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GALACTOLIPID TRANSFORMATIONS AND PHOTOCHEMICAL ACTIVITIES OF SPINACH CHLOROPLASTS

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SUMMARY

1. When spinach chloroplasts are isolated in media containing high concentrations of NaCl, monogalactolipase activity can be observed. In media containing sucrose or mannitol, there is also an enzymatic transacylation which results in a decrease in digalactolipid and the formation of acylated derivatives of the mono- and digalactolipids. The rate of release of free fatty acids is slower under the latter conditions.

2. In order to establish a relation between these phenomena and the photochemical capacities of the chloroplasts, several light reactions have been measured in fresh and aged chloroplasts.

3. Hill reactions in Photosystem II and $NADP^+$ reduction in Photosystem I are not directly influenced by the lipid transformations.

4. Photophosphorylation and Hill reaction at limiting light intensities are very labile under the experimental conditions. Lipid transformations may contribute to this effect.

5. Serum albumin has no effect on the transformations of chloroplast galactolipids. It has a stimulating and stabilizing effect on photophosphorylation and improves the efficiency of photophosphorylation. Only a part of this effect can be explained by the known capacity of serum albumin to bind free fatty acids.

INTRODUCTION

When it was found by SASTRY AND KATES[1] that isolated bean chloroplasts, which are notoriously bad in photochemical activities, were further characterized by the presence of a very active enzyme hydrolyzing the galactolipids, it became of interest to consider the galactolipase activity as one of the possible reasons for low and labile photochemical performance of isolated chloroplasts. MCCARTY AND JAGENDORF[2] established that linolenic acid was released due to hydrolysis of galactolipids and that it was inhibitory to the Hill reaction with 2,6-dichlorophenolindophenol (DCIP) and ferricyanide and to cyclic photophosphorylation. Galactolipase activity in spinach chloroplasts was observed by these authors at pH 6 but not at pH 8. WASSERMAN AND FLEISCHER[3] have observed that the stability of spinach chloroplast fragments could be improved by storage at high suspension density and by the addition of serum albumin. The latter compound has been found to protect the activity

Abbreviation; DCIP, 2,6-dichlorophenolindophenol.

of isolated mitochondria, probably due to the binding of fatty acids[4]. Since WASSERMAN AND FLEISCHER[3] found no change in the pattern of polar lipids they remained uncertain as to the mechanism of protection by bovine serum albumin.

It has been established, however, that the activity of galactolipid transforming enzymes in spinach chloroplasts is by no means negligible, also at higher pH[5,6]. The pattern of transformation depends more on the main osmotic component of the isolation medium than on pH. It seemed to be of interest, therefore, to compare the rate and pattern of galactolipid transformations with the rate of release of free fatty acids and with the rate of decay of several photochemical reactions under comparable conditions. Furthermore, the influence of the addition of serum albumin on galactolipid breakdown and on some photochemical reactions was studied. The results may contribute to an understanding of the relation between galactolipid transformations and chloroplasts reactions and of the protective action of serum albumin.

After completion of the experimental work described here, we took notice of a paper by CONSTANTOPOULOS AND KENYON[7] in which the rate of release of free fatty acids and the decay of photoreduction of trichloroindophenol in aging spinach chloroplasts were studied. Their results, which are to a certain extent at variance with ours, will be discussed.

MATERIALS AND METHODS

Chloroplasts from freshly collected spinach leaves were prepared at 4° in the following media: 0.35 M NaCl *plus* 0.067 M Tris–HCl (pH 7.5); 0.35 M NaCl *plus* 0.05 M phosphate buffer (pH 6.0); 0.5 M sucrose *plus* 0.01 M sodium pyrophosphate–HCl (pH 7.5); 0.5 M mannitol *plus* 0.01 M sodium pyrophosphate–HCl (pH 7.5) and 0.5 M mannitol *plus* 0.05 M phosphate buffer (pH 6.0). Whole unwashed chloroplasts were suspended in the medium used for isolation, to which streptomycin sulfate was added until the concentration was 1 mg/ml. Aliquots were analyzed immediately, *i.e.* about 45 min after homogenization, and after dark storage at room temperature for various periods as indicated.

Lipids and pigments were extracted with boiling 96 % ethanol until the residue was colorless. The extract was taken to dryness on a rotatory vacuum evaporator, and the lipids were taken up in chloroform–methanol (2:1, v/v) followed by three washings with water, to remove nonlipid materials. Later the more convenient extraction method of BLIGH AND DYER[8] was adopted. Interfacial material was always dissolved in methanol and pooled with the lipid extract. The purified lipids were dried in a stream of N_2, weighed and dissolved in chloroform–methanol (2:1, v/v) to make a solution of 4 % (w/v). Chlorophyll was measured separately in 96 % ethanol using the extinction coefficients given by WINTERMANS AND DE MOTS[9].

For quantitative thin-layer chromatography, 4–8 mg of lipid were separated on silica gel with the following solvents, chloroform–methanol–water (65:25:3.5, by vol.); chloroform–methanol (10:1, v/v). For the separation of free fatty acids, we used petroleum ether 40–60°–diethyl ether–formic acid (60:40:1.5, by vol.). Reference chromatograms on the same plate were stained with periodate and Schiff's reagent[10], the carbohydrate-containing fractions were removed by aspiration and were eluted with chloroform and methanol. After alkaline methanolysis according to DAWSON[11], dilute H_2SO_4 and chloroform were added, and aliquots from the aqueous supernatant

were used for sugar determination with anthrone–sulfuric acid[12], using galactose as a standard. Free fatty acids were determined according to HEINEN AND DE VRIES[13]. Alternatively, free fatty acids were esterified with BF_3 in methanol, and ester groups were determined according to RENKONEN[14].

The following photochemical reactions were measured. Photoreduction of DCIP at pH 7.0 was measured by spectrophotometry at 610 nm using extinction coefficients according to ARMSTRONG[15]. Photoreduction of ferricyanide was measured according to JAGENDORF AND SMITH[16]. $NADP^+$ reduction was measured under anaerobic conditions. Reaction mixtures contained in a total volume of 3 ml: 0.25 mmole Tris–HCl (pH 7.0), 4.5 μmoles $NaHCO_3$, 0.75 μmole $NADP^+$, 0.5 ml of ferredoxin and chloroplasts containing 15–70 μg of chlorophyll. For experiments in which Photosystem II was to be by-passed, reaction mixtures also contained: 0.3 μmole DCIP, 10 μmoles ascorbic acid and 3.0 μmoles hydroxylamine–HCl. Reactions were stopped by the addition of 1.5 ml of 1 M NaOH, followed by 1.35 ml of satd. $(NH_4)_2SO_4$ and 0.15 ml of 1 M triethanolamine–HCl[17,36]. NADPH was measured spectrophotometrically in the supernatant.

Ferredoxin was prepared according to SAN PIETRO AND LANG[18] to the stage of extract of acetone precipitate, and contained 1.0–1.2 mg protein per ml.

Photophosphorylation was measured according to WHATLEY AND ARNON[19]. Reaction mixtures for cyclic photophosphorylation contained in a total volume of 2.5 ml: 15–25 μg chlorophyl; 80 μmoles of Tris–HCl (pH 8.3); 10 μmoles $MgCl_2$; 10 μmoles ascorbic acid; 0.05 μmole phenazine methosulfate; 10 μmoles ADP and 10 μmoles KH_2PO_4 containing 1 μC of carrier-free $H_3{}^{32}PO_4$. For the measurement of noncyclic photophosphorylation, phenazine methosulfate and ascorbic acid were omitted and replaced by 15 μmoles $K_3Fe(CN)_6$.

Chemicals used were Merck A.R.D. quality. ADP and $NADP^+$, Boehringer; phenazine methosulfate, Sigma; bovine serum albumin, Calbiochem; streptomycin sulfate, Mycofarm (Delft); $H_3{}^{32}PO_4$, Radiochemical Centre.

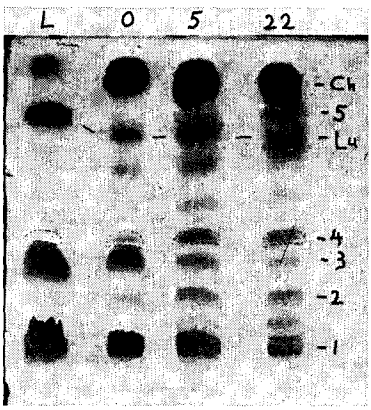
EXPERIMENTAL RESULTS

Lipid transformations

Different patterns of galactolipid breakdown had been established for spinach chloroplasts, isolated and aged in media containing either NaCl or sucrose[5,6]. In the present experiments, it was established that under our conditions, no significant differences were observed when the pH was lowered from 7.5 to 6.0, although this change has great influence on the activity of purified galactolipase[20]. Also the replacement of sucrose by mannitol in equimolar amounts caused no change in the pattern or rate of lipid transformations.

In media containing NaCl, mainly the monogalactolipid is hydrolyzed. In media containing sucrose or mannitol, the transformations are more complex. Both mono- and digalactolipid are transformed. Thin-layer chromatography in chloroform–methanol (10:1, v/v) reveals the formation of three new spots containing neutral sugar (Fig. 1). By paper chromatography of the deacylated lipids in phenol–water (3:1, w/v), it was established that one of the new components is derived from digalactolipid and two new components are derived from monogalactolipid. HEINZ[21,22] has investigated an enzyme from spinach which catalyzes the transacylation of one fatty acid residue

from galactolipid to one of the hydroxyl groups of galactose of the monogalactolipid. Activities of this type may help one to understand the present phenomena. The digalactolipid compound running ahead of normal digalactolipid has an ester/galactose molar ratio of 1.6 or higher and may be an acylated derivative (GGDG-X in Fig. 1). The compound GDG-X, running slightly beyond the monogalactosyl diglyceride is usually present in minor amounts. The component GDG-Y may finally contain the major part of the original monogalactolipid. We suppose it to be the acylated monogalactolipid described by HEINZ[21] but found the ester/galactose ratio quite variable and usually well above 3; hence, further investigation is required.



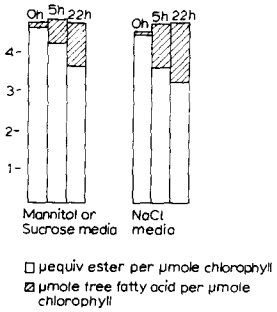


Fig. 1. Thin-layer chromatogram of lipids from spinach leaves (L) and from chloroplasts isolated in 0.5 M mannitol pyrophosphate (pH 7.5) and extracted immediately (0) and after storage at room temperature for the time indicated. Solvent: chloroform–methanol (10:1, v/v). Detection: $NaIO_4$ and Schiff's reagent[10]. 1, digalactosyl diglyceride; 2, GGDG-X, derivative from digalactosyl diglyceride; 3, monogalactosyl diglyceride; 4, GDG-X; 5, GDG-Y; 4 and 5 are derivatives from monogalactosyl diglyceride; Ch, chlorophyll; Lu, lutein.

Fig. 2. Changes in amounts of fatty acids esterified to galactolipids and of free fatty acids in spinach chloroplasts, isolated and aged in different media.

It may be remarked that these new derivatives are regularly found in freshly isolated chloroplasts, especially when the medium for isolation contains mannitol. Their concentration increases little after 5 h at room temperature, whereas the hydrolysis due to galactolipase continues for longer periods. As a result of these events, chloroplasts suspended in sucrose or mannitol lose relatively more lipid-bound sugar during the first h of aging, whereas chloroplasts suspended in media high in NaCl release free fatty acids at a higher rate during this period (Table I).

Upon the assumption that the ester/galactose molar ratios for the monogalactosyl diglyceride and GGDG-X are 1 and 1.5, respectively, and that those for digalactosyl diglyceride, GDG-X and GDG-Y are 2, 2 and 3, respectively, we have calculated the decrease in esterified fatty acid and have compared it with the increase in free fatty acids (Fig. 2). For chloroplasts in mannitol or sucrose media, the observed increase agrees well with what was expected, whereas in NaCl-containing media some extra fatty acids are produced which may be due to breakdown of phospholipids.

In one experiment chloroplasts were isolated in mannitol medium (pH 7.5), and part of the chloroplasts were resuspended in mannitol medium and another part in

TABLE I

CONCENTRATION OF THE MAIN CHLOROPLAST GALACTOLIPIDS AND THEIR DERIVATIVES AND OF TOTAL FREE FATTY ACIDS IN SPINACH CHLOROPLASTS

Spinach chloroplasts isolated and suspended in two types of media, immediately after isolation and after storage at room temperature for 5 and 22 h. Concentrations in μmoles/mg chlorophyll, from measurement of galactose and free fatty acids. GGDG-X, a derivative from digalactosyl diglyceride. GDG-X and GDG-Y, derivatives from monogalactosyl diglyceride.

Medium	*Aging period (h)*	*Digalactosyl diglyceride*	*GGDG-X*	*Monogalactosyl diglyceride*	*GDG-X*	*GDG-Y*	*Free fatty acids*
Mannitol or sucrose	0	0.77	0.13	1.05	0.15	0.30	0.14
	5	0.41	0.21	0.36	0.21	0.63	0.80
	22	0.28	0.21	0.20	0.21	0.69	1.15
NaCl	0	0.90	—	1.35	—	0.15	0.10
	5	0.90	0.02	0.78	—	0.16	1.15
	22	0.92	0.07	0.42	0.02	0.20	1.55

NaCl medium at the same pH. In this case the rates and patterns prevailing in the mannitol medium were observed also in the NaCl medium.

Using gas chromatography it was further established that the free fatty acids present in freshly isolated chloroplasts consist mainly of C_{16} and C_{14} saturated acids, with very little C_{18} acids present (but *cf.* ref. 7). The acids released during aging consisted mainly of C_{18} and to some extent of C_{16} unsaturated acids. The same acids are released in mannitol and NaCl (both at pH 7.5).

The upper part of Fig. 3 shows the time course of release of free fatty acids in concentrated chloroplast suspensions, containing 1.5–3 mg chlorophyll per ml in mannitol and NaCl media.

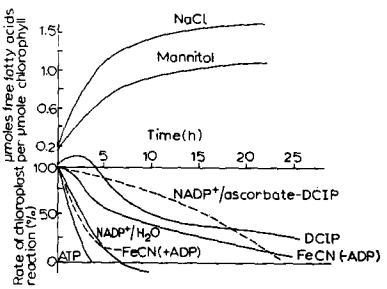


Fig. 3. Time course of release of free fatty acids in isolated chloroplasts and of the relative rates of several chloroplast reactions after aging in mannitol-based media. Chloroplast reactions took place at high light intensity.

Photochemical reactions

The rate of decay of the photochemical reactions studied, using chloroplasts isolated and aged in mannitol media (pH 7.5), is summarized in the lower part of Fig. 3.

The Hill reactions show complex decay curves. With DCIP (measured at pH 7.0), an initial increase in rate was usually found and may be correlated with uncoupling of photophosphorylation (*cf.* ref. 2). With ferrycyanide this effect was not seen, even when no ADP was present. In the presence of ADP, initial rates of ferricyanide reduction were higher, but decay was more rapid (*cf.* also refs. 2, 3).

Photoreduction of $NADP^+$ with water as electron donor, and in the presence of ferredoxin, decays at a rather uniform rate, much more rapidly than the Hill reaction. However, $NADP^+$ reduction with ascorbate–indophenol as the electron donating system was quite stable. This is in agreement with observations by McCARTY AND JAGENDORF[2] using intact and damaged bean chloroplasts.

Photophosphorylation, both cyclic (with phenazine methosulfate as cofactor) and noncyclic (with ferricyanide present) decayed very rapidly with half-times between 1.5 and 2 h.

Effects of serum albumin

The rate and patterns of galactolipid transformations were not changed by the addition of serum albumin to the suspension medium. Also, no effect was observed upon rate and time course of DCIP reduction at high light intensity. It could be confirmed, however, that albumin stimulates both cyclic and noncyclic photophosphorylation and enhances and stabilizes the phosphorylation efficiency (*cf.* refs. 2, 3, 23). Representative results are given in Table II.

TABLE II

INITIAL RATES OF PHOTOPHOSPHORYLATION (μmoles ATP/mg CHLOROPHYLL PER h), PHOTOREDUCTION (μmoles FERRICYANIDE/mg CHLOROPHYLL PER h) AND PHOSPHORYLATION EFFICIENCY (P/2*e*), AS INFLUENCED BY THE ADDITION OF SERUM ALBUMIN TO SPINACH CHLOROPLASTS

Spinach chloroplasts isolated and suspended in 0.35 M NaCl–0.02 M Tris–HCl (pH 7.5).

Experiment	*mg albumin/mg chlorophyll*			
	0	*3*	*6*	*27*
1. Cyclic photophosporylation	200	250	359	493
2. Cyclic photophosporylation	232	276	364	464
Noncyclic photophosporylation	56	89	159	182
3. Cyclic photophosporylation	94	191	277	434
Noncyclic photophosporylation	70	112	139	252
Ferricyanide reduction	190	284	291	438
P/2*e*	0.74	0.79	0.96	1.15

The initial stimulation is especially pronounced when control rates are low, whereas maximal rates differ less between experiments. The quality of the starting material seems to be quite variable, probably being dependent on season and growth conditions. With chloroplasts isolated from leaves grown in spring, rates of cyclic photophosphorylation of 500 μmoles ATP/mg chlorophyll per h could be obtained without addition of serum albumin.

Fig. 4 shows the time course of decay of noncyclic photophosphorylation of Expt. 3 (Table II). Fig. 5 shows the change in P/2*e* in the same experiment. These figures show that the enhanced rates and tight coupling are stabilized for 1–2 h at most and only with high albumin/chlorophyll ratios. After this time, and when relatively less albumin is present, rates of decay are not much different from those of the control.

Influence of the density of chloroplast suspensions

When chloroplast suspensions of varying density were compared, it was observed that the rate of release of fatty acids increased in dilute suspensions in mannitol or

sucrose media and approached the rate observed in NaCl media. In the latter, no such increase was observed.

Cyclic photophosphorylation was measured with chloroplasts from suspensions containing between 0.3 and 4.5 mg chlorophyll per ml. Highest initial rates were obtained when suspensions contained 1.5–2 mg chlorphyll per ml (*cf.* also ref. 3). Such high rates, however, tended to decay somewhat more rapidly; hence, this effect cannot be similar to the effect of albumin reported above.

The stability of DCIP reduction was found to be very strongly influenced by the density of the suspension in which chloroplasts were stored. Whereas Fig. 4 represents the decay of this Hill reaction in dense suspensions, more rapid rates of decay were seen with more dilute suspensions; half-times of 2–4 h were common for suspensions containing 0.1–0.2 mg chlorophyll per ml.

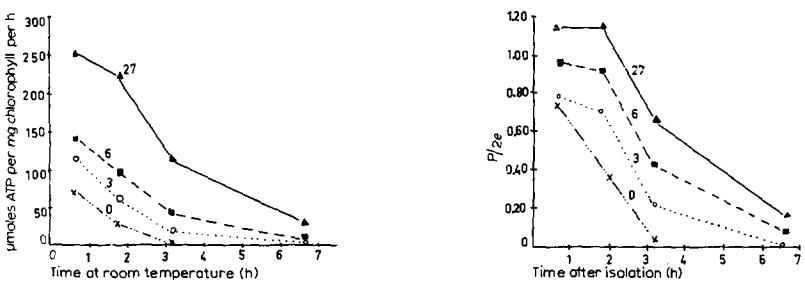


Fig. 4. Time course of the capacity for noncyclic photophosphorylation of spinach chloroplasts, isolated and suspended in 0.35 M NaCl (pH 7.5), as influenced by serum albumin in concentrations of 0, 3, 6 and 27 mg protein per mg chlorophyll. Experiment of 28.6.68.

Fig. 5. Time course of change in efficiency of noncyclic photophosphorylation (P/2*e*) in aging spinach chloroplasts, as influenced by serum albumin. Experiment of 28.6.68.

Influence of suspension medium on photochemical reactions

An influence of the suspension medium in which chloroplasts were stored and aged was studied especially with the most labile photoreactions, since the greatest relative difference in content of free fatty acids may be expected during the first h of storage at room temperature. No significant difference was noticed for photophosphorylation so far. However, the decay of DCIP reduction by chloroplasts from very thin suspensions proved to be influenced by the medium used for aging. In this case, mannitol or sucrose proved beneficial, and the time required to halve the initial rate was increased from about 2 h for chloroplasts stored in NaCl to about 4 h for those stored in

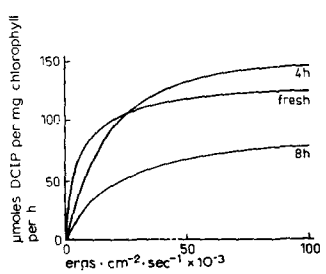


Fig. 6. Rates of DCIP reduction at different light intensities, in fresh and aged spinach chloroplasts, isolated and suspended in 0.35 M NaCl–Tris (pH 7.5).

mannitol or sucrose. It may be pointed out here that in such dilute suspensions, the rate of release of free fatty acids was about equal in both suspension media (*cf.* preceding section).

Photoreduction at low light intensities

The photoreduction of DCIP was followed over a wide range of light intensities. Fig. 6 shows the contrasting changes in rates under limiting and saturating light intensities. It appears that the quantum efficiency decreases much more rapidly during aging than do saturated rates. A rapid increase in quantum requirement of DCIP reduction, after incubation of spinach chloroplasts with bean galactolipase, has been observed by BAMBERGER AND PARK[25].

DISCUSSION

The experiments reported demonstrate that complex changes occur during and after isolation of chloroplasts by standard techniques. Activity of galactolipase could always be observed in spinach chloroplasts. The hydrolysis of mono- and digalactolipids by isolated spinach galactolipase shows different pH optima[20]. But in suspensions of whole spinach chloroplasts, lowering pH from 7.5 to 6.0 had little influence on galactolipid transformations. Important differences arise when different osmotic components of the medium are compared. In media containing 0.35 M NaCl, monogalactolipid is hydrolyzed with release of the equivalent amount of free fatty acid. In media containning 0.5 M sucrose or mannitol, the activity of galactolipid transacylating enzymes becomes evident already in freshly isolated chloroplasts. Since isolation in NaCl solutions results in the loss of stroma protein[26], it may be supposed that the transacylating enzyme discovered by HEINZ[22] is localized in the plastid stroma and is easily lost. As a result of these events, the rate of appearance of free fatty acids is lower in chloroplasts isolated in mannitol or sucrose media than in media containing NaCl. Since, however, many other changes take place in aging chloroplasts, it remains a question how much influence the observed changes in the main lipid fractions have on the photochemical activities.

In work done with mitochondria, WOJTCZAK AND LEHNINGER[27] observed that fatty acids caused swelling and uncoupling of oxidative phosphorylation. These effects could be overcome by serum albumin and by ATP. BJÖRNTORP *et al.*[28] reported damage to mitochondrial membranes caused by fatty acids, resulting in loss of respiratory cofactors. Respiratory processes could be protected by serum albumin or by the addition of cofactors for respiration. Swelling and damage in chloroplasts caused by fatty acids was first reported by MOLOTKOVSKY AND ZHESTKOVA[29], who also noted that the effect could be reversed by serum albumin or by ATP. Similar observations were made by other authors[2,3,23], but effects of serum albumin were also observed when there was no evidence of lipid breakdown[3].

From the present results it is seen that there is a great difference in the stability of the various photochemical reactions of chloroplasts.

Photoreductions in System II, represented by DCIP reduction[30], seem to be resistant to a certain amount of fatty acids. Strong inhibition was only observed when free fatty acids approached a concentration of 1 mole/mole of chlorophyll. Similar amounts were required in the work by MOLOTKOVSKY AND ZHESTKOVA[29]. However,

CONSTANTOUPOULOS AND KENYON[7] found severe inhibition of photoreduction of trichloroindophenol after incubation during 1 h at room temperature under conditions in which the rate of release of fatty acids was only half that observed in the present experiments. The main experimental difference seems to have been the very low osmotic value of the suspension medium used by CONSTANTOPOULOS AND DYER[7]. Also, working with fragmented chloroplasts, WASSERMAN AND FLEISCHER[13] found photochemical reactions to be very labile at room temperature without evidence of lipid breakdown.

Further, in our experiments we found that the rate of photoreduction of DCIP decayed more rapidly as the chlorophyll content of the suspension was lowered. This more rapid decay was not paralleled by changes in galactolipase activity. This points to a mode of decay in which a necessary factor is lost more easily when chloroplast stroma is removed or when suspensions are diluted. The activity of galactolipase seems not to be concerned with the decay of photoreduction in these cases.

Photoreduction in System I, represented by $NADP^+$ reduction with ascorbate–DCIP, also seems to be rather independent of lipid components. It can be performed by very small particles, poor in colorless lipid components[31,32]. Our results are in agreement with those of MCCARTY AND JAGENDORF[2] and indicate that this system is not directly affected by galactolipase.

Photoreductions in which the Systems I and II are both engaged ($NADP^+$ and ferricyanide Hill reactions[30]) were more labile and especially the rates observed under phosphorylating conditions (Fig. 4). Still more labile was photophosphorylation. The rapid decrease in the ratio $P/2e$ indicates that the latter process is first affected during aging and that fatty acids may contribute to this uncoupling. We found no difference in decay of cyclic and noncyclic photophosphorylation. Both were about 50 % inhibited when about 0.1 mole fatty acid had been released per mole of chlorophyll. This is close to the amounts required for a similar percentage of inhibition in the work of MCCARTY AND JAGENDORF[2] (Fig. 9 l.c.). However, in our work full inhibition was reached at much lower concentrations of fatty acids than in experiments in which fatty acid was added externally[2,23].

The effects of serum albumin were in agreement with those found by other authors[3,23]. In our experience, serum albumin did not influence the changes in rate of DCIP reduction at saturating light intensities (but *cf.* ref. 7). The effects on photophosphorylation, illustrated in Figs. 4 and 5, were most pronounced at a moment when very little fatty acid had been released. Moreover, this initial stimulation was seasonally variable in contrast to galactolipase activity. Hence, at least the initial effect may be caused by properties of the protein other than the binding of fatty acids (*cf.* ref. 3). The binding of fatty acids may be one of the factors which contribute to the stability of photophosphorylation, since the rate of release of fatty acids was not affected by serum albumin.

From the results of electron microscopy and galactolipase action, BAMBERGER AND PARK[25] have concluded that a substantial part of the chloroplast lipids, notably the galactolipids, are not associated with chlorophyll but do constitute a continuous matrix in which protein particles containing pigment and lipids are embedded. It is this matrix which must be responsible for properties of the thylakoid membrane which may play a large role in photophosphorylation. For noncyclic photophosphorylation, a high degree of structural integrity of the thylakoid seems to be required[33,34]. Cyclic

photophosphorylation has been observed in digitonin particles which are poor in galactolipids, although higher concentrations of the detergent were inhibitory[32]. Since lipid is also present in the pigmented particles, attention may be paid to remarks made by ROSENBERG[35] who pointed out that the steric configurations of the plant linolenic acid and of the phytol residue of the chlorophylls permit a very close pairing of the chains of these compounds and may thus contribute to the efficient spacing of the pigment molecules. The efficiency of energy transfer between pigment molecules may be sensitive to very small changes in lipid composition, and electron transport at low light intensities (quantum yield) may be more sensitive to galactolipase action than rates at saturating intensities. This may explain the results illustrated in Fig. 6.

In conclusion, it is not clear whether the concentrations of free fatty acids in aging chloroplasts may reach levels required to block electron flow through Photosystems I and II at saturating intensities. It remains possible, however, that already very low concentrations of fatty acid may contribute to the uncoupling of photophosphorylation and may be responsible for low quantum yield of the Hill reaction.

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