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P_{700} Activity and Chlorophyll Content of Plants with Different Photosynthetic Carbon Dioxide Fixation Cycles[1]

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ABSTRACT

Representative plants containing either the reductive pentose phosphate cycle or the C_4 dicarboxylic acid cycle of photosynthetic carbon dioxide fixation have distinctly different contents of P_{700} and chlorophylls *a* and *b*. With leaf extracts and isolated chloroplasts from C_4 cycle plants, the mean value of the relative ratio of P_{700} to total chlorophyll was 1.83 and the mean value of the ratio of chlorophyll *a* to *b* was 3.89. The respective values in similar extracts and chloroplasts from pentose cycle plants are 1.2 and 2.78.

It seems likely that these results are indicative of a more active Photosystem I or a different size photosynthetic unit in C_4 cycle plants than in the reductve pentose phosphate cycle plants.

In 1965, Kortschak *et al.* (14) reported that photosynthetically sugarcane leaves incorporated carbon dioxide primarily into malic and aspartic acids. These initial observations on sugarcane were confirmed by Hatch and Slack (7), who subsequently extended this type of experimental observation to include specific grasses, sedges, and dicotyledonous plants. Furthermore, Hatch and Slack (7) demonstrated that oxaloacetic acid was labeled. (In the Kortschak *et al.* (14) experiments, oxaloacetic acid presumably was destroyed in the initial extraction procedure and thus was not detected.) Hatch and Slack (7) proposed that the initial CO_2-fixation reaction is the carboxylation of phosphoenolpyruvate to form oxaloacetate, which rapidly equilibrates with malate and aspartate. These investigators have proposed a C_4 dicarboxylic acid cycle of photosynthesis, and they have partially substantiated their proposal with subsequent data on enzyme activities and labeling patterns. Although the details of the mechanism of the C_4 cycle are not yet understood, the data clearly indicate that a major, previously unobserved cycle of photosynthesis is present in such plants, whereas in other plants the reductive pentose phosphate cycle seems to predominate (3, 15).

One distinctive characteristic of the C_4 plants of interest to us was the response of photosynthesis in representative plants to light intensity. Photosynthesis in plants with the C_4 cycle does not saturate until intensities near 8,000 to 10,000 ft-c are reached (which is roughly equivalent to full sunlight), whereas pentose cycle plants saturate near 2,000 to 3,000 ft-c (5). Also the maximal rates of photosynthesis in plants with the C_4 cycle, as measured by CO_2 consumption, are 2- to 3-fold higher than pentose cycle plants at full sunlight (5, 6). We recently calculated a theoretical stoichiometry for CO_2 fixation in C_4 cycle plants of 5 moles of ATP and 2 moles of NADPH per mole of CO_2 (6). In contrast, the stoichiometry in pentose cycle plants is: 3 ATP: 2 NADPH: 1 CO_2 (3). We reasoned that the higher ATP requirements of C_4 cycle plants could be supplied by cyclic photophosphorylation catalyzed by Photosystem I and recently presented some data to support this hypothesis (6). The reaction-center pigment for Photosystem I is P_{700} according to current ideas (13). Thus this manuscript reports our findings on P_{700} and other pigments in these two distinct groups of higher plants.

Materials and Methods

Spinacia oleracea L. (spinach) was purchased from the local market for use in these studies. The other plants were field grown and usually harvested by 9:00 AM in midsummer in Yellow Springs, Ohio. The species utilized were *Zea mays* L. (corn); *Amaranthus hybridus* L. (pigweed); *Phytolacca americana* L. (pokeweed); *Cynodon dactylon* (L.) Pers. (coastal bermudagrass); *Beta vulgaris* L. (sugar beet); *Beta vulgaris* L. (Swiss chard); *Triticum vulgare* L. (wheat); and *Digitaria sanguinalis* (L.) Scop. (crabgrass).

Preparation of Chloroplasts. Sodium chloride chloroplasts were isolated fresh for each experiment in 0.35 M NaCl, 0.05 M Tricine-NaOH[2] buffer, pH 7.8, as previously described (19). Chloroplasts were finally suspended in 0.035 M NaCl, 0.005 M Tricine, pH 7.8. Sucrose chloroplasts were isolated and suspended in a similar manner substituting 0.4 M sucrose for NaCl in the media. Total chlorophyll was determined by the method of Arnon (1). All isolations and extracts were made in a cold room (about 4 C) and stored in an ice bucket through each experiment.

Preparation of Crude Leaf Extracts. Twenty grams of freshly harvested leaves were chopped with a razor and then vigorously handground for 1 to 5 min in a cold (4 C) mortar and pestle with 1 g of acid-washed sea sand, plus 45 ml of 0.35 M NaCl, 0.1 M Tricine-NaOH buffer, pH 7.8. The slurry was passed through four layers of cheesecloth and then centrifuged at 200*g* for 1 min. The pellet was discarded, and the supernatant fluid was passed through a cold (4 C) French Press at 4000 to 6000 p.s.i. of pressure. The resulting suspension was designated as a crude leaf extract.

Assay of P_{700} Activity. The standard reaction mixture contained in 2.1 ml: 70 μmoles of sucrose; 100 μmoles of Tricine-

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[2] Abbreviations: Tricine: tris (hydroxymethyl)methylglycine (obtained from CalBiochem); DCMU: 3-(3,4-dichlorophenyl)-1,1-dimethylurea (obtained from DuPont Co.).

NaOH buffer, pH 7.8; 40 μmoles of KCl; 0.05 μmole of pyocyanine; 0.06 μmole of DCMU; and 15 to 50 μg of chlorophyll, either as chloroplasts or crude leaf extract.

Measurements of absorption changes in the standard reaction mixture were performed at room temperature in a quartz cuvette with a commercial version of the Britton Chance dual wavelength spectrophotometer (Aminco Corp., Silver Springs, Md.) in the split beam mode. Interference filters (normally 702 nm) were placed in front of the photomultiplier to protect the detector from the actinic light and to reduce the fluorescence artifact, which may be observed in the absence of these precautions (16). The irradiating light source was a 650 w "Sungun" lamp (Sylvania, DWY). Light intensity varied with a variable transformer. The reaction mixtures were irradiated 90 degrees to the measuring beam with a broad band of blue light isolated with Corning Filters No. 9762 and 4694. The standard irradiation intensity used was 10^{-4} ergs/cm^{-2}·sec^{-1}.

RESULTS

P_{700} Studies. The reversible P_{700} absorbance change, discovered and studied in detail in spinach and algae by Kok (12, 13), was observed readily in all of the leaf and chloroplast preparations. The kinetics of the light-induced absorbance changes at 702 nm of corn or wheat, a C_4 and pentose cycle plant, respectively, were the same, within the detection limits of the dual-beam spectrophotometer. Similar observations were made with all of the preparations in these experiments. In subsequent discussions, the magnitude of the absorption change refers to the change in absorbance from the initial dark value to the maximal light-on value (usually obtained in 5–15 sec).

The difference spectra of the reversible absorption change in crude leaf extracts from corn and pigweed (Fig. 1) are similar to the difference spectra for spinach (Fig. 1, ref. 13) and algae (12, 13).

The more striking observation in Figure 1 is the magnitude of the P_{700} absorption change in the two C_4 cycle plants, pigweed and corn, compared to spinach. Note that the pigweed reaction mixture contained 21 μg of chlorophyll and the spinach, 34 μg, and yet the magnitudes of the absorption changes are identical in the two reaction mixtures. This observation prompted us to examine this phenomenon in other plants, and in Tables I and II the results of a more extensive study are summarized. These data are presented as the reversible absorbance change at 700 nm expressed in arbitrary units, divided by the chlorophyll concentration (relative ratio of P_{700} to chlorophyll concentration). Although some variations in the relative ratio of P_{700} to total chlorophyll were observed in different experiments

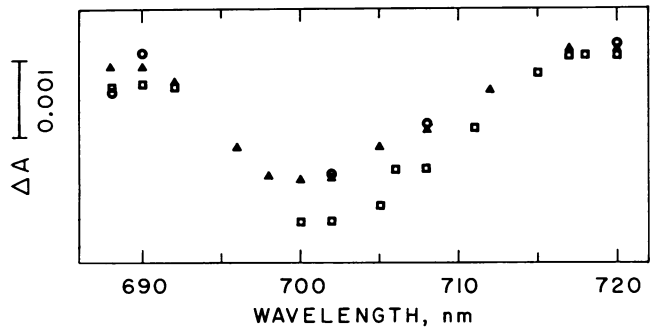

FIG. 1. Difference spectra of the light-induced reversible absorption changes in a crude leaf extract of pigweed, *Amaranthus hybridus* L., 21 μg of chlorophyll per reaction mixture (▲); corn, *Zea mays* L., 24 μg of chlorophyll per reaction mixture (□); and spinach, *Spinacia oleracea* L., 34 μg per reaction mixture O. Experimental conditions are described in text.

Table I. *Ratio of P_{700} to Total Chlorophyll in Crude Leaf Extracts from Plants with Different CO_2 Fixation Cycles*

Experiment No.	Plant	Major CO_2 Fixation Cycle	Relative P_{700}/Total Chlorophyll
1[1]	Corn	C_4	1.90
	Crabgrass	C_4	1.72
	Pigweed	C_4	2.09
	Swiss chard	Pentose	1.25
2	Corn	C_4	1.71
	Pigweed	C_4	1.89
	Pokeweed	Pentose	1.26
	Spinach	Pentose	1.30
3	Corn	C_4	1.70
	Pigweed	C_4	2.26
	Wheat	Pentose	1.10
	Pokeweed	Pentose	1.30
4	Corn	C_4	1.73
	Spinach	Pentose	1.05
5	Corn	C_4	1.70
	Pigweed	C_4	1.61
	Spinach	Pentose	1.07
	Wheat	Pentose	1.27

[1] These extracts were not passed through a French pressure cell.

Table II. *Ratio of P_{700} to Total Chlorophyll in Once-washed Chloroplasts Isolated from Plants with Different CO_2 Fixation Cycles*

Experiment No.	Plant	Major CO_2 Fixation Cycle	Relative P_{700}/Total Chlorophyll
1	Corn[1]	C_4	1.92
	Pigweed[1]	C_4	2.21
	Swiss chard[1]	Pentose	1.25
2	Bermuda grass[1]	C_4	2.14
	Swiss chard[2]	Pentose	1.30
	Sugar beets[1]	Pentose	1.33
3	Corn[2]	C_4	1.97
	Spinach[2]	Pentose	1.05

[1] Chloroplasts isolated in 0.35 M NaCl.
[2] Chloroplasts isolated in 0.4 M sucrose.

(for example, in corn crude leaf extracts values of the ratio ranged from 1.70:1.90) plants with the C_4 cycle of photosynthesis have more P_{700} on a total chlorophyll basis than plants with the pentose cycle of CO_2 fixation. Thus, some difficulty also is evident in trying to obtain an exact value for the differences in these two groups of plants, but the C_4 cycle plants were never less than 25% greater than pentose cycle plants and were as much as 100% greater in some experiments (Tables I and II). The mean of the relative P_{700} absorption change to total chlorophyll with pentose cycle plants was 1.2 with a standard deviation of 0.11. The corresponding values with C_4 cycle plants were 1.83 and 0.21.

It is appropriate to note some of the experimental controls used in the course of this study. Each reported value for P_{700} and chlorophyll in all of these experiments was obtained on the linear portion of plots of chloroplasts or extract concentration *versus* change in absorbance. In the P_{700} studies, each sample was repeatedly (two to five times) illuminated to be sure the change in absorption was reversible and of the same magnitude. All P_{700} values were obtained at saturating light intensities. Instrumentation controls were also routinely employed to check

Table III. *Chlorophyll Concentration in Extracts from Plants with Different CO_2 Fixation Cycles*

Preparation	Major CO_2 Fixation Cycle	A		Chlorophyll Ratio a:b
		645 nm	663 nm	
Spinach chloroplasts	Pentose	0.074	0.180	2.42
		0.072	0.184	2.72
		0.112	0.292	2.84
		0.188	0.510	3.10
		0.075	0.202	3.06
Swiss chard chloroplasts	Pentose	0.046	0.110	2.41
		0.083	0.197	2.33
		0.160	0.394	2.52
Corn chloroplasts	C_4	0.152	0.465	4.43
Pigweed chloroplasts	C_4	0.080	0.235	3.78
Bermuda grass chloroplasts	C_4	0.045	0.132	3.80
Spinach crude leaf extract	Pentose	0.059	0.153	2.92
		0.087	0.210	2.43
		0.097	0.247	2.67
Pokeweed crude leaf extract	Pentose	0.192	0.550	3.52
		0.075	0.194	2.80
Wheat crude leaf extract	Pentose	0.163	0.448	3.20
Pigweed crude leaf extract	C_4	0.097	0.297	4.23
		0.095	0.304	4.80
		0.047	0.124	2.96
		0.020	0.056	3.38
Corn crude leaf extract	C_4	0.077	0.230	3.93
		0.152	0.448	3.86
		0.066	0.185	3.40
		0.083	0.263	4.65

problems such as light scattering and fluorescence artifacts. For example, the distance of the phototube from the sample was varied (from 1–16 cm) and the absorption changes were not affected.

Chlorophyll Concentrations. During the P_{700} studies we noted that plants containing the C_4 cycle of photosynthesis consistently had higher ratios of absorbances at 663:645 than pentose cycle plants. Table III contains the observed absorbance values in 80% acetone and the ratio of chlorophyll *a*:*b* for chloroplast and leaf extracts in several experiments. Plants with the C_4 cycle of CO_2 fixation consistently have higher concentrations of chlorophyll *a* than pentose cycle plants both in isolated once-washed chloroplasts and in crude leaf extracts. The mean chlorophyll *a*:*b* ratio in the pentose cycle plants was 2.78 with standard deviation of the samples of 0.35. The corresponding values with C_4 cycle plants were 3.89 and 0.60.

This mean chlorophyll *a*:*b* ratio in pentose cycle plants of 2.78 agrees very closely with data of other workers such as Wintermans (20), who examined leaves from 10 pentose cycle plants and obtained a mean value of 2.80. Comparable data for C_4 cycle plants could not be located by the authors. The authors realize that chlorophyll *a*:*b* ratios in plants vary depending upon such factors as the nutritional status or other environmental growth conditions. In the present study such factors were not controlled. However, all plants, except spinach, were grown in full sunlight in a natural environment and the differences were recorded consistently.

DISCUSSION

The experiments reported in this manuscript are in agreement with the hypothesis (6) that cyclic photophosphorylation should be quite active to support the high photosynthetic capacity of C_4 cycle plants (5, 6). Several ideas are involved in proposing that cyclic photophosphorylation may be exceptionally active in C_4 cycle plants. First, is the theoretical stoichiometry to conduct photosynthesis in C_4 cycle plants of 1 mole of CO_2:5 moles of ATP:2 moles of NADPH (6) contrasted to 3 moles of ATP and 2 moles of NADPH in pentose cycle plants. It seems reasonable that cyclic ATP synthesis could supply this extra ATP, particularly considering the fact that studies on photophosphorylation in a C_4 cycle plant, bermudagrass, indicated that the molar ratio of ATP to 2 electrons in noncyclic electron flow approaches unity (6). Second, photosynthesis in plants with the C_4 cycle does not saturate until the light intensity is near that of full sunlight (5, 6). In chloroplast studies only cyclic photophosphorylation responds in a similar fashion to increasing light intensity (2, 6, 17, 19). Third, all studies on noncyclic electron transport indicate that saturation is reached at 2000 to 3000 ft-c in both C_4 (6) and pentose cycle plants (2, 6, 17, 19) and thus noncyclic electron transport appears an unlikely source of ATP at higher light intensities.

The extensive studies of Kok (12, 13) and others on P_{700} indicate that this pigment is the reaction center pigment for Photosystem I. Certainly the general consensus is that cyclic photophosphorylation in plants is catalyzed by Photosystem I. Therefore, the present experiments strongly support the possibility that plants with the C_4 cycle of CO_2 fixation contain more Photosystem I than pentose phosphate cycle plants as evidenced by the increased amount of P_{700} and chlorophyll *a*. One should also note that these differences in pigment concentration may reflect on a molecular level the striking differences in chloroplast and cell anatomy which have been observed in these two groups of plants (4, 10; Black, C. C. and Mollenhauer, H. H., manuscript in preparation). Furthermore, the data suggest that the photosynthetic unit in C_4 cycle plants may have a distinct size and/or composition, *i.e.*, C_4 cycle plants appear to have a smaller photosynthetic unit than pentose cycle plants.

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