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Spectral, Physical, and Electron Transport Activities in the Photosynthetic Apparatus of Mesophyll Cells and Bundle Sheath Cells of *Digitaria sanguinalis* (L.) Scop.[1]

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ABSTRACT

Isolated mesophyll cells and bundle sheath cells of *Digitaria sanguinalis* were used to study the light-absorbing pigments and electron transport reactions of a plant which possesses the C_4-dicarboxylic acid cycle of photosynthesis. Absorption spectra and chlorophyll determinations are presented showing that mesophyll cells have a chlorophyll *a–b* ratio of about 3.0 and bundle sheath cells have a chlorophyll *a–b* ratio of about 4.5. The absorption spectrum of bundle sheath cells has a greater absorption in the 700 nm region at liquid nitrogen temperature, and there is a relatively greater amount of a pigment absorbing at 670 nm in the bundle sheath cells compared to the mesophyll cells. Fluorescence emission spectra, at liquid nitrogen temperature, of mesophyll cells have a fluorescence 730 nm–685 nm ratio of about 0.82 and bundle sheath cells have a ratio of about 2.84. The reversible light-induced absorption change in the region of P_{700} absorption is similar in both cell types but bundle sheath cells exhibit about twice as much total P_{700} change as mesophyll cells on a total chlorophyll basis. The delayed light emission of bundle sheath cells is about one-half that of mesophyll cells. Both mesophyll cells and bundle sheath cells evolve oxygen in the presence of Hill oxidants with the mesophyll cells exhibiting about twice the activity of bundle sheath cells, and both activities are inhibited by 1 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea. Ferredoxin nicotinamide adenine dinucleotide phosphate reductase is present in both cells although it is about 3- or 4-fold higher in mesophyll cells than in bundle sheath cells. Glyceraldehyde 3-P dehydrogenases, both nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, are equally distributed in the two cell types on a chlorophyll basis. Malic enzyme is localized in the bundle sheath cells.

We interpret the data as evidence for the presence of a complete chloroplast electron transport system from oxygen evolution to pyridine nucleotide reduction in both mesophyll and bundle sheath cells. However, there is a quantitative difference in the distribution of photosystem I and photosystem II components in the two photosynthetic cells with about a 3-fold higher photosystem I–II ratio in the bundle sheath cells than in the mesophyll cells. A scheme is proposed to accommodate photosynthetic CO_2 fixation and electron transport activities in the mesophyll cells via a β-carboxylation and in the bundle sheath cells via carboxylation of ribulose-1,5-diphosphate.

Recently higher plants have been divided into at least two groups on the basis of their initial products of photosynthetic carbon dioxide fixation. One group fixes carbon dioxide via the reductive pentose phosphate cycle while the other group appears to fix carbon dioxide primarily via the C_4 cycle[3] (3, 20, 29). In addition to the difference in their primary carbon fixation pathways, the two groups of plants differ in other ways such as: CO_2 compensation point, rate of photosynthesis at high light intensity, and ease of detection of photorespiration (5, 21, 23).

In plants which fix carbon dioxide via the C_4 cycle there are two major types of chloroplast containing cells (19, 22); mesophyll cells which may fix carbon via the C_4 cycle and bundle sheath cells which may fix carbon via the pentose cycle (16, 21). Recently we developed a method for separating the two cell types (14, 15), and have undertaken an investigation of the properties of each cell type. This paper is a report on some spectral and physical measurements, Hill reactions, and enzyme activities of the photosynthetic apparatus of these two distinct cell types isolated from *Digitaria sanguinalis* leaves.

MATERIALS AND METHODS

The mesophyll and bundle sheath cells of "Large Crabgrass," *Digitaria sanguinalis* (L.) Scop. were separated by the grinding and filtration method of Edwards and Black (14). Freshly harvested leaves of greenhouse or field-grown crabgrass were used. For the absorption spectra, fluorescence spectra, and P_{700} content determinations, the isolated cell preparations were sus-

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[3] Abbreviations: C_4 cycle: C_4-dicarboxylic acid cycle; diquat: 1,1′-ethylene-2,2′-dipyridylium dibromide; MOPS: morpholinopropane sulfonic acid; pentose cycle: reductive pentose phosphate cycle; PEP: phosphoenolpyruvate; RuDP: ribulose-1,5-diphosphate; F: fluorescence.

pended in the isolation medium (sorbitol, 0.33 M; sodium Tricine buffer, pH 8.0, 0.05 M; $NaNO_3$, 2 mM; EDTA, 2 mM; $MnCl_2$, 1 mM; $MgCl_2$, 5 mM and K_2HPO_4, 5 mM) or sorbitol, 0.33 M in sodium Tricine buffer, pH 7.8, 0.05 M, and passed through a French press three times at 6000 lb psi. The reason for the rather drastic treatment was the desire to prevent any fractionation of the chloroplast material at this time and to obtain a clear nonscattering suspension of all the chl in the cells. This suspension was then centrifuged for 5 min at 1,000*g* to remove the larger pieces of cell debris. The debris contained very little chl. The bundle sheath strand cells are very difficult to break due to their toughness and their tendency to settle out on the lower plug of the press. The latter problem is partially alleviated by breaking the strands with a Ten-Broeck homogenizer.

The absorption spectra were measured on a Cary 14 spectrophotometer. In the case of the low temperature absorption spectra measurement, the spectrophotometer was modified for measurement of highly scattering samples.

The fluorescence spectra were obtained with a spectrofluorometer which consisted of a Bausch and Lomb 0.5 M monochromator and an EMI photomultiplier tube (No. 9558C). A Corning filter (No. 2412) was placed between sample and monochromator to reduce the scattering light reaching the phototube. The sample, 0.17 mm thick, was irradiated from the front and the fluorescence collected at an angle from the back. The measuring light was isolated from the output of a Sylvania DWY "sungun" lamp by a Baird Atomic interference filter with a peak at 435 nm and a 1 cm thick saturated acid copper sulfate solution. The fluorescence spectra were corrected for the variation with wavelength of the transmission of the monochromator-filter combination used and the sensitivity of the photomultiplier tube. This correction curve was obtained by dividing the response of the system to the radiation of a lamp of known color temperature by the number of quanta emitted at the wavelength of interest, as outlined by Clayton (13).

Delayed light emission was measured with a modification of a Becquerel phosphoroscope described previously (13). The tungsten lamp was replaced with a flash lamp with a half peak height time of 20 μsec. The triggering circuit of the lamp was designed so that the lamp could fire only when the front window of the phosphoroscope was open. In the experiments reported here, the lamp fired every 5.4 sec. The delayed light emission was measured from approximately 0.5 msec to 4.5 msec.

The P_{700} absorbance change was measured in the same manner as reported previously with an Aminco-Chance dual beam spectrophotometer (7). Chl determinations were made by the method of Arnon (1) or Wintermans and DeMots (34). In some cases, the cells suspended in the isolation medium were stored over solid carbon dioxide prior to use in spectral studies with essentially the same results as with fresh cells.

The activity of ferredoxin $NADP^+$-reductase was measured in crude extracts by several methods since this enzyme exhibits a number of enzymatic activities (for a more detailed discussion of this enzyme see Ref. 26). Its transhydrogenase activity was measured with deamino-$NADP^+$ as described by Keister *et al.* (26) and various NADH and NADPH diaphorase activities were assayed as described by Avron and Jagendorf (2). Crude extracts were prepared by sonicating isolated mesophyll cells for 2 min and bundle sheath cells for 4 min, then each preparation was filtered through a 20 μ nylon net. The filtrates did not contain whole cells and were used as crude extracts. Cells were sonicated in 0.1 M tris buffer, pH 7.8, in an ice bath with a Bronwill Biosonik at a probe intensity of 95.

Glyceraldehyde 3-P dehydrogenase activity with NAD^+ and $NADP^+$ was assayed as described by Gibbs (18) with the addition of 4 mM dithiothreitol as a sulfhydryl reagent. Malic enzyme was assayed as described by Ochoa (32).

Oxygen metabolism with each cell type was assayed with a Clark-type oxygen electrode purchased from Chemtronics, Inc., San Antonio, Texas. Illumination intensity was about 3,000 ft-c. The light was passed through a Corning No. 3-70 filter and a solution of $CuSO_4$ (2% w/v).

RESULTS

Absorption Spectra. The room and liquid nitrogen temperature absorption spectra of extracts, prepared with the French pressure cell, from the two cell types are shown in Figures 1 and 2. A cursory examination in the red region of the room temperature absorption spectra shows an increase in absorption at the 650 nm region of the mesophyll cell extract relative to the bundle sheath cell extract. This is in agreement with the different chl *a*–*b* ratio in the two cell types and the greater ΔA at 702 nm in the bundle sheath cells (see results below).

The low temperature absorption spectra show a greater difference between the two cell types. The chl *b* peak is more prominent in the mesophyll cell extract spectrum than in the bundle sheath cell extracts. The 75% peak height band width of the bundle sheath cell extracts was approximately twice as wide as that of the mesophyll cell extract and the 700 nm absorption shoulder twice as large. The increase in the 75% peak height band width of bundle sheath cell extracts appear to be due to a relatively greater amount of a component absorbing at approximately 670 nm in the bundle sheath cells.

In the blue absorption band, the spectra differ in the wavelength of the "chl *a* peak," 438 nm in mesophyll cell extracts and 444 nm in bundle sheath cell extracts. The chl *a* blue peak also was higher in mesophyll cell extract relative to the other peaks than in the bundle sheath cell extracts. These spectra probably deserve a more extended analysis such as that of French and Prager (17).

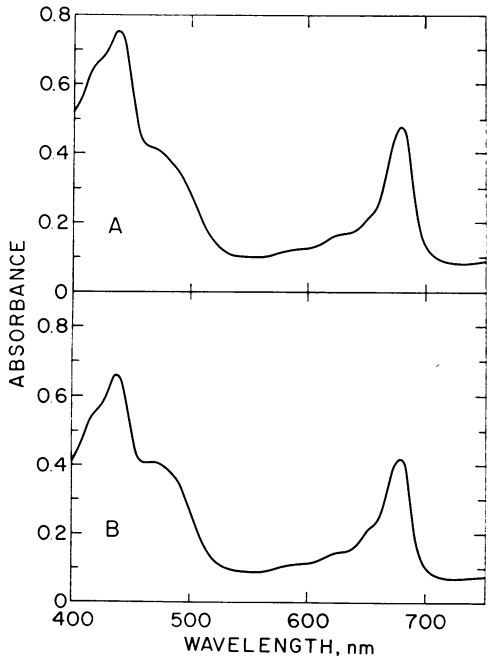

FIG. 1. Room temperature absorption spectra of cell extracts. A: Bundle sheath cell extract; B: mesophyll cell extract.

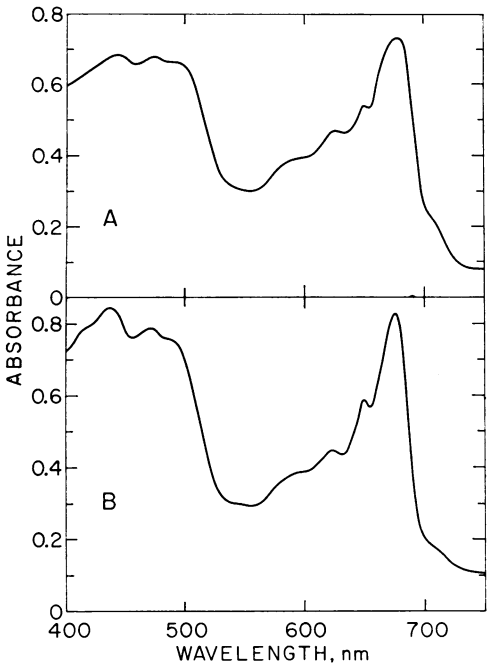

FIG. 2. Liquid nitrogen temperature absorption spectra of cell extracts. A: Bundle sheath cell extract; B: mesophyll cell extract.

Fluorescence Spectra. The room temperature fluorescence spectra of the two types are similar but the fluorescence of the mesophyll cell extract is always larger than the fluorescence of the bundle sheath cell extract. On the other hand the low temperature fluorescence (liquid nitrogen temperature) spectra of the cell extracts show a striking difference between the two cell types (Fig. 3). The bundle sheath cell extracts showed a much greater fluorescence in the long wavelength band at 730 nm than the mesophyll cells. Although there was some variation in the ratio of the peaks, in day-to-day experiments, in all cases the bundle sheath cells have a much greater F_{730}–F_{685} than the mesophyll cells, with the respective values in Figure 3 being 2.84 and 0.82. The small size of the particles and the low absorbance of the sample, approximately 0.02 at the red peak, would minimize the distortion of the fluorescence spectra due to self-absorption.

Amaranthus hybridus L. (a C_4 cycle plant) chloroplasts treated in the same manner as crabgrass cell extracts had a fluorescence spectrum at low temperature intermediate (ratio of F_{730}–F_{685} of 1.8) between that of the two cell types as would be expected for the unseparated chloroplasts from the distinct cell types of a C_4 cycle plant leaf.

P_{700} Content. It was previously reported that plants which fix carbon via the C_4 cycle had a larger light-induced P_{700} absorbance change on a total chlorophyll basis than plants which utilize the pentose cycle (7). In view of this, we were particularly interested in the measurement of the reversible 700 nm absorption change in extracts from the two cell types. Figure 4 shows that although both cells have essentially the same reversible absorption spectrum in the region of P_{700} absorption, the bundle sheath cells on a chl basis clearly have a greater ΔA at 700 nm.

Delayed Light Emission. Figure 5 shows the delayed light emission of isolated cells of crabgrass. This figure is a photograph of an oscilloscope screen. It appears from this experiment that the amount of delayed light emission is greater in mesophyll cells but that there is no difference in the kinetics of the decay.

Ratio of Chl *a*–*b*. It was previously found that C_4 cycle plants including crabgrass had a higher chl *a*–*b* ratio than pentose plants (7). The extension of these measurements to the two cell types of crabgrass shows that the difference between C_4 and pentose plants, in chl *a*–*b* ratio, is due primarily to the increased chl *a*–*b* ratio of the bundle sheath cells (Table I).

Ferredoxin $NADP^+$ Reductase. In the process of photochemically reducing $NADP^+$ with spinach chloroplasts, ferredoxin $NADP^+$ reductase is the terminal enzyme (26, 28). Current ideas would place this enzyme in photosystem I. The data in Table II show that the enzyme is in both mesophyll and bundle sheath cells. The NADPH enzyme activity by several assays appears to be 2- to 4-fold higher in the mesophyll cells than in the bundle sheath cells.

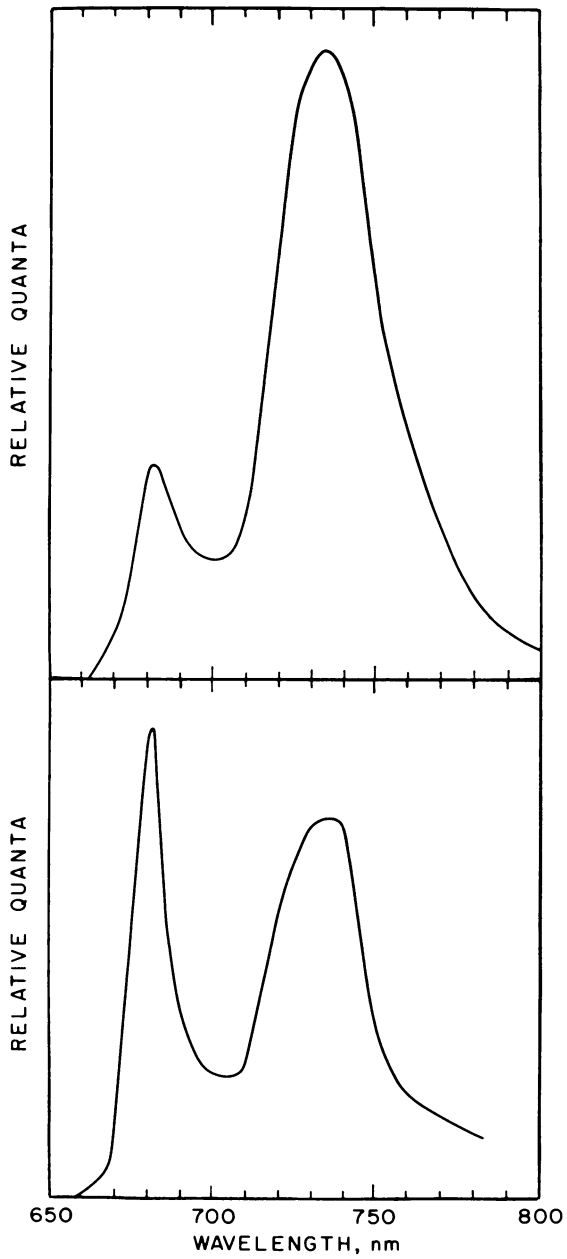

FIG. 3. Fluorescence emission spectra of cell extracts at liquid nitrogen temperature. Upper trace is an extract of bundle sheath cells. Lower trace is an extract of mesophyll cells. Both samples were adjusted to an equal absorption at 675 nm.

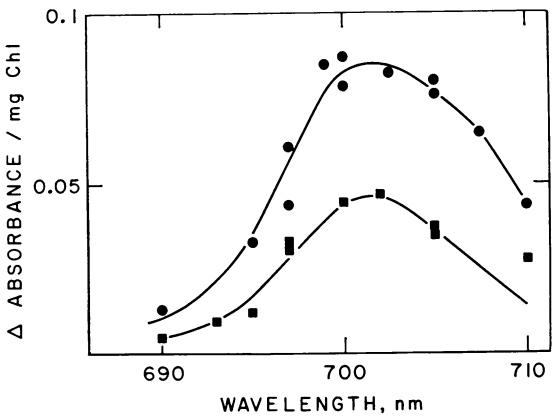

FIG. 4. Difference spectra of the light-induced reversible absorption change near 700 nm in cell extracts: ●: bundle sheath cells, 9 μg chl per ml; ■: mesophyll cells, 8.5 μg chl per ml. The reaction mixture was the isolation medium plus 14 μM pyocyanine and 50 μM DCMU.

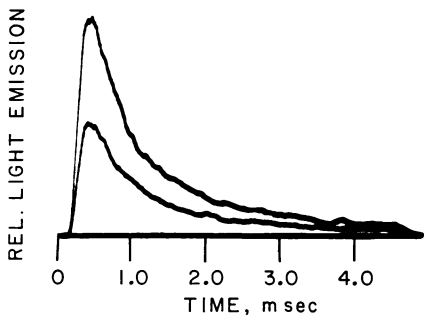

FIG. 5. Delayed light emission from mesophyll cells (upper trace) and bundle sheath cell (lower trace). The cells were suspended in isolation medium to which 0.17 g Ficoll per ml was added to prevent the cells from settling out during the experiment. Chl content was 14 μg in 2 ml for both cell types.

Table I. *Chl a-b Ratios in Isolated Mesophyll Cells and Bundle Sheath Cells of Digitaria sanguinalis*

The chl was determined by extracting each cell type for 20 min with 96% ethanol. The absorbance of the ethanol extract was determined with a Cary 14 spectrophotometer and the chl *a-b* ratio was calculated using the extinction coefficients of Wintermans and DeMots (34).

Experiment No.	Mesophyll Cells			Bundle Sheath Cells		
	649 nm	665 nm	Chl *a-b*	649 nm	665 nm	Chl *a-b*
	absorbance					
1	0.305	0.705	3.12	0.217	0.564	4.90
2	0.170	0.384	2.94	0.275	0.708	4.72
3	0.290	0.650	3.00	0.224	0.551	3.94
4	0.260	0.580	2.83	0.109	0.265	3.82
5	0.316	0.726	3.08	0.191	0.498	5.15
Average ratio of chl *a-b*			2.99			4.51

Glyceraldehyde 3-P Dehydrogenase. This very critical enzyme which catalyzes the only known carbon reduction step in photosynthesis was assayed and the results are presented in Table III. Both the $NADP^+$ and NAD^+ dependent glyceraldehyde 3-P dehydrogenase are almost equally distributed on a chl basis between the mesophyll cells and the bundle sheath cells.

Malic Enzyme. Table III also presents data on malic enzyme activity assayed in the direction of decarboxylation with $NADP^+$. Malic enzyme is localized in the bundle sheath cells.

Oxygen Evolution. Table IV presents the results of an oxygen evolution study with the isolated cell types in the presence of exogenous electron acceptors. It is assumed that benzoquinone is a typical noncyclic Hill oxidant and that diquat also is a typical Hill oxidant which catalyzes a Mehler reaction. Some variation in absolute rates of oxygen production are observed when comparing day-to-day experiments but these data are presented to demonstrate that both cells do evolve oxygen and that on a chl basis the mesophyll cells are about twice as active as bundle sheath cells in these typical noncyclic electron transport reactions. All of these reactions were sensitive to DCMU with 90 to 100% inhibition being observed at 1 μM DCMU.

DISCUSSION

Plants which utilize the C_4 cycle as a portion of their photosynthetic CO_2 fixation process also are distinguished from other plants by a high rate of net photosynthesis and a tendency for photosynthesis to reach saturation only at light intensities near full sunlight (roughly 10,000 ft-c in many areas of the world where C_4 plants are common). We previously have postulated a theoretical stoichiometry of 5 moles of ATP–2 moles of NADPH per mole of CO_2 fixed during C_4 photosynthesis (11). We theorized that cyclic photophosphorylation (hence photosystem I) may be very active or have a high level in C_4 plants. The following data on C_4 plants partially support these theories:

Table II. *Ferredoxin Pyridine Nucleotide Reductase Activities in Extracts from Leaves, Mesophyll Cells, and Bundle Sheath Cells of Digitaria sanguinalis*

Preparation	Activity Measured	Electron Donor	
		NADPH	NADH
		μmoles/mg chl·hr	
Mesophyll cells	Diaphorase (Ferricyanide)	774	208
Bundle sheath cells		224	380
Whole leaf		586	398
Mesophyll cells	Diaphorase (DCIP)	46	12
Bundle sheath cells		12	16
Whole leaf		19	16
Mesophyll cells	Menadione reductase	122	0
Bundle sheath cells		28	6
Whole leaf		34	5
Mesophyll cells	Transhydrogenase (deamino-$NADP^+$)	40	
Bundle sheath cells		9	
Whole leaf		6	

Table III. *Glyceraldehyde 3-P Dehydrogenase and Malic Enzyme Activities in Mesophyll Cells and Bundle Sheath Cells from Digitaria sanguinalis*

Enzyme	Mesophyll Cells	Bundle Sheath Cells
	μmoles/mg chl·hr	
Glyceraldehyde 3-P dehydrogenase $NADP^+$	206	284
Glyceraldehyde 3-P dehydrogenase NAD^+	92	101
Malic enzyme	26	548

Table IV. *Photochemical Oxygen Production by Isolated Mesophyll Cells and Bundle Sheath Cells of Digitaria sanguinalis*

Cells were isolated in 0.4 M sorbitol and 0.02 M MOPS buffer, pH 6.5. The assays employed the same medium and were performed at 30 C.

Electron Acceptor Added	Experiment No.	Mesophyll Cells	Bundle Sheath Cells	Ratio of Mesophyll-Bundle Sheath
		μmoles O_2 evolved/mg chl·hr		
None		0–2	3–5	
Benzoquinone (Air) 0.25 mM	1	102	36	
	1	86	42	
	2	82	63	
	6	75	33	
Average		85	43	1.97
Benzoquinone (N_2) 0.25 mM	1	89	42	
	2	122	99	
	3	190	125	
	4	185	115	
	5	170	30	
	6	108	32	
Average		145	74	1.96
Diquat (Air) 0.2 mM	1	35[1]	21	
Plus 2 mM KCN	2	77	45	
	6	63	27	
Average		58	31	1.87

[1] With diquat as an acceptor oxygen uptake was measured.

the high levels of P_{700} (7); the high ratio of chl *a–b* (7); the high levels of ferredoxin (30, 31); and a lower apparent K*m* for P_i and ADP during cyclic photophosphorylation (11). These experiments were conducted on leaf extracts and crude chloroplast preparations.

It is well documented that C_4 plants contain two major types of photosynthetic cells (19, 22). Electron microscopy studies have demonstrated that crabgrass mesophyll cell chloroplasts have highly developed grana and that bundle sheath cell chloroplasts have only rudimentary grana. It has also been shown that both types of cells accumulate starch as a photosynthetic storage product (6). Studies with isolated cells of the two types have shown that the mesophyll cells fix CO_2 via a β-carboxylation and that the bundle sheath cells fix CO_2 via the carboxylation of RuDP (16).

The present study with these distinct cell types isolated from crabgrass leaves allows a more detailed characterization and understanding of photosynthesis in C_4 plants. The following is our interpretation of photosynthesis in mesophyll and bundle sheath cells in C_4 cycle plants.

First, both cell types contain photosystems I and II. Photosystem II activity is shown by the following: bundle sheath cells are capable of evolving oxygen in the presence of typical Hill oxidants (Table IV) which we interpret as indicating that a photosystem II (*e.g.*, a water splitting reaction) is present in the chloroplasts in both cells. The oxygen evolution activity is sensitive to DCMU. The low temperature fluorescence emission spectra in chloroplast extracts from both cells peak around 685 nm (Fig. 3) which also indicates that photosystem II is operative (9). The millisecond delayed light emission, which is another indicator of photosystem II activity (4, 13), also shows that there is photosystem II activity in both cell types.

Photosystem I activity also is present in both cell types. The commonly accepted reaction center pigment for photosystem I, P_{700}, is present in both cell types (Fig. 4). The absorption spectra and direct measurements indicate that chl *a* is present in both cell types (Table I, Figs. 1 and 2). The fluorescence emission spectra (Fig. 3) with peaks near 730 nm clearly show photosystem I activity in both cells. Diquat probably accepts electrons from photosystem I and is active in oxygen metabolism with both cells (Table IV). It is debatable whether or not ferredoxin $NADP^+$-reductase is a component of cyclic electron flow (27) but the data in Table II indicate that this enzyme, which reduces pyridine nucleotides photochemically for carbon metabolism to proceed, is active in both cell types. Thus we interpret the present data as evidence that the two photosynthetic cell types of crabgrass leaves each contain a complete electron transport system from oxygen evolution to the reduction of pyridine nucleotides.

Second, there is a difference in the quantitative distribution in the two cell types of the photosynthetic electron transport components. The following data are consistent with the mesophyll cells containing more noncyclic electron flow than the bundle sheath cells: the oxygen evolution data show about a 2:1 ratio of mesophyll activity–bundle sheath activity (Table IV); the ferredoxin $NADP^+$-reductase activity is three to four times higher in mesophyll cells than in bundle sheath cells (Table II); the intensity of low temperature fluorescence emission at 684 nm (Fig. 3) indicates a high ratio of photosystem II–I activity in the mesophyll cells (possibly two or three times higher than in bundle sheath cells); and approximately twice as much delayed light emission is observed in mesophyll cells (Fig. 5). On the other hand, the bundle sheath cells have much higher photosystem I activity than the mesophyll cells as indicated by: the higher P_{700} change in bundle sheath cells (about 2:1 on a chl basis, (Fig. 4); the higher chl *a–b* ratio in bundle sheath cells (about 3:2, Table I); the increased absorption in the region of 700 to 705 nm, shown by Butler and Bishop (10) to be related to photosystem I activity, in bundle sheath cell extracts (Figs. 1 and 2); and the very high fluorescence emission at 730 nm indicating photosystem I activity (8, 9, 25) relative to F_{685} in bundle sheath cell extracts (Fig. 3). It is possible that part of the increased amount of P_{700} of the bundle sheath cells is due to the form of P_{700} recently reported to be linked to stroma lamellae (33) and alternatively the electron transport data can be explained within the frame work of Knaff and Arnon's (27) electron transport scheme rather than two photosystems (8).

We conclude that the mesophyll cells are about two to four times more active than bundle sheath cells in noncyclic electron flow, which results in the production of reducing power, NADPH, and the associated ATP. Also, the bundle sheath cells generate some reducing capacity and ATP via noncyclic electron flow; however, they have a two to three times greater capacity for cyclic electron flow than mesophyll cells, which results in more cyclic ATP production in bundle sheath cells than in mesophyll cells.

We now can propose a scheme to accommodate the CO_2 fixation and subsequent carbon metabolism reactions of the mesophyll cells which fix CO_2 via the carboxylation of PEP and bundle sheath cells which fix CO_2 via the carboxylation of RuDP (16). In the mesophyll cells CO_2 is fixed into oxaloacetate and then NADPH is utilized to produce malate. The malate moves to the bundle sheath cells where malic enzyme is localized (Table III). There malate is decarboxylated by malic enzyme producing pyruvate, CO_2, and NADPH. The fate of pyruvate is uncertain. The CO_2 released in the bundle sheath cell is fixed via the pentose cycle and the NADPH produced by the malic enzyme is utilized by the glyceraldehyde 3-P dehydrogenase to reduce the PGA to glyceraldehyde 3-P.

If the bundle sheath cell operates by the classical reductive pentose cycle 3 ATP and 2 NADPH are needed per CO_2 fixed. The malic enzyme would provide one of the NADPH needed to fix the CO_2 released. Therefore, the noncyclic electron flow in the bundle sheath cells would need to provide only one NADPH per CO_2 fixed. To operate the pentose cycle the energy requirements of the bundle sheath chloroplasts would therefore be about 3 ATP–1 NADPH per CO_2 fixed. The theoretical energy requirement of the C_4 cycle in the mesophyll cells are 2 ATP–1 NADPH per CO_2 fixed if pyruvate is the precursor for PEP (21). The present data which indicate more cyclic electron flow in the bundle sheath cells relative to mesophyll cells and more noncyclic electron flow in mesophyll cells than in bundle sheath cells, are consistent with above theoretical energy requirements.

The theoretical energy requirements of the two cell types would be altered to the extent which PGA may be transported from bundle sheath cells to mesophyll cells and reduced there to form starch. At this point it is important to note that both bundle sheath cells and mesophyll cells contain about an equal amount of glyceraldehyde 3-P dehydrogenase (Table III), and that both synthesize starch (6). Therefore we propose that PGA or 2-PGA also can move from the bundle sheath cells back to the mesophyll cells to be either reduced and produce starch or regenerate PEP (data in a manuscript in preparation supports this hypothesis). Thus in the bundle sheath cells PGA or 2-PGA have alternative fates and the same may be true for pyruvate which could be metabolized by the very prominent mitochondria in the bundle sheath cells (6) or return to the mesophyll cells and generate PEP. Bundle sheath cells also may fix some atmospheric CO_2 (about 15% of the total CO_2 fixed by the leaf) via the regular pentose cycle (14).

In brief, experimental data show that in crabgrass leaves both cell types fix CO_2 and both reduce carbon and both synthesize starch. We therefore propose that the cells are only partially dependent since the bundle sheath cells appear to lack the capacity to produce sufficient reduced pyridine nucleotides to operate the pentose cycle catalytically.

Although this hypothesis for C_4 cycle photosynthesis may be correct for crabgrass, it appears that other C_4 plants such as bermudagrass may lack sufficient malic enzyme to operate this exact cycle and an alternative pathway involving aspartic acid has been proposed (12).

During processing of this manuscript the results of an investigation with isolated chloroplasts from C_4 plants by Woo *et al.* (35) were published. They reported the absence of photosystem II activity in isolated bundle sheath chloroplasts of *Sorghum bicolor* and *Zea mays*. These results with isolated chloroplasts from plants which are structually similar to crabgrass are contrary to those reported here with whole cells. The reason(s) for their failure to detect photosystem II activity in their isolated bundle sheath chloroplasts preparations from these two plants is unknown. Karpilov *et al.* (24) also recently presented some fluorescence and Hill activity data on C_4 plants which qualitatively agree with the data in this manuscript.

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