Plant Physiol. (1973) 52, 257–262

Photochemical Properties of Mesophyll and Bundle Sheath Chloroplasts of Maize[1]

Received for publication January 22, 1973

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ABSTRACT

Several photochemical and spectral properties of maize (*Zea* mays) bundle sheath and mesophyll chloroplasts are reported that provide a better understanding of the photosynthetic apparatus of C_4 plants. The difference absorption spectrum at 298 K and the fluorescence excitation and emission spectra of chlorophyll at 298 K and 77 K provide new information on the different forms of chlorophyll *a* in bundle sheath and mesophyll chloroplasts: the former contain, relative to short wavelength chlorophyll *a* forms, more long wavelength chlorophyll *a* form (*e.g.* chlorophyll *a* 693 and chlorophyll *a* 705) and less chlorophyll *b* than the latter. The degree of polarization of chlorophyll *a* fluorescence is 6% in bundle sheath and 4% in mesophyll chloroplasts. This result is consistent with the presence of relatively high amounts of oriented long wavelength forms of chlorophyll *a* in bundle sheath compared to mesophyll chloroplasts. The relative yield of variable, with respect to constant, chorophyll *a* fluorescence in mesophyll chloroplasts is more than twice that in bundle sheath chloroplast. Furthermore, the relative yield of total chlorophyll *a* fluorescence is 40% lower in bundle sheath compared to that in mesophyll chloroplasts. This is in agreement with the presence of the higher ratio of the weakly fluorescent pigment system I to pigment system II in bundle sheath than in mesophyll chloroplast. The efficiency of energy transfer from chlorophyll *b* and carotenoids to chlorophyll *a* are calculated to be 100 and 50%, respectively, in both types of chloroplasts. Fluorescence quenching of atebrin, reflecting high energy state of chloroplasts, is 10 times higher in mesophyll chloroplasts than in bundle sheath chloroplasts during noncyclic electron flow but is equal during cyclic flow. The entire electron transport chain is shown to be present in both types of chloroplasts, as inferred from the antagonistic effect of red (650 nm) and far red (710 nm) lights on the absorbance changes at 559 nm and 553 nm, and the photoreduction of methyl viologen from H_2O. (The rate of methyl viologen photoreduction in bundle sheath chloroplasts was 40% of that of mesophyll chloroplasts.)

Plants which fix CO_2 via the C_4 dicarboxylic acid pathway usually have two distinct types of chloroplasts contained in separate cells: bundle sheath and mesophyll (16). Chloroplasts of the mesophyll cells contain grana, but those of the bundle sheath cells show various degrees of granal development, depending on the species, age, and growth conditions. Several papers have been published on the photochemical activities of the two types of chloroplasts (2, 5, 20, 24). In spite of some of the controversial reports, it is now clear that BS Chlp[2] are enriched in PS I relative to PS II: there is about 3-fold higher PS I/PS II ratio in these chloroplasts than in M Chlp.

In this study, we provide further evidence, in addition to the new information on the different forms of Chl *a*, for enrichment of BS Chlp in PS I relative to PS II by new types of measurements: the difference absorption spectrum at 298 K, the excitation spectra of Chl *a* fluorescence at 77 K and 298 K, and the degree of polarization of Chl *a* fluorescence. Furthermore, new information is presented on the relative efficiencies of energy transfer from the accessory pigments to Chl *a*. We also provide new and direct evidence, aside from enzymatic assay for system II activity, for the relative deficiency in BS Chlp of system II from the measurements of the time course of Chl *a* fluorescence, and of the fluorescence quenching of the membrane probe atebrin that reflects the energy state leading to ATP production (18). We also present new data on the photooxidation of cytochromes by 710 nm in the presence and in the absence of 650 nm light. This result confirms earlier findings (6, 24) of the intactness of the electron transport chain in BS Chlp.

MATERIALS AND METHODS

Chloroplast Preparation. Seedlings of *Zea mays* (single cross-hybrid GSC 50) were grown in a temperature-controlled greenhouse in a photoperiod (16 hr light, 8 hr darkness) and a temperature of 21 C (day) and 16 C (night) for 4 to 5 weeks. Bundle sheath chloroplasts were separated from M Chlp according to a modified method of Woo *et al.* (26). The isolation medium contained 20 mM tris-HCl buffer, pH 7.8, 0.33 M sorbitol, 1 mM $MgCl_2$, and 6 mg of Carbowax 4000 per ml of reaction mixture. This method yields chloroplast fragments. Examination of the iodine-stained preparations under the light microscope indicated less than 2% contamination of BS Chlp with M Chlp and vice versa.

Absorption Spectra. Absorption measurements were made with a Bausch and Lomb (Spectronic 505) recording spectrophotometer equipped with an integrating sphere.

Pigment Content. Total chlorophyll and the individual concentrations of Chl *a* and *b* were determined in 80% acetone ac-

[1] This work was supported by the National Science Foundation.

[2] Abbreviations: BS Chlp: bundle sheath chloroplasts; atebrin: 9-(4-diethylamino-1-methyl-butylamino)-6-chloro-2-methoxy acridine; DCPIP: 2,6-dichlorophenolindophenol; diquat: N,N'-ethylene-2,2'-dipyridilium dibromide; DPC: diphenylcarbazide; M Chlp: mesophyll chloroplasts; PMS: phenazine methosulfate; PSI: pigment system I; PS II: pigment system II.

cording to the method of Arnon (3). For the determination of carotenoids, see Daves (12).

Electron Transport. Electron transport was measured by following H_2O to methylviologen reaction at 20 C. (Excess catalase and 2% ethanol were added to remove H_2O_2 formed in the reaction.) Chloroplasts were illuminated with 4×10^5 ergs cm^{-2} sec^{-1} white light, and O_2 uptake by reduced methylviologen was recorded by a Clark electrode.

Absorbance changes at 559, 553, and 563 nm (for cytochromes) were measured by a split beam differential spectrophotometer (25). Absorbance changes at 540 nm were measured and subtracted from the above changes to correct for scattering and volume changes. The actinic light passed through interference filters with peak transmissions at 650 nm (half band width, 37 nm; light I + II) and 710 nm (half band width, 10.5 nm; light I). The intensities of the incident beams, measured with a calibrated photocell placed directly in the sample position, were 4.8×10^4 ergs cm^{-2} sec^{-1} for 650 nm and 0.15×10^4 ergs cm^{-2} sec^{-1} for 710 nm beam. The measuring beams (559, 553, 563, and 540 nm) had half band widths of 5.0 nm. The photomultiplier (Amperex 56 AVP with a S11 photocathode) was protected by two Corning filters (C.S. 4-96 and C.S. 4-94).

Fluorescence Characteristics. The emission and the excitation spectra of Chl *a* fluorescence were measured with a spectrofluorometer described earlier (23). Excitation spectra were corrected for the variations in the incident quanta at different wavelengths. The emission spectra were corrected for the spectral sensitivity of the photomultiplier (EMI 9558B) and the transmission efficiency of the analyzing monochromator. Methods for measuring fluorescence at 77 K were as described earlier (15).

The time course of fluorescence and the degree of polarization of fluorescence were measured as described by Munday and Govindjee (21) and Mar and Govindjee (19), respectively.

Quenching of Atebrin Fluorescence. The light-induced quenching of atebrin fluorescence (18) was measured at 505 nm (half band width of analyzing monochromator slit, 6.6 nm; plus C.S. 4-72) and excited at 420 nm (half band width of exciting monochromator slit, 13.2 nm; plus C.S. 5-60). The high intensity actinic beam (white light) was filtered through a red corning filter (C.S. 2-62).

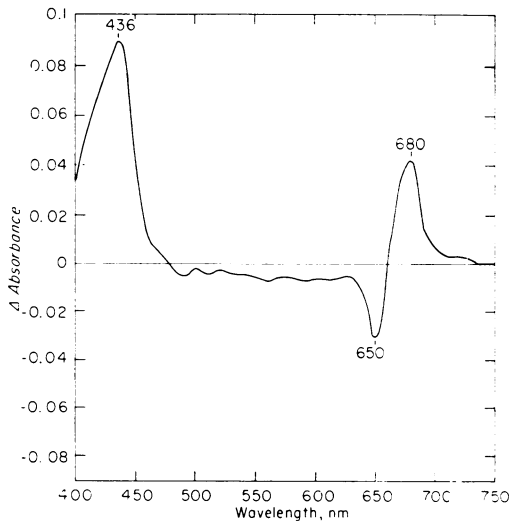

FIG. 1. Room temperature difference absorption spectra of bundle sheath minus mesophyll chloroplast fragments.

Table I. *Absorbance Decrease Induced by 710 nm Light in the Absence and in the Presence of 650 nm Light in Bundle Sheath and Mesophyll Chloroplasts*

The reaction mixture contained 60 μg of Chl suspended in 0.02 M Tricine, pH 7.8, 0.4 M sorbitol, 0.01 M NaCl, 0.001 M $MgCl_2$, 2% bovine serum albumin, 0.5 mM DPC, and 0.1 mM methylviologen.

Wavelength	A		B		B-A	
	$[\Delta A(710 + 650) - \Delta A(650)] \times 10^4$		ΔA 710 $\times 10^4$		Bundle sheath chloroplasts	Mesophyll chloroplasts
	Bundle sheath	Mesophyll	Bundle sheath	Mesophyll		
nm						
553–540	3.4	6.1	6.3	12.0	+2.9	+5.9
559–540	0.2	5.2	7.0	13.9	+6.8	+8.7
563–540	3.4	5.2	2.7	13.3	−0.7	+8.1

RESULTS AND DISCUSSION

Pigment Composition and *in vivo* Absorption Spectra. To characterize our system, we first measured the pigment ratios. The ratio of Chl *a*/*b* was 5.0 in BS Chlp compared to 3.0 for M Chlp in agreement with the published ratios (20). The total carotenoid(s) content was almost equal (0.16 mg/mg Chl) in both BS and M Chlp.

The absorption difference spectrum (Fig. 1) of bundle sheath minus mesophyll chloroplasts, having the same absorbance at 660 nm, shows positive absorption between 660 and 730 nm with a peak at 680 nm (Chl *a*); a negative absorption between 625 and 660 nm with a peak at 650 nm (Chl *b*). In the blue-green region, the positive absorption between 400 and 480 nm, with a peak at 436 nm, is attributed mainly to Chl *a* absorption. This large band must have masked the negative Chl *b* band which is seen only as a shoulder in the 450 to 500 nm region.

The positive band between 660 to 730 nm in the difference absorption spectrum has a half band width broader than for one form of Chl *a* and is asymmetric, suggesting the presence of several additional forms of Chl *a* (see ref. 14 for *S. sudanense*) in BS Chlp compared to M Chlp of *Zea mays*. Other results obtained from the study of fluorescence excitation spectra, presented later, provide information on the identity of these forms.

Intactness of Electron Transport Chain. The intactness of the electron transport demonstrated for bundle sheath chloroplasts by Smillie *et al.* (24), was confirmed in our laboratory: by (*a*) the use of methyl viologen as electron acceptor, and (*b*) the presence of antagonistic effect of light I and II on cytochrome changes.

The rate of methyl viologen reduction, measured as microequivalent of O_2 consumed/mg Chl·hr was 140 and 56 for M Chlp and BS Chlp, respectively. In contrast, the rate of electron flow in system I reaction (DCPIPH_2 to methyl viologen) was 241 and 778 microequivalents O_2 consumed/mg Chl·hr for M Chlp and BS Chlp, respectively.

In Table I, data are presented on absorbance changes induced by 710 nm light in the presence (column A) and in the absence (column B) of 650 nm light. To obtain Cyt changes free of light-induced scattering and volume changes, absorbance changes at 540 nm induced by 710 nm light or 710 + 650 nm lights were subtracted from total changes measured at 553, 559, and 563 nm separately. The absorbance changes thus measured (553–540 nm, 559–540 nm, and 563–540 nm) are

attributed to Cyt *f* and *b*, since difference spectra in the blue and the green regions of the spectrum in BS and M Chlps showed changes typical of these cytochromes (data not shown). Such spectra had positive bands around 405 nm (Cyt *f*) and 445 nm (Cyt b_6), a negative band with peaks around 422 nm (Cyt *f*), 428 nm (Cyt b_3), and 430 nm (P700), and a second negative band with peaks around 553 and 559 to 563 nm.

The results in Table I show that in BS and M Chlp oxidation of cytochromes was induced by 710 nm light in the absence and in the presence of 650 nm light. However, except at 563 nm in BS Chlp, much less oxidation was induced by 710 nm in the presence of 650 nm light than in its absence. These results indicate that 650 nm light and 710 nm light affect Cyt changes in an antagonistic manner, implying that a good interaction between PS I and PS II exists in BS as well as in M Chlp. Antagonistic effect of PS I and PS II on Cyt b_6 oxidation was reported by Hind and Olson (17). In M Chlp, there is 2.5-fold higher oxidation at 563 nm by 710 nm light in the absence of 650 nm light than in its presence, suggesting that antagonistic effects of PS I and PS II on Cyt b_6 are present. However, in BS Chlp no significant difference was observed between the changes induced by 710 nm in the absence or in the presence of 650 nm light. It may be that our preparation of BS Chlp, during separation, lost a soluble component involved in the side chain where PS I and PS II are connected through Cyt b_6 (17). On the other hand, the electron transport chain involving Cyt b_3 (559–540 nm) and Cyt *f* (553–540 nm) is intact in both types of chloroplasts.

Chlorophyll Fluorescence Transients. Fluorescence changes with the time of illumination is another indication of the interaction of PS I and PS II (21). The time course of Chl *a* fluorescence (at 685 nm) was measured in suspensions of BS and M Chlp of *Z. mays* (Fig. 2). The two curves are normalized at 0 (or F_0, the initial level of fluorescence which is due to constant fluorescence from the bulk pigment of PS I and PS II). The yield of total fluorescence $(F_\infty - F_0)/F_0$ for M Chlp is 2.35 and 1.0 for BS Chlp. However, the quantum yield of the constant fluorescence in M Chlp is 1.3 times that of BS Chlp.

The rise in Chl *a* fluorescence intensity of isolated chloroplasts upon illumination has been attributed to the photoreduction, by PS II, of the electron acceptor Q of PS II (21). Reduced Q is reoxidized in darkness or by far red light absorbed

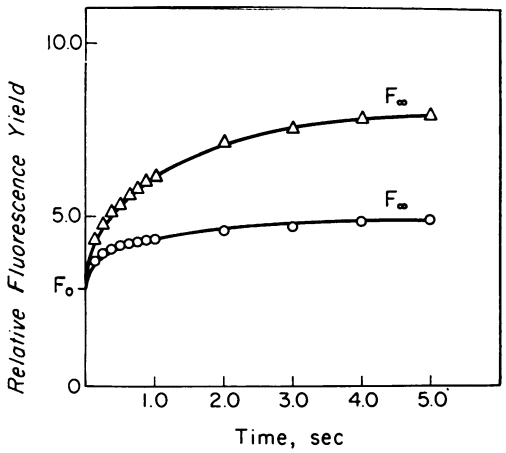

FIG. 2. Time course of Chl *a* fluorescence of mesophyll (△) and bundle sheath (○) chloroplast fragments normalized at F_0. The exciting beam (2×10^5 ergs cm^{-2} sec^{-1}) was provided by white light passed through two Corning filters (C.S. 3-75 and C.S. 4-96). A Corning C.S. 2-58 filter was placed at the entrance slit of the analyzing monochromator.

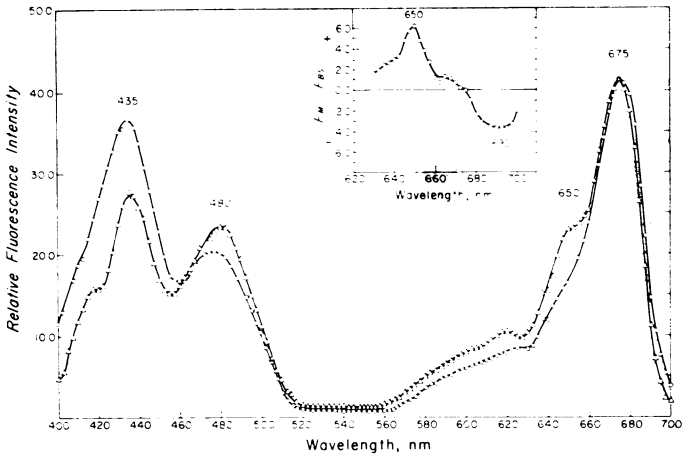

FIG. 3. Fluorescence excitation spectra of F740 of mesophyll (△) and bundle sheath (○) chloroplast fragments, normalized at 675 nm. Insert, the difference excitation spectrum (M minus BC Chlp). Chloroplasts (absorbance at 678 nm, 0.17) were suspended in 0.02 M tris-HCl buffer pH 7.8, 0.4 M sorbitol and 0.01 M NaCl. The exciting slit had a half band width of 4.95 nm and the measuring slit of 9.9 nm. A Corning filter (C.S. 7-69) was placed before the analyzing monochromator.

predominantly by PS I. The relatively lower yield of variable fluorescence in BS Chlp can be explained by the unequal distribution of light quanta between PS I and PS II which results from the presence of more chlorophyll molecules associated with PS I relative to PS II in these chloroplasts.

Excitation (or Action) Spectra of Chl *a* Fluorescence. Figure 3 shows room temperature excitation spectra of Chl *a* fluorescence at 740 nm (F740) for M and BS Chlp fragments normalized at 675 nm for Chl *a*. The ratio of fluorescence at 740 nm excited by 675 nm light (F^{740}_{675}) to that excited by 650 nm light (F^{740}_{650}) is 1.7 in M Chlp compared to 2.4 for BS Chlp. At 675 nm, Chl *a* is the main pigment contributing to F740 while at 650 nm Chl *b* contribution is dominant. The above fluorescence results are consistent with the higher ratio, in BS Chlp, of Chl *a*/*b* obtained from acetone extractions, and also from the *in vivo* measurement of the absorption spectra of these chloroplasts.

The inset in Figure 3 shows the difference excitation spectrum (M minus BS Chlp) of F740 of the two curves normalized at 675 nm. The negative band with a peak maximum at 692 nm has a half band width of 22 nm. Due to the wide half band widths, several long wavelength forms of Chl *a* contribute to F740 in BS Chlp than in M Chlp. These results again show clearly that in comparison to M Chlp, BS Chlp are enriched in Chl *a* forms absorbing at long wavelengths relative to Chl *b*. The shoulder at 665 nm suggests that besides Chl *b*, BS Chlp also contain different amounts of Chl *a* form absorbing at 660 nm.

In the blue region, the excitation peaks are at 435 and 480 nm in both chloroplasts. However, the ratio of F^{760}_{435} to F^{760}_{480} is different in the two cases; it is 1.2 in M Chlp compared to 1.6 in BS Chlp. Qualitatively, these ratios are consistent with those obtained in the red portion of the fluorescence spectrum for Chl *a*/*b*, but the numerical value of the ratios differ because of the carotenoid contribution in this portion of the spectrum. It is interesting to compare the ratio of the intensity of fluorescence at 740 nm excited by 435 and 678 nm ($F^{740}_{435}/F^{740}_{678}$) in BS Chlp and in M Chlp; it is 0.9 in the first and 0.67 in the second. The difference in these values between the two types of chloroplasts could be due to: (*a*) more carotenoids in BS Chlp; this,

however, is not the case because BS and M Chlp contained almost equal amounts of carotenoids; (*b*) an increased efficiency of energy transfer from carotenoids to Chl *a* in BS Chlp; this, however, is not the case either (see below); (*c*) the Soret bands of the long wavelength forms of Chl *a;* this interpretation is in agreement with the rest of the data presented here.

The Relative Quantum Yield of Fluorescence (F740) of Chl *a*. The relative quantum yields of Chl *a* fluorescence measured at 740 nm and excited by Chl *b* and carotenoids were calculated from the fluorescence excitation spectra of Chl *a* (F740). The fluorescence intensities of Chl *a* at 740 nm excited by light absorbed in various pigments were divided by the percentage of absorption of the samples used at the wavelength of excitation. From these values, the efficiency of energy transfer from carotenoids and Chl *b* to Chl *a* was calculated. The ratio of the relative fluorescence yield at 740 nm, which is excited by 500 nm to that excited by 675 nm, represents approximately the efficiency of energy transfer from carotenoids to Chl *a*, since at 500 nm 90% of incident, quanta is absorbed by carotenoids, while at 675 nm absorption by Chl *a* is dominant. The ratios were 0.51 for M Chlp and 0.48 for BS Chlp. Thus, the efficiency of energy transfer from carotenoids to Chl *a* is as high as 50% (it compares well with 50% in algae [13]). The ratio of these values is 1.06, which indicates that the efficiency of energy transfer from carotenoids to Chl *a* is almost the same in both types of chloroplasts. The ratios of the fluorescence yield at 740 nm, which is excited by 480 nm (Chl *b*) to that excited by 440 nm (Chl *a*), were 0.99 for BS Chlp and 1.1 for M Chlp; the ratio of these values is 1.11, which again indicates the similarity in the efficiency of energy transfer from Chl *b* to Chl *a* in both types of chloroplasts. Furthermore, the ratio $F_{650}^{740}/F_{675}^{740}$ was 1.0 in both cases; thus, the efficiency of energy transfer from Chl *b* to Chl *a* is 100% in maize chloroplasts.

Excitation Spectra of Chl *a* Fluorescence in Thick Samples. Excitation spectra of fluorescence at 760 nm of thick samples (100% absorption at 678 nm) of M Chlp and BS Chlp at 77 K were measured in order to detect the differences in the amount of Chl *a* 705 between the two types of chloroplasts in *Zea mays*. This band cannot be observed in thin suspensions because of its low concentration (9, 15). In spite of the use of thick samples, reabsorption of fluorescence, when measuring at long wavelength, is not a problem because there is no significant absorption at 760 nm. Figure 4 shows fluorescence excitation spectra at 760 nm of M and BS Chlp at 77 K. Since both samples have 100% absorption at 678 nm, equal absorption in both samples is assumed and hence, the intensity of fluorescence at 760 nm excited by 680 nm and shorter wavelengths is the same. However, one can observe the difference in the two samples at wavelengths longer than 690 nm; the difference between assumed curves for Chl *a* 678 and the experimental curves are 20 and 5 for BS Chlp and M Chlp, respectively. This clearly shows that the former have more than twice Chl *a* 705 than the latter. This result is consistent with the higher fluorescence emission at 735 nm relative to 685 nm at 77 K in BS Chlp compared to M Chlp.

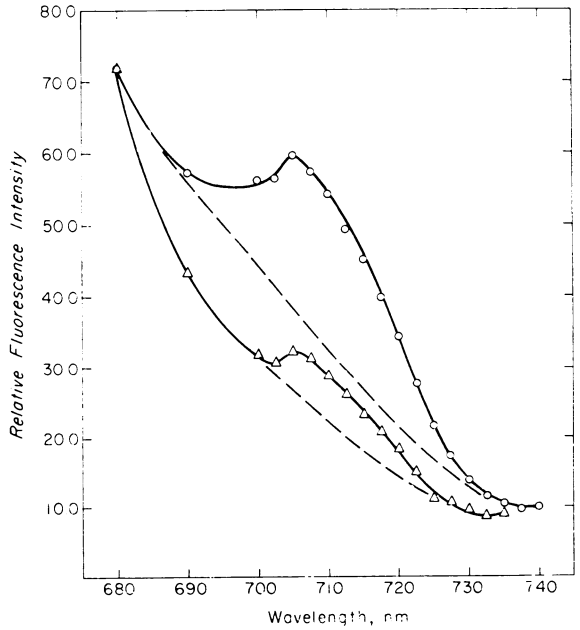

FIG. 4. Excitation spectra of F760 at 77 K of thick samples (100% absorption at 680 nm), of mesophyll (△) and bundle sheath (○) chloroplast fragments from maize. Conditions are the same as in Fig. 3.

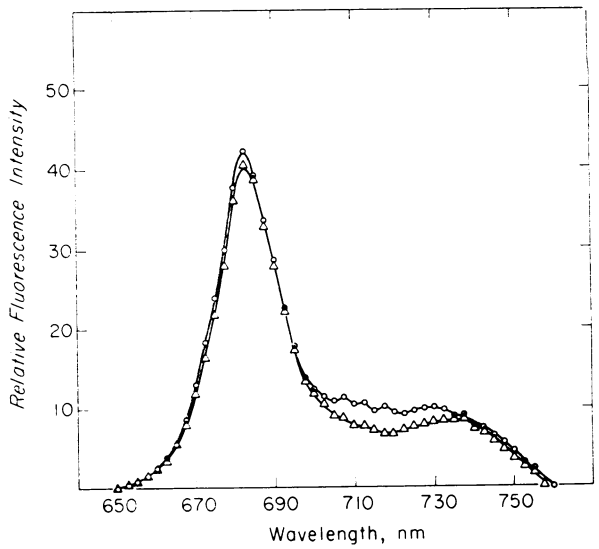

FIG. 5. Fluorescence emission spectra at 298 K of mesophyll (△) and bundle sheath (○) chloroplast fragments (from maize). Details are the same as Fig. 2. Exciting wavelength, 440 nm. The exciting slit had a half band width of 6.6 nm and the measuring slit of 3.3 nm.

Fluorescence (or Emission) Spectra at 77 K and 298 K. Fluorescence spectra of thin suspensions (absorbance at 678 nm, 0.004 for measurement at 77 K, and 0.15 for room temperature measurement) of fragments of M Chlp and BS Chlp, excited by 440 nm, were measured at 77 K (not shown, ref. 37 presents evidence for *D. singuinalis)* and 298 K (Fig. 5; normalized at 685 nm).

At 77 K, in both chloroplasts, we obtained three banded spectra, characteristic of fluorescence spectra of species with granal chloroplasts like spinach (15), with maxima at 685, 695, and 735 nm. However, the ratio of fluorescence at 735 nm to that emitted at 685 nm was different in the two types of chloroplasts; it was 1.6 in M Chlp compared to 5.5 for BS Chlp. From earlier work (7, 10), it has been concluded that the emission band at 735 nm originates mainly from PS I, while bands at 685 nm and 695 nm come mainly from PS II. Thus, BS Chlp are enriched in PS I pigments (estimated to be about 3-fold) relative to PS II pigments in comparison with M Chlp.

At 298 K, the F715/F683 ratio in BS Chlp is 0.29 compared to 0.17 for M Chlp (Fig. 5). The F740/F683 ratio is

Table II. *Light-induced Quenching of Atebrin Fluorescence in Mesophyll and Bundle Sheath Chloroplasts of Maize*

The reaction mixture consisted of 0.02 M TES buffer (pH 7.8), 0.4 M sucrose, 0.01 M NaCl, 5 μM atebrin, and chloroplasts containing 70 μg/ml of Chl. Atebrin fluorescence was excited with 420 nm monochromatic beam (half band width, 13.2 nm), and emission was measured at 505 nm. Samples containing chloroplasts were excited with actinic beam filtered through (C.S. 2-62) filter. Intensity of actinic beam was 5×10^5 ergs/cm^{-2} sec^{-1}.

Additions to Reaction Mixture	Quenching of Atebrin Fluorescence Induced by Cyclic (PMS) or Noncyclic (Diquat) Electron Transport in Chloroplasts	
	Mesophyll	Bundle sheath
	%	
4 μM Diquat	85.0	8.0–14.0
4 μM Diquat and 5 μM DCMU	0.0	0.0
5 μM DCMU + 0.2 mM ascorbate + 5 μM PMS	80.0	78.0
ATP formed, μmoles/mg Chl·hr		
Cyclic	614 (4);[1] 520 (1)	570 (4); 96 (1)
Noncylic	237 (4); 96 (1)	23 (4); 15 (1)
Proton transport extent (μmoles/mg Chl)	0.48 (4)	0.05 (4)

[1] Numbers in parentheses indicate references.

0.25 for BS Chlp compared to 0.18 for M Chlp. It is known (8, 11) that F715–F730 is mainly excited by PS I, while F740–F760 is excited by both PS I and PS II. From the above ratios, it is clear that BS Chlp are enriched in long wavelength forms of Chl *a* with respect to short wavelength forms. The slightly higher ratio of F715/F683 to F740/F683 in BS Chlp confirms that there is more PS I with respect to PS II in these chloroplasts. These ratios, however, do not differ significantly in the case of M Chlp.

Degree of Polarization of Fluorescence. Further support for higher content of long wavelength form of Chl *a* relative to short wavelength form in BS Chlp than the M Chlp comes from the polarization of fluorescence data. The degree of polarization of Chl fluorescence was 6% in BS Chlp and 4% in M Chlp. This implies that there is a higher ratio of oriented to unoriented chlorophylls in BS Chlp. Long wavelength forms of Chl *a* are known to be highly oriented.

Fluorescence Quenching of Atebrin. The quenching of fluorescence of the uncoupler atebrin in isolated chloroplasts (18) has been shown to be induced by the operation of the electron transport, ATP hydrolysis, or a pH gradient. Bundle sheath chloroplasts have been shown (4) to have a reduced ability for light-induced pH change compared to mesophyll chloroplasts. However, BS Chlp performed cyclic phosphorylation at a rate comparable to M Chlp (1, 4). Thus, BS Chlp and M Chlp are useful systems to test whether the quenching of atebrin fluorescence (induced by cyclic and noncyclic electron transport) is correlated directly with the energy state (X ~ I [18]) or with the proton gradient (22) induced upon illumination.

When diquat is added to the chloroplasts, the proton gradient across the membrane is assumed to be due to noncyclic electron transport. When DCMU, ascorbate, and PMS are added to the chloroplasts, the proton gradient is due to cyclic electron transport. Bundle sheath chloroplasts (Table II) showed 8 to 14% quenching of atebrin fluorescence compared to 85% in M Chlp when 4 μM diquat was added. This quenching is sensitive to DCMU as both types of chloroplasts showed the absence of atebrin quenching upon the addition of DCMU and diquat. When noncyclic electron flow from PS II was stopped by DCMU and ascorbate was added as the electron donor to PS I with PMS as a "cyclic" electron carrier, both chloroplasts showed similar percentage of atebrin quenching. However, one could expect the BS Chlp to show higher levels of cyclic proton gradient because they are enriched in PS I compared to PS II. The above results on "% quenching of atebrin fluorescence" agree with ATP measurements (1, 4). They disagree with the direct proton gradient measurements (4), indicating that fluorescence quenching of atebrin reflects some "energy state" that leads to ATP production (18) but not proton gradient (22).

Acknowledgment—We thank Dr. Prasanna K. Mohanty for his valuable help in our experiments with atebrin.

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