SPECIFICITY OF GALACTOLIPIDS IN PHOTOCHEMICAL REACTIONS COUPLED WITH CYTOCHROME C REDUCTION*

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Galactolipids (monogalactosyl diglyceride and digalactosyl diglyceride) have been found in the various forms of photosynthetic organisms (Benson, et. al., 1959; Wintermans, 1960; Sastry and Kates, 1963; Levin et.al., 1964; Bloch and Chang, 1964). These lipids constitute a major part of chloroplast lipids and are known to contain a high percentage of polyunsaturated fatty acids, especially, α-linolenic acid (9, 12, 15-octadecatrienoic acid) and 4, 7, 10, 16-hexadecatetraenoic acid (Sastry and Kates, 1963; Bloch and Chang, 1964). Recently Carter reviewed thoroughly on the subject (Carter, et. al., 1965). The possible function of α-linolenic acid in the photosynthetic electron transport has been indicated by Erwin and Bloch (1962, 1963) from their observation that α-linolenic acid was the major fatty acid in light-grown cells and the synthesis of the fatty acid was inhibited specifically by Hill reaction inhibitors. The light induced-synthesis of galactolipids has been observed in the etiolated cells of Englena gracilis as these cells become green (Rosenberg, 1963; Rosenberg, et. al., 1964).

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**All the communications to be addressed to this author. The following abbreviation is used:

PMS (Phenazine methosulfate), FMN (Flavin mononucleotide), CoQ_6 (Coenzyme Q_6) Vit. K_1 (Vitamin K_1), Mono-G.L. (Monogalactosyl diglyceride), Di-G.L. (Digalactosyl diglyceride), DCMU (3-(2, 4-dichlorophenyl) -1, 1-dimethyl urea).

Nichols (1963) has shown with cabbage that the tissues which have been exposed to the light most contain the largest amount of galactolipids and those which have been exposed to the light least contained the least amount of galactolipids. The present investigation concerns with the specific function of galactolipids obtained from spinach chloroplast in the photochemical reaction of intact spinach chloroplasts coupled with cytochrome C reduction.

Materials and Methods

In all experiments fresh chloroplasts were used. The chloroplasts were prepared from spinach leaves as described by Avron, Jagendorf, and Evans (1957) by grinding the leaves in a solution of sucrose (0.4M) and NaCl (0.05M). The determination of chlorophylls was done by the method of Mackinney (1941). Galactolipids were extracted from the chloroplasts with $CHCl_3$-CH_3OH (2:1 $^{V}/_{V}$) and were purified by thin layer chromatography with a solvent system of $CHCl_3$-CH_3OH-AcOH (8:3:1 $^{V/V/V}$). For the detection of galactolipids a diphenylamine spray reagent was used (Levine, et.al., 1964, Wagner, et. al., 1961). For sugar analysis the lipids were hydrolyzed under acidic condition (Levine, et. al., 1964).

Galactose was identified by borate impregnated thin layer plates (Jacin, et. al., 1965). Quantitative analysis of the lipids was accomplished by determining the amount of galactose present in the acid hydrolysate by means of Nelson's method. (Nelson, 1944).

Illumination for the photochemical reaction was accomplished with a reflector lamp, 150 W., 120 V. (Sylvania Electric Products Co., Inc., Salem, Mass.). A cold-water filter (13.5 cm.) was used between the light source and the reaction vessels. The rate of cytochrome C reduction was determined spectrophotometrically by measuring the absorbance before and after the illumination. Calculation of the rate was done by taking the molar absorptivity of cytochrome C at 550 mμ as 21,000 (Vernon et.al., 1965). Cytochrome C (Type III), PMS, FMN, CoQ_6, and Vit. K_1 were purchased from

Sigma Chemical Co. (St. Louis, Mo.). α-tocopherol was purchased from Calbiochem. (Los Angeles, Calif.). DCMU was a generous gift of Dr. G. Constantopoulos.

TABLE I

Analysis of galactolipids by thin layer chromatography

Compounds	R_f values
Mono-G.L.	0.90*
Di-G.L.	0.47*
Galactose	0.33**
Glucose	0.38**
Sugar from G. L. Acid Hydrolysates	0.33**

*On silicagel G thin layer plates, solvent system, $CHCl_3$-CH_3OH-AcOH (8:3:1 V/V/V)

**On borate impregnated thin layer plates, solvent system, BuOH-AcOH-H_2O (5:4:1 V/V/V)

Results

Monogalactosyl diglyceride and digalactosyl diglyceride were separated from the total lipids as shown in Table I. Molar ratio of monogalactolipid to digalactolipid was about 2 to 1. The sugar moiety in the lipids was identified as galactose (Table I). The rate of photoreduction of cytochrome C by spinach chloroplasts was stimulated by both monogalactolipid and digalactolipid (Table II). On equal molar basis, these lipids showed about equal stimulatory effect on the rate of cytochrome C photoreduction. Several

TABLE II

Stimulation of cytochrome C photoreduction by galactolipids

Lipids Added*	Concentration, (μM in 3.0 ml.)	Cytochrome C Reduced (μM/hr./mg. Chl)
Chloroplast		26
" + Mono-G.L.	.040	99
" + Mono-G.L.	.020	44
" + Mono-G.L.	.010	37
" + Di-G.L.	.015	49
" + Di-G.L.	.010	37
" + Di-G.L.	.005	37

Total 3.0 ml. of reaction mixture contained chloroplast equivalent to 22 μg. of chlorophyll, 0.15 μ mole of cytochrome C, 0.05 M phosphate buffer, P^H7.0, 0.25M sucrose, and the galactolipids as indicated.

* The lipids were dissolved in a minimum volume of ethanol and an aliquot of the ethanol solution was used for the photochemical reaction.

TABLE III

Stimulation of cytochrome C photoreduction by redox compounds

Redox Compounds	Concentration (μM in 3.0 ml.)	Cytochrome C Reduced (μM/hr./mg.chlorophyll)
Chloroplast	-	26
" + PMS	0.10	204
" + FMN	0.20	108
" + CoQ_6	0.20	52
" + Vit. K_1	0.20	69
" α-Tocopherol	0.20	45

The condition for the reaction is same as Table II

TABLE IV

Effect of DCMU on the rate of cytochrome C photoreduction in the presence of galactolipids

Lipids added	Concentration (μM. in 3.0 ml.)	Cytochrome C Reduced (μM/hr./mg.Chlorophyll) Control	DCMU (0.5×10^{-5} M)
Chloroplast	-	26	0
" + Mono-G.L.	0.02	44	0
" + Di-G.L.	0.01	37	0

The condition for the reaction is same as Table II.

TABLE V

Effect of photophosphorylation on the rate of cytochrome C photoreduction in the presence of galactolipids

Lipids Added	Concentration (μM. in 3.0 ml.)	Cytochrome C reduced (μM/hr./mg.chlorophyll) Control*	Under** Photophosphorylation
Chloroplast		24	24
" + Mono-G.L.	0.02	44	37
" + Di-G.L.	0.01	37	34

*The condition for the reaction is same as Table II.

**Total 3.0 ml. of the reaction mixture contained 1.5μ M of ADP, 5 μ M of $Mgcl_2$, 0.15 μ M of Cyt. C, 0.05 M Phosphate buffer, $pH_{7.0}$, and the galactolipids as indicated.

known redox compounds also showed the stimulatory effect on the rate of cytochrome C photoreduction under the present experimental conditions (Table III). DCMU inhibited completely the photoreduction of cytochrome C in the presence of galactolipids (Table IV). This effect by DCMU was also true with all the cofactors mentioned in Table III. Photophosphorylating condition had no effect on the stimulatory function of galactolipids in the rate of cytochrome C photoreduction (Table V).

Discussion

Although galactolipids comprise a major fraction of chloroplast lipids the specific function of these lipids in the photochemical reactions is not known except the fact that they contribute a part of surfactant structures in the chloroplast. The difficulty with galactolipids is obvious. "Extraction and adding-back" approach is not feasible with galactolipids as in the case of quinones in chloroplasts (Bishop, 1959; Krogomann, et. al., 1962) since one has to use quite polar solvents to extract galactolipids. This extraction procedure obviously will damage chloroplast structure more severely than do more non-polar solvents used for the extraction of quinone compounds. Another approach seems to be desirable for the problem. Recently, Vernon, and his coworker (1965) showed that several quinones had stimulated the rate of photoreduction of cytochrome C by intact chloroplasts.

The present investigation shows clearly that galactolipids have stimulatory effect on the rate of cytochrome C photoreduction by intact chloroplast obtained from fresh spinach. DCMU inhibits completely the galactolipids mediated cytochrome C photoreduction indicating that galactolipids may participate in the light reaction involving the short-wave length system. Several known redox compounds also stimulated the rate of photoreduction of cytochrome C under the condition of the present investigation. The stimulatory effects of PMS, FMN, and low activity with CoQ_6 have been reported previously (Vernon, et. al., 1965). Photophosphorylating condition has been shown to

enhance the stimulatory effect by a certain cofactor in the photoreduction of cytochrome C (Keister, et. al., 1963; Vernon, et. al., 1965). In the present investigation photophosphorylating condition seems to have no effect on the activity of galactolipids in cytochrome C photoreduction (Table V). The activity of galactolipids reported in the present communication leads us to believe that α-linolenic acid, a major fatty acid in galactolipids may have specific function in photosynthetic electron transport as suggested by Erwin and Bloch (1962, 1963) rather than this fatty acid is required simply as a part of lipoprotein in the chloroplast structure as indicated by some investigators (O'Brien, and Benson, 1964).

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