids has two broad peaks at about 455 and 483 nm. Participation of the individual pigments in the absorbance spectrum of a single leaf can be illustrated as Fig. 2.

Participation of pigments in the absorbance spectrum of leaf

Absorbance spectra of the normal and mutant rice leaves are indicated in Fig. 3. The leaf samples are identical with those used for the absorbance measurement. Fig. 4 represents the absorbance spectra due to the individual pigments in a single leaf obtained from the curves shown in Fig. 3. The absorbance curve of chlorophyll a has a broad maximum at 680 nm and the second peak at 630 nm, while that due to chlorophyll b has a distinct peak at 645 nm. The mean absorbance of chlorophyll b in the spectrum was as low as 1/10 of that of chlorophyll a. No absorbance peak was found in the blue in either curve of chlorophyll a or b, unlike those observed in the absorbance spectra (Fig. 1). The participation of carotenoids in the leaf absorbance was comparable to the sum of chlorophylls a and b as far as in the blue region.

The spectral participation of the individual pigments in the absorbance spectrum of a single leaf can be illustrated as Fig. 5. The absorbance of a single leaf in the green is known to be variable with the leaf characteristics, especially with the chlorophyll content per unit leaf area^{3,7}. The uppermost curve in the Fig. 5 is identical with the total absorbance by a leaf of N 8(2), which has a chlorophyll a+b content, 1.84 mg dm⁻², and an absorbance at 555 nm, 55.0%. Most leaves of plants have

Fig. 1. Absorbance spectra due to chlorophylls a, b and carotenoids in a single leaf obtained from the difference absorbance between the indicated two mutant strains of rice. The absorbance was adjusted to zero at 800nm.

Fig. 2. Participation of individual pigments in the absorbance spectrum of a single leaf. The uppermost curve corresponds to the total absorbance of a N 8(2) leaf.

greater contents of chlorophyll^{3,7)} and higher absorbances in the green^{3,7,12,25)}. So, the absolute value of the absorbance due to the each pigment may be variable in some extent.

Contribution of individual pigments to photosynthetic action spectrum

According to the general definition, an action spectrum gives the reciprocal of the

number of quanta needed to bring about an effect of a certain size, as a function of wavelength of light. An action spectrum is often compared with the absorbance spectrum to see the relative or absolute activity for absorbed quanta as a function of wavelength. So, the interpretation of the shape of photosynthetic action spectrum for unit incident quanta was attempted using the absorbance spectra due to the individual pigments in a single leaf. In the normal plant (Fig. 6, A), the sum of the relative absorbance curve of chlorophyll a+b+carotenoids $\times 0.5$ (curve 2) coincides well with the relative action spectra for rice (curve 4) and other species (curve 5) in the wavelengths 450–680 nm, whereas the action is higher in the blue end and lower at around 700 nm than the absorbance.

The absorbance by chlorophyll a+b indicated here was obtained from the difference between N 8(1), which has a chlorophyll a+b content (2.42 mg dm^{-2}) and absorbance at 555 nm (61.2%) higher than those of N 8(2), and CM 213, but that by carotenoids was identical with that shown in Fig. 4. For the comparison of curves between the absorbance and the action, the leaves used for the curve 5 were selected so that the chlorophyll content and the absorbance at 555 nm are comparable to those of the leaves used for the curves 1–3. However, the correspondence between the curves of absorbance and action did not vary so much even in the action (curve 4) for the leaves having more chlorophyll and higher absorbance at 555 nm. Thus, the curves of photosynthetic action spectra for the normal leaves, which have the pigment system of chlorophyll a+b+carotenoids, can be interpreted by the all of the absorption by chlorophylls and a half of the absorption by carotenoids.

In the chlorophyll b-deficient plant (Fig. 6, B), the action spectra (curves 4 and 5) in the wavelengths longer than 480 nm run closely together with the absorbance spectrum (curve 1) due to chlorophyll a. Divergence between the curves at around 700 nm is smaller than that found in the normal

Fig. 3. Absorbance spectra of normal and mutant rice leaves. Contents (mg dm^{-2}) of chlorophylls a and b of the leaves were as follows: N 8(1), 1.82 and 0.60; N 8(2), 1.39 and 0.45; and CMV 44, 1.09 and 0.02; respectively. The curve of CM 53 was revised for calculating the difference absorbance between CM 213 and CM 53.

Fig. 4. Absorbance spectra due to chlorophylls a and b and carotenoids in a single leaf obtained from the difference absorbance between the indicated two mutant strains of rice.

Fig. 5. Participation of individual pigments in the absorbance spectrum of a single leaf. The uppermost curve corresponds to that of N 8(2) in Fig. 3.

plant (Fig. 6, A). A high photosynthetic action in the blue and near ultraviolet (<480 nm), however, can not be explained by either absorption of chlorophyll a or

carotenoids.

Contribution of individual pigments to photosynthetic enhancement spectrum

The enhancement spectrum for photosystem II, which obtained from the photosynthetic rate by superimposing a 700-nm signal beam on a background beam of a number of different shorter wavelengths¹³⁾, was compared with the absorbance spectrum due to different pigments in a single leaf (Fig. 7). Curves of the enhancement spectra for the normal plant coincide well with the sum of the absorbance of chlorophyll b + carotenoids $\times 0.3$ in the wavelengths 400–700 nm, despite of a considerable difference in both the chlorophyll content and the leaf absorbance at 555 nm between the leaves (Fig. 7, A). The enhancement in the wavelengths 520–700 nm seems to be attributable to the absorption by chlorophyll b, whereas that in the shorter wavelengths is mainly due to the absorption by carotenoids.

In the leaves of chlorophyll b-deficient plants, the absorbance spectrum due to carotenoids $\times 0.3$ roughly corresponds to the enhancement in the blue, but the lower enhancement in the wavelengths 500–700 nm can not be interpreted by the absorption of carotenoids. These findings suggest that photosynthetic enhancement for photosystem II can be attributed mostly to a large part of the absorption by chlorophyll b and a small part of the absorption by carotenoids.

Discussion

For determining the absorption spectrum due to a certain pigment by means of difference spectrum between two definite leaves, all of the optical properties other than the pigment must be identical between them. In this study, however, no particular procedure was made to unify such optical properties of the leaves, except the treatment made to reduce chlorophyll content for the normal leaves, N 8(2). Among the normal and mutant rice leaves used for the measurement, deviation of the absorbance and absorbance values at 800 nm was 0.36–0.39 and 6–10%, respectively,

Fig. 6. Comparison between the absorbance spectra due to certain pigments in a single leaf and the photosynthetic action spectra for unit incident quanta. All these curves are shown as the relative values normalized to 1.0 for the red peak.

A: 1, Absorbance by chlorophyll a+b obtained from N 8(1); 2, absorbance by chlorophyll a+b+carotenoids $\times 0.5$; 3, absorbance by chlorophyll a+b+carotenoids; 4, action for normal rice; 5, average action for finger millet, soybean and cucumber. Chlorophyll a+b content (mg dm^{-2}) and absorbance (%) at 555 nm of the leaves were as follows: 1—3, 2.42 and 61.2; 4, 3.12 and 70.1; 5, 2.30 and 60.1 in average; respectively.

B: 1, Absorbance by chlorophyll a obtained from N 8(2); 2, absorbance by chlorophyll a+carotenoids $\times 0.5$; 3, absorbance by chlorophyll a+carotenoids; 4 and 5, action for chlorophyll b-deficient rice (CMV 44). Chlorophyll a content (mg dm^{-2}) and absorbance (%) at 555 nm of the leaves were as follows: 1—3, 1.11 and 46.0; 4, 1.40 and 49.8; 5, unmeasured and 53.7; respectively.

and both of the absorbance and the absorbance of the mutant, CM 53 lacking almost all of pigments were nearly constant in the wavelengths 500—800 nm. These data suggest that the absorption properties other than pigments were not so much different among those leaves.

Fig. 7. Comparison between the absorbance spectra due to certain pigments in a single leaf and the photosynthetic enhancement spectra for photosystem II.

A: 1, absorbance by chlorophyll b obtained from N 8(2); 2, absorbance by chlorophyll b+carotenoids $\times 0.3$; 3, enhancement for normal rice¹³⁾; 4, average enhancement for 9 plant species¹³⁾. The curves of 2—4 are shown as the relative values normalized to 1.0 for the blue peak. Chlorophyll a+b content (mg dm^{-2}) and absorbance (%) at 555 nm of the leaves were as follows: 1 and 2, 1.84 and 55.0; 3, 3.12 and 70.2; 4, 2.81 and 64.3 in average; respectively.

B: 1, absorbance by carotenoids $\times 0.3$ indicated with the same scale as that (curve 2 minus curve 1) in A; 2, enhancement for chlorophyll b-deficient rice (CMV 44) indicated as the relative value to those of the normal plant (curves 3 and 4) in A. The leaves used for curve 2 were the same as those for curve 4 in Fig. 6, B.

The curves of absorbance and absorbance spectra due to chlorophyll a obtained from the difference between CMV 44 and CM 213 leaves may be reliable in the wavelengths longer than 550 nm, but may involve some error in the shorter wavelengths because it has been not ascertained whether the carotenoid content per unit leaf area was the same or not between the two mutant leaves. To obtain the accurate absorption

curves due to chlorophyll b from the difference between the normal and CMV 44 leaves, the latter must be chlorophyll b-free and either content of chlorophyll a or carotenoids should be equal in the leaf area basis between the two strains.

It was confirmed that CMV 44 strain entirely lacks chlorophyll b by a thin-layer chromatography¹⁴⁾, though a trace of it was observed spectrophotometrically (Fig. 3). As for carotenoids, it has been reported that the content ($\mu\text{g}/\text{mg}$ fresh leaves) ratios of carotenoids to chlorophyll a in the normal and the chlorophyll b-deficient mutant, M-5 rice are 0.19 and 0.21, respectively¹⁵⁾, and that the relative carotenoid content of CMV 44 to the normal (N 8) leaves is 1.16 in leaf area basis, which was obtained from the relative content per fresh weight (1.45) and the relative leaf thickness (0.8)²¹⁾. From these data and chlorophyll a contents shown in Fig. 3, the difference of carotenoid content per unit leaf area between N 8(2) and CMV 44 strains might be kept within $\pm 15\%$. Thus, the absorbance and absorptance spectra due to chlorophyll b obtained here seems to be practically acceptable.

The absorption curves due to carotenoids obtained from the difference between CM 213 and CM 53 leaves must depend on the carotenoid content in the former, but some underestimation will be involved owing to a very small absorption by CM 53 leaves. In conclusion, the curve shapes of the absorbance and absorptance spectra due to individual pigments obtained in this study probably be reliable for the most part, even if some modification will be involved.

The curves of absorptance spectra due to certain pigments in a single leaf were used to interpret the shapes of photosynthetic action and enhancement spectra. The action spectrum in the normal plant (chlorophyll a+b+carotenoid system) could be interpreted by all of the absorption of chlorophylls a and b and a half of carotenoid absorption, whereas in the chlorophyll b-deficient plant (chlorophyll a+carotenoid system) the absorption by carotenoids seems to have no effect on the action in the wavelength about 480—540 nm. This sug-

gests that chlorophyll b has an essential role to energy transfer from carotenoids to photosynthetic units. A great divergence in the blue end showing a much higher action compared with the absorptance by chlorophylls can not be explained well by any combination of the pigments, though it has been described that the peak at about 435 nm in the action spectrum is attributable to the absorption by the Soret maximum of the action of chlorophyll a^{2,12)}.

The curve of enhancement spectrum for photosystem II corresponded with the sum of the absorptance of chlorophyll b and about 1/3 of carotenoids. The enhancement in the wavelengths 520—700 nm can be attributable mostly to the absorption of chlorophyll b, whereas that in the shorter region must be due largely to carotenoid absorption and partially to chlorophyll b absorption. This finding supports the conclusion made in the previous paper¹³⁾.

Already it has been known that the efficiency of energy transfer from chlorophyll b to chlorophyll a probably be 1.0⁴⁾, whereas the transfer from carotenoids to chlorophyll a is 0.4—0.5⁴⁾ or 0.2—0.5⁶⁾ in green plants. Moreover, it has been found that in green algae and etiolated bean leaves, light energy absorbed by β -carotene occurs in both photosystems I and II to be transferred to chlorophyll with an efficiency approaching 1.0, but the energy absorbed by xanthophylls is not transferred to chlorophyll⁸⁾. On the other hand, the content ratio of β -carotene to xanthophylls in the normal rice leaves has been reported to be 0.47¹⁵⁾, corresponding to 0.32 in the ratio of β -carotene to total carotenoids.

According to these literature, the results of this study suggest that in the normal plant light absorbed by chlorophylls a and b contributes to photosynthesis with an equal efficiency in the wide spectral region, but light absorbed by carotenoids with an efficiency of 0.3—0.5 as compared with that of chlorophylls. From these, it is also demonstrated that the absorptance spectra due to individual pigments obtained by the present method can be reliable in the most part of the spectrum.

Summary

Absorbance ($\log 1/T$) and absorptance $[1-(R+T)]$ spectra due to individual pigments in a single leaf were analyzed by means of difference spectrum using the leaves of a normal strain, Norin No. 8 and three mutant strains, i.e. CMV 44 (lacking chlorophyll b), CM 213 (lacking chlorophylls) and CM 53 (lacking chlorophylls and most of carotenoids), of rice plant (*Oryza sativa* L.).

The absorbance spectra due to individual pigments were characterized by the following maxima: chlorophyll a, 437 and 678 nm; chlorophyll b, 473 and 649 nm; carotenoids, 455 and 483 nm; respectively. The absorbance at the peaks of chlorophyll b and carotenoids was about 1/3.5 of that of chlorophyll a. The absorptance spectra due to chlorophylls a and b had the maxima at 680 and 645 nm, respectively, but no clear peak in the blue region. The mean absorptance due to chlorophyll b was as low as 1/10 of that due to chlorophyll a. Absorptance due to carotenoids was roughly comparable to that due to chlorophyll a+b in the blue.

In the normal plant (chlorophyll a+b+carotenoid system), the shape of photosynthetic action spectrum could be interpreted by the sum of the absorptance of chlorophyll a+b+carotenoids $\times 0.5$, while the enhancement spectrum for photosystem II by the sum of the absorptance of chlorophyll b+carotenoids $\times 0.3$, for the most part of the spectrum. In the chlorophyll b-deficient plant (chlorophyll a+carotenoid system), the action spectrum corresponded well with the absorptance spectrum of chlorophyll a exclusively except for the blue end of the spectrum, while the enhancement spectrum depends mostly on the absorptance of carotenoids $\times 0.3$.

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〔和 文 摘 要〕

生葉における色素の分光吸収特性とその光合成への寄与

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生葉中の個々の色素に基づく吸光度 ($\log 1/T$) および吸収率 $[1-(R+T)]$ スペクトルを、イネの正常品種、農林8号 (N 8) および人為突然変異系統を用いて差スペクトル法で解析した。ここで、 T は透過率、 R は反射率を示す。供試した突然変異系統は、N 8 に起源をもつ CMV 44 (クロロフィル b を欠く)、CM 213 (クロロフィル全部を欠く) および CM 53 (クロロフィル全部とカロチノイドの大部分を欠く) の3系統で、次の組合せを用いて差スペクトルを測定し、各色素に基づく吸収特性とした：クロロフィル a = CMV 44 - CM 213；クロロフィル b = N 8 - CMV 44；カロチノイド = CM 213 - CM 53；および色素以外の物質 = CM 53 - 空気。

各色素に基づく吸光度スペクトルの極大値は、クロロフィル a が 437 と 678 nm；クロロフィル b が 473 と 649 nm；カロチノイドが 455 と 483 nm の各波長にみられた(第1図)。クロロフィル b とカロチノイドの吸光度極大値はクロロフィル a の極大値の約 $1/3.5$ であった。単一葉層の吸光度スペクトルに対する各色素の関与の仕方は第2図に示すようである。一方、各系統の葉の吸収率スペクトル(第3図)の差から求めた各色素に基づく吸収率スペクトルの結果では、クロロフィル a は 680 nm に、クロロフィル b は 645 nm にそれぞれ極大値を示したが、両クロロフィルとも青色域に明らかな極大値を示さなかった(第4図)。測定波長域の平均吸収率を比較すると、クロロフィル b はクロロフィル a の約 $1/10$ で、吸光度におけるピークの比率より著しく低かった。カロチノイドの吸収率はクロロフィル $a+b$ の青色部における吸収率にはほぼ匹敵していた。単一葉層の吸収率スペクトルに対する各色素の関与程度を示すと第5図のようになる。

光合成の作用スペクトルおよび enhancement (増進) スペクトルの形を単一葉層中の各色素に基づく吸収率スペクトルと比較した。その結果、正常植物(クロロフィル $a+b$ + カロチノイド系)では、作用スペクトル曲線はクロロフィル $a+b$ + カロチノイド $\times 0.5$ の合計吸収率スペクトルによって(第6図A)、また、光反応系IIの増進スペクトルはクロロフィル b + カロチノイド $\times 0.3$ の合計吸収率スペクトルによって(第7図A)ほぼ説明することができた。クロロフィル b を欠く植物(クロロフィル a + カロチノイド系)では、作用スペクトルは青色域末端を除くともっぱらクロロフィル a のみの吸収率スペクトルと一致し(第6図B)、増進スペクトルは主にカロチノイドの吸収率 $\times 0.3$ と対応した(第7図B)。これらの結果から、クロロフィル a と b によって吸収された光は、広い波長域にわたって等しい効率で光合成に寄与しているが、カロチノイドによって吸収された光の光合成に対する効率はクロロフィルに比べて $0.3-0.5$ であることが示唆された。