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Photosynthetic Apparatus in Chilling-sensitive Plants

II. Changes in Free Fatty Acid Composition and Photoperoxidation in Chloroplasts following Cold Storage and Illumination of Leaves in Relation to Hill Reaction Activity

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Abstract. The composition of free fatty acids (FFA) in relation to Hill reaction activity and photoperoxidation of lipids was studied in chloroplasts isolated from fresh, cold and dark-stored as well as illuminated leaves of *Lycopersicon esculentum Mill.*, *Phaseolus vulgaris L.* and *Cucumis sativus L.* Following the cold and dark-storage of leaves the loss of Hill reaction activity is accompanied by approximately a 5-fold increase in the amount of FFA and by an increase in the percentage of unsaturated FFA, particularly that of linolenic acid. Illumination of the cold- and dark-stored leaves restores both Hill reaction activity and the content and composition of chloroplast FFA. Following the second and third cycles of cold storage and illumination of leaves the percentage of unsaturated fatty acids in chloroplasts increases while that of saturated ones decreases despite of the significant restoration of Hill reaction activity. Since the illumination of cold-stored leaves results in peroxidation of inhibitory fatty acids it seems likely that this phenomenon could, at least partially, be responsible for the restoration of Hill reaction activity. Inhibition of Hill reaction activity by exogenous linolenic acid in chloroplasts of fresh, cold-stored as well as cold-stored and illuminated leaves could be reversed following the incubation of chloroplast suspension with BSA, however only to a value measured in the absence of unsaturated fatty acid. All these results indicate that the inhibition of Hill reaction activity due to the cold and dark storage of leaves is caused by both inhibitory FFA released from chloroplast lipids as well as by damage to the thylakoid structure affecting the electron transport within photosystem II.

Abbreviations: BSA = bovine serum albumin; DCIP = 2,6-dichlorophenolindophenol; DGDG = digalactosyl diglyceride; HEPES = 2-(4(2-hydroxyethyl)-piperazinyl) ethanesulfonic acid; FFA = free fatty acids; MDA = malondialdehyde; MGDG = monogalactosyldiglyceride; TBA = thiobarbituric acid; Tris = *tris*-(Hydroxymethyl)aminomethane

Key words: Chilling – Chloroplasts – Cold storage – *Cucumis* – *Lycopersicon* – *Phaseolus* – Photosynthesis – Temperature (chilling).

Introduction

The ability to withstand chilling injury in both poikolothermic animals and chilling-sensitive plants has been associated with the degree of unsaturation of fatty acids of the membranes (Lyons, 1973; Raison, 1973). The higher the degree of unsaturation, the lower the temperature at which the phase change occurs in the lipid portion of the membrane. However, recent research has cast doubts on whether the degree of unsaturation of the fatty acids plays a dominant role in determining the temperature at which the phase change occurs in the lipid portion of the cellular membranes (Wilson and Crawford, 1974). It was found that leaves of chilling-resistant plants did not contain more unsaturated fatty acid than chilling-sensitive plants. Moreover, on hardening of the later ones there was no increase in the amount of unsaturated fatty acids or in the total weight of fatty acids in the leaf. On the other hand, chilling increased the percentage of linolenic acid and the total weight of fatty acids in chloroplasts of chilling-sensitive species (Wilson and Crawford, 1974).

In the accompanying paper (Kaniuga et al., 1978) it was reported that cold storage of leaves of chilling-sensitive species results in an increased sensitivity of Hill reaction activity to the exogenous linolenic acid. This suggests that unsaturated fatty acids were released during the cold treatment of leaves. Upon the illumination of leaves both Hill reaction activity and its sensitivity to linolenic acid were greatly restored, probably due either to incorporation of fatty acid into the thylakoid lipids or to photoperoxidation. This paper deals with the changes in chloroplast free

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fatty acid composition of the cold-stored and illuminated leaves as well as with photoperoxidation of chloroplast fatty acids upon illumination of leaves. These two processes were studied in relation to Hill reaction activity. Preliminary results were reported previously (Kaniuga et al., 1975).

Materials and Methods

Plant Material

Leaves of chilling-sensitive plants – tomato (*Lycopersicon esculentum* var. Eurocross and Revermoon) and cucumber (*Cucumis sativus* var. Skierniewickie) – were grown under greenhouse conditions used for commercial purpose while bean (*Phaseolus vulgaris* var. Piękny Jaś) was grown using our own cultivation procedure described previously (Kaniuga et al., 1978). Leaves of spinach (*Spinicia oleracea* var. Matador) were purchased in the local market.

Table 1. Free fatty acid content in chloroplasts from fresh, cold-stored and illuminated tomato leaves

Source of chloroplasts	FFA content (μmol mg chlorophyll^{-1})		
	Expt. A	Expt. B	Expt. C
Fresh leaves (F)	1.02	1.10	0.97
Leaves stored 3 days (A_3)	5.20	6.03	5.97
Leaves stored 3 days and then illuminated (R_3)	1.49	1.79	1.35

Detached leaves were stored at 0° C in dark for 3 days (A_3) then illuminated (8000 lx) for 2 h (R_3)

Cold Storage of Leaves, Reactivation with Light, Isolation of Chloroplasts, and Determination of Hill Reaction Activity

The procedures for all these treatment are described in the previous paper (Kaniuga et al., 1978).

Extraction of Chloroplast Lipids

A suspension of chloroplasts (about 5 mg of chlorophyll) was heated with isopropanol at 40–50° C for 3–5 min and extracted according to the classical method of Folch et al. (1957) as modified by Sastry and Kates (1964).

Separation of Free Fatty Acids

The FFA were prepared from the low Folch phase by the solvent extraction (Radin, 1969). The sample of the lipid fraction was gently concentrated at about 50° C and redissolved in 3 ml of diethyl ether, followed by four extractions with 1 ml of 4% aqueous disodium carbonate. The combined disodium carbonate solution containing FFA was washed twice with 2 ml of diethyl ether, and then the aqueous low phase was acidified with sulfuric acid and again extracted 4–5 times with 1 ml of diethyl ether. The combined extracts were dried with anhydrous disodium sulfate. The FFA were converted to their methyl esters with a 14% solution of boron trifluoride in methanol.

Determination of Free Fatty Acid Content

The content of FFA (extracted from Folch phase with 4% aqueous disodium carbonate) was determined using the colorimetric rhodamine 6G method described by Anderson and McCarty (1972). The following fatty acids were used as endogenous and exogenous

Table 2. Changes in free fatty acid composition of chloroplast of cold- and dark-stored and illuminated leaves of chilling-sensitive

Species	Source of chloroplast[a]	Hill reaction activity[b]	Fatty acid (% of total free fatty acid)												
			$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$	$C_{20:0}$	$C_{20:1}$	$C_{20:2}$	$C_{22:0}$	$C_{22:1}$
Chilling-sensitive															
Bean	F	81.8	3.2	7.5	26.9	6.7	14.3	9.7	10.7	2.3	8.7	1.4	1.2	7.4	T[d]
	A_3	9.8	2.4	3.3	29.6	8.7	13.9	13.5	7.5	7.8	7.4	0.3	T	3.8	1.8
	R_3	63.8	3.6	6.1	31.3	2.3	14.8	9.1	9.3	3.3	6.0	1.0	1.3	9.8	2.1
Cucumber	F	47.4	0.5	5.4	28.5	6.1	12.2	16.3	4.9	2.2	8.3	1.0	5.6	4.4	4.6
	A_3	10.3	0.6	4.5	24.6	3.7	10.0	13.3	18.1	9.3	6.2	T	5.0	T	4.7
	R_3	49.3	3.6	5.1	26.5	3.5	13.9	12.8	6.5	5.9	6.6	T	7.5	8.1	T
Tomato	F	84.0	1.1	5.8	25.5	0.8	16.9	3.8	11.6	3.4	6.2	3.6	10.7	5.7	4.9
	A_3	20.4	0.4	3.2	22.0	0.6	11.5	6.3	12.1	16.1	7.3	4.0	8.9	1.5	6.2
	R_3	82.0	0.9	2.9	27.3	1.5	15.4	4.9	6.9	4.2	7.8	4.9	10.9	6.1	6.3
Chilling-resistant															
Spinach	F	205.0	1.2	5.1	27.6	7.0	15.4	10.7	10.2	1.9	2.4	12.0	0.5	T	6.0
	A_3	237.0	1.9	8.8	29.5	6.8	12.3	16.3	5.6	2.1	1.3	10.0	2.5	T	2.9
	R_3	240.0	1.8	5.4	27.9	6.3	14.0	16.0	6.4	2.1	2.0	10.8	1.5	T	5.8

[a] F = fresh leaves; A_3 = leaves stored for 3 days at 0° C in dark; R_3 = leaves stored for 3 days at 0° C in dark and then illuminated
[b] μmol DCIP red mg chl^{-1} h^{-1}
[c] Δ in relation to the control (F)
[d] T = trace

standards: palmitic, $C_{16:0}$, stearic, $C_{18:0}$, linoleic, $C_{18:2}$, and linoleic, $C_{18:3}$. The experimentally estimated absorption coefficient was found to be 12.5 mM^{-1} cm^{-1}.

Gas Liquid Chromatography

The methyl esters of fatty acids were separated on Pye-Unicam 104 model 24 chromatograph using a 7-foot (2.14 m) ×4 mm column with 10% PEGA on 100/120 mesh Diatomite CAW. The column temperature was 200° C with the flow rate of argon of about 40 ml min. The relative quantity of fatty acids was estimated by the areas under the peaks.

Determination of Lipid Peroxidation

The peroxidation of lipids was followed by the thiobarbituric acid (TBA) method in which TBA reacts with malondialdehyle, a decomposition product of the oxidation of polyunsaturated fatty acids (Kwon et al., 1965). For determination of lipids peroxidation the method of Heath and Packer (1968a) was used applying an extinction coefficient of 155 mM^{-1} cm^{-1} at 532 nm, corrected for nonspecific absorbance at 600 nm. The amount of MDA was determined in chloroplast preparations obtained from either fresh or cold-stored and illuminated leaves, usually about 1.5 h after the end of illumination.

Reagents

Linolenic acid and thiobarbituric acid were purchased from Koch and Light while HEPES was obtained from Sigma. Fatty acid standards were obtained from Pye-Unicam and Serva. All other reagents were provided by P.O.Ch. (Poland). BSA (fraction V) was defatted by the method of Chen (1967).

and chilling-resistant plants

% of Total FFA		Ratio: saturated/ unsaturated	Δ unsaturated[c]	Δ $C_{18:3}$[c]
Saturated	Unsaturated			
68.0	32.0	2.13		
60.4	39.6	1.52	+ 7.6	+ 5.5
71.6	28.4	2.52	− 3.6	+ 1.0
59.3	40.7	1.45		
45.9	54.1	0.85	+13.4	+ 7.1
63.2	36.2	1.74	− 4.5	+ 3.7
61.2	38.8	1.58		
45.9	54.1	0.85	+15.4	+12.7
60.4	39.6	1.53	+ 0.1	+ 0.8
51.7	48.3	1.07		
53.8	46.2	1.16	− 2.1	+ 0.2
51.1	48.9	1.04	+ 0.6	− 0.2

for 2 h

Results

Changes in the Amount and Composition of Free Fatty Acid in Chloroplasts Following Cold Storage of Leaves in the Darkness and Their Illumination

Data presented in Table 1 show that the cold and dark storage of tomato leaves results in the 5-to-6-fold increase in amount of FFA. Upon illumination of leaves the amount of FFA declines to the level somewhat above that found in fresh leaves.

The cold and dark storage of leaves produces an increase in the percentage of free unsaturated fatty acids in chloroplasts while on the illumination of these leaves results in a decrease in the proportion of unsaturated fatty acids observed in all three species studied (Table 2). The ratio of the total saturated: unsaturated fatty acids decreases upon the colike storage and returns to the original value after illumination of leaves. Upon cold storage of leaves, the most significant changes occur in the level of linolenic acid, which increases by 5.5, 7.1 and 12.7% of the total FFA in bean; cucumber, and tomato chloroplasts, respectively. Upon illumination of leaves linolenic acid decreases in bean and tomato chloroplasts to its level in the chloroplasts from fresh leaves, while in cucumber chloroplasts it exceeds that of the control value. In chloroplasts of the cold-stored cucumber leaves linoleic acid ($C_{18:2}$) greatly increases also (by 13.2% of the total FFA). Although it is known that hexadeca-7,10,13-trienoic acid ($C_{16:3\omega_3}$) is a component of MGDG of cauliflower chloroplasts (Schwertner and Biale, 1973) as well as of MGDG in lipid fraction from leaves of several plant species (Jamieson and Reid, 1971; Smoleńska and Kuiper, 1977), there are no detectable amounts of this fatty acid in chloroplasts isolated from either fresh, cold-stored, or illuminated leaves of bean, cucumber and tomato. Moreover, the level of both $C_{18:0}$ and $C_{18:1}$ fatty acids in chloroplasts of cold-stored and illuminated leaves of chilling-sensitive plants do not change essentially, except for some increase (by 3.4%) of $C_{18:1}$ in bean chloroplasts isolated from cold-stored leaves.

None of these changes in the composition of FFA occur in spinach chloroplasts a chilling-resistant species, following cold storage and illumination of leaves. Both, the ratio of saturated: unsaturated FFA and the proportion of linolenic acid are almost the same in chloroplasts from fresh, cold-stored as well as illuminated leaves. Cold storage results only in an increase in the oleic acid level and in a decrease in both stearic and linoleic acid contents.

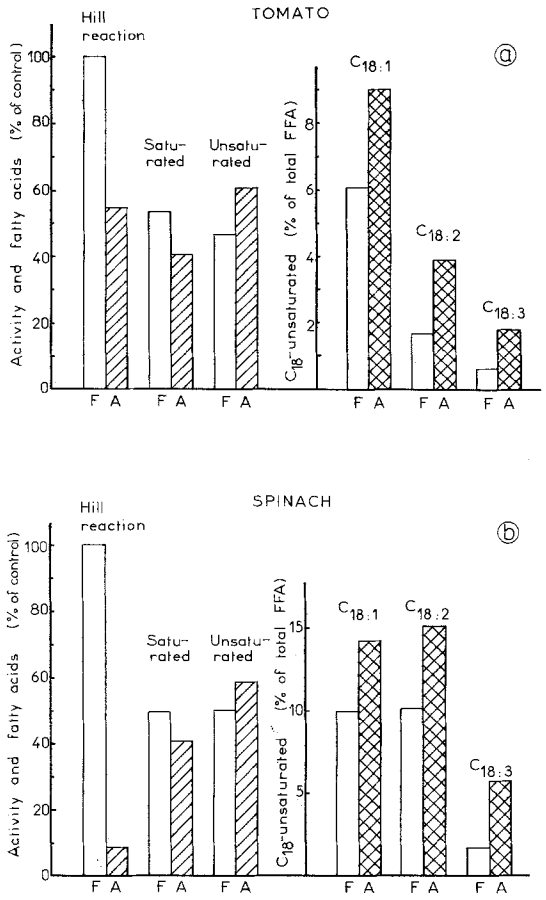

Fig. 1a and b. Changes in FFA composition of cold-stored tomato **a** and spinach **b** chloroplasts. Chloroplast preparations of tomato (2 mg of chlorophyll/ml) and spinach (2.8 mg of chlorophyll/ml) were stored at 4° C in dark for 116 and 95 h, respectively. Hill reaction activity in control tomato and spinach chloroplast preparations was 202 and 85 µmol DCIP reduced mg chlorophyll^{-1} h^{-1}, respectively. F and A, control and aged chloroplast preparations, respectively

Changes in Free Fatty Acid Composition Following Cold Storage of Isolated Chloroplasts in the Dark

Cold storage of isolated tomato and spinach chloroplasts results in essentially the same pattern of changes in FFA composition as does the cold and dark storage of leaves i.e., a decrease in saturated and an increase in unsaturated fatty acids (Fig. 1a, b). However, there are two differences: Firstly, the illumination of the cold-stored chloroplast suspension in the dark does not restore Hill reaction activity. Secondly, the ratio of total free saturated: unsaturated fatty acids of the cold-stored spinach and tomato chloroplast preparations (0.70 and 0.68 respectively) are lower than those observed in chloroplasts isolated from the leaves following their cold storage in the dark (cf. Table 2).

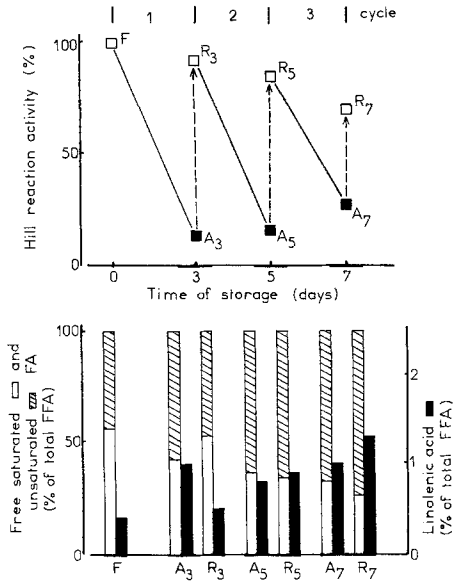

Fig. 2. Hill reaction activity and FFA composition of chloroplasts of tomato leaves following repeated cold storage and illumination. Detached leaves were stored at 0° C in dark for 3 days then illuminated (8000 lx) for 2 h and again cold-stored and illuminated at the time indicated. Hill reaction activity (84 µmol DCIP reduced mg chlorophyll^{-1} h^{-1} in the control) and fatty acid composition were determined in chloroplast preparations from fresh leaves (F), leaves stored for 3, 5 and 7 days (A_3, A_5 and A_7) and illuminated leaves (R_3, R_5, R_7)

Relationship Between Chloroplast Free Fatty Acid Composition and Hill Reaction Activity of Tomato Leaves Following Repeated Cold Storage and Illumination

The relation between the FFA composition and Hill reaction activity in chloroplasts following cold storage and illumination of tomato leaves is shown in Figure 2. The first cycle (3 days of cold storage in the dark followed by illumination of leaves) results in typical changes in FFA composition: (1) an increase of the ratio of unsaturated: saturated fatty acids accompanied by the loss of Hill reaction activity upon cold storage of leaves in the dark; (2) a decrease of the unsaturated: saturated fatty acids ratio accompanied by the restoration of this activity upon illumination of leaves. Following the second and the third cycles of cold storage and illumination of leaves, the percentage of saturated fatty acids gradually decreases while that of unsaturated ones (particularly that of linolenic acid) increases. These changes take place independently of the significant reactivation of Hill reaction activity on the 5th and even on 7th day of cold storage of leaves followed by their illumination. The lack of correlation between Hill reaction activity and the level of unsaturated fatty acids seems

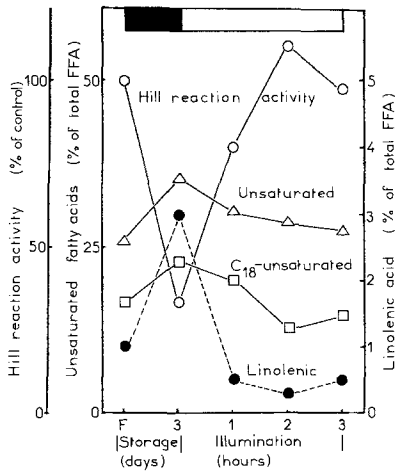

Fig. 3. Time course of light-stimulated disappearance of chloroplast unsaturated fatty acids and the restoration of Hill reaction activity in tomato leaves. Detached leaves were stored at 0° C in dark for 3 days followed by illumination (8000 lx) at 25° C for the time indicated

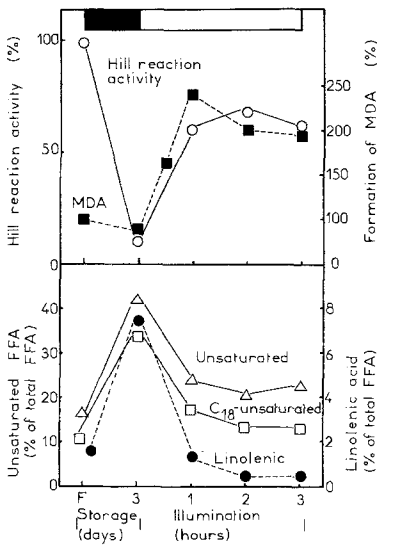

Fig. 4. Reactivation of Hill reaction activity accompanied by the formation of malondiadehyde (*upper*) and a decrease of unsaturated fatty acids (*bottom*) in chloroplasts of cold-stored and illuminated tomato leaves. Leaves were stored for 3 days at 0° C in dark followed by illumination (8000 lx) at 25° C for the time indicated. Hill reaction activity of the control was 82 μmol DCIP reduced mg chlorophyll^{-1} h^{-1} while the initial level of MDA was 134.7 μmol mg chlorophyll^{-1}

to indicate that, after aging of leaves in the cold and dark for a period of more than 3 days, the illumination of leaves results in restoration of Hill reaction activity despite accumulation of fatty acids in chloroplasts.

Peroxidation of Chloroplast Fatty Acids and the Restoration of Hill Reaction Activity During Illumination of the Cold-stored Leaves

Figure 3 shows the stimulatory effect of light on the disappearance of FFA in chloroplasts. After 3 days of cold and dark storage of tomato leaves, Hill reaction activity and the level of unsaturated fatty acids in chloroplasts reach a minimum and a maximum, respectively. Following illumination of leaves for 1–1.5 h, Hill reaction activity is greatly restored whereas the percentage of unsaturated fatty acid declines. The prolonged illumination does not essentially affect either fatty acid level or Hill reaction activity. These results suggest that similarly to isolated chloroplasts (Heath and Packer, 1968a, b; Hoshina et al., 1975; Takahama and Nishimura, 1975, 1976) the illumination of leaves induces peroxidation of unsaturated fatty acids and formation of MDA, a product of the decomposition of tri-unsaturated fatty acids poroxides. To check this possibility, experiments of the type shown in Figure 4 were carried out. It was found that there is a close relation between Hill reaction activity, linolenic acid disappearance and MDA formation during illumination of the cold-stored leaves in the darkness. An almost constant level of MDA during cold storage of leaves in the absence of light is accompanied by a fast increase of unsaturated fatty acids, specifically $C_{18:3}$. A rapid linear phase of MDA formation upon illumination of leaves for the first hour is accompanied by a rapid decrease in the linolenic acid content while after one hour of illumination both MDA and linolenic acid levels slowly decrease. Thus, in contrast to isolated chloroplasts, in which light results in destruction of the photosynthetic reaction due to the peroxidation of membrane bound (Heath and Packer, 1968a) or free (Hoshina et al., 1975) fatty acids, the illumination of leaves of chilling-sensitive plants following their storage in the dark causes the restoration of Hill reaction activity, probably resulting from the peroxidation of inhibitory fatty acids.

Reversal of Linolenic Acid Inhibition of Hill Reaction Activity by Bovine Serum Albumin

Data presented in Table 3 show that Hill reaction activity inhibited completely with the exogenous linolenic acid can be restored upon incubation of the chloroplasts with BSA, probably due to its ability to bind long-chain fatty acids. In chloroplast preparations obtained from fresh as well as cold-stored and illuminated leaves the addition of BSA restores Hill reaction activity (in the presence of added linolenic

Table 3. Reversal of linolenic acid inhibition of Hill reaction activity by BSA. Tomato leaves were stored at 0° C in the dark followed by 2 h illumination (8000 lx) at 25° C where indicated. Methanolic solution of linolenic acid in the amount to produce the complete inhibition of Hill reaction activity was added in darkness to the reaction mixture containing chloroplast preparation and incubated for 1 min. Then defatted BSA was added (6–7 mg/ml of the reaction mixture) and after 3 min of incubation the sample was illuminated for 0.5–1 min and the reduction of DCIP was measured. Hill reaction activity is expressed in μmol of DCIP reduced mg chl^{-1} h^{-1}

Source of chloroplast	Hill reaction activity	Linolenic acid added (nmol mg chl^{-1})	Hill reaction activity restored by BSA
Fresh leaves	54.2	17.3	64.3
Leaves stored 2 days	22.6	12.0	20.6
Leaves stored 3 days	11.3	10.2	9.0
Leaves stored 3 days and illuminated	54.8	16.4	63.8

acid) and even stimulates this activity by about 18% in comparison with the control value (measured without this fatty acid). However, when chloroplasts from the cold-stored leaves are incubated with linolenic acid, Hill reaction activity in the presence of BSA does not exceed the value measured in the absence of linolenic acid.

Discussion

There are few data concerning the changes in FFA in chloroplasts following cold treatment of chilling-sensitive species. The chilling of bean and cucumber at 5° C (Wilson and Crawford, 1974) and the storage of cucumber leaves at 1° C in the dark (De Kok and Kuiper, 1977) results in an increase of the percentage of linolenic acid in chloroplasts. A different pattern of change in fatty acid composition is observed in whole leaves. On the chilling of bean and cucumber (Wilson and Crawford, 1974) the percentage of linolenic acid and the total weight of fatty acids decrease while the storage of cucumber leaves and leaf discs in the dark at low temperature does not alter their fatty acid composition (De Kok and Kuiper, 1977). Increased sensitivity of Hill reaction activity to exogenous linolenic acid upon cold storage of leaves of chilling-sensitive plants in dark is diminished following illumination of leaves, suggesting some changes in lipid composition of the chloroplasts (Kaniuga et al., 1978). As indicated in Tables 1 and 2, cold storage of leaves of bean, cucumber and tomato in the dark induces significant changes in chloroplasts FFA content and composition. Ratio of total saturated:unsaturated fatty acids decreases upon cold storage and returns to the original value upon illumination of leaves. The most evident changes occur in the level of linolenic acid. Increase of FFA during the cold storage probably originates from galactolipid degradation, since chloroplasts are known to contain both substrate (MGDG and DGDG) and galactolipase activity (Sastry and Kates, 1964; Helmsing, 1967; Wintermans et al., 1969; Kates, 1970; Mazliak, 1973; Anderson et al., 1974; Hoshina et al., 1975). The observations indicating that: (1) free $C_{16:3}$ fatty acid is not detectable and (2) the level of free $C_{18:0}$ and $C_{18:1}$ fatty acids does not change following the cold storage and illumination of laves, may suggest that these fatty acids are either minor components of galactolipids present in chloroplasts of chilling-sensitive species or that galactolipase specifically releases only $C_{18:3}$ during the cold and dark storage of leaves.

There is no essential difference in the composition of FFA in chloroplasts of cold-stored leaves and isolated chloroplasts following such a treatment. However, the loss of Hill reaction activity in isolated chloroplasts is not restored upon illumination although the removal of fatty acids due to its peroxidation might occur (Heath and Packer, 1968a, b; van Hasselt 1974; Hoshina et al., 1975; Takahama and Nishimura, 1975, 1976).

Protective action of BSA on the mitochondrial as well as on the chloroplast electron transport against the inhibition by unsaturated fatty acids is well known (Constantopoulos and Kenyon, 1968; Friedlander and Neumann, 1968; Wasserman and Fleischer, 1968; Siegenthaler, 1972; Anderson et al., 1974; Kulandaivelu and Hall, 1976). As can be concluded from Table 3, BSA binds both exogenous and endogenous fatty acids in chloroplast preparations obtained from fresh as well from cold-stored and illuminated leaves resulting in a stimulation of Hill reaction activity. However, the addition of BSA to chloroplasts isolated from the cold-stored leaves is not sufficient to restore the Hill reaction activity suggesting that some structural changes in the thylakoid membranes are also involved. This also seems to be evident from Figure 2. Only the first cycle of inactivation/reactivation of Hill reaction is a completely reversible process. Thus, there are two interrelated processes responsible for the observed changes in Hill reaction activity: (1), the inhibitory effect of both added and endogenous fatty acids, which can be reversed by BSA treatment; (2), the effect of cold storage of leaves in the dark on the structure of chloroplast membranes as manifested by a decrease of manganese content in

chloroplasts (manuscript in preparation). Moreover, an increased percentage of unsaturated fatty acids in chloroplasts, even upon illumination of leaves during the second and the third cycles, suggests a disorder in fatty acid peroxidation.

It has been suggested that peroxidation of lipids occurs in thylakoid membranes and is followed by release of peroxidation products into the incubation medium (Heath and Packer, 1968a, b). However, the experiments of Hoshina et al. (1975) indicate that MDA formation occurs in both the medium and in the thylakoid membranes. The major fatty acids lost by peroxidation are $C_{16:1}$ $C_{16:3}$ and $C_{18:3}$ (Heath and Packer, 1968a). The data shown in Figure 4 suggest a correlation between the disappearance of free linolenic acid and the formation of MDA. Light-induced peroxidation of fatty acids as measured by MDA formation in isolated chloroplast preparations was studied in detail (Heath and Packer, 1968a, b; van Hasselt, 1974; Hoshina et al., 1975; Takahama and Nishimura, 1975, 1976). The formation of MDA is stimulated when the electron transport between water and photochemical reaction center II is blocked by Tris-washing or by aging of chloroplast preparations at 4° C in dark (Takahama and Nishimura, 1975). It is also more effective in cucumber leaf discs when studied at 1° C than at higher temperatures (van Hasselt, 1974). Very rapid formation of MDA during the first hour of illumination of the cold-stored tomato leaves in the dark (Fig. 4) may be ascribed to damage of electron transfer on the oxidizing side of photosystem II following the cold treatment of leaves (Kaniuga et al., 1978), as does also washing of chloroplasts with Tris (Yamashita and Butler, 1969).

Both the previous (Kaniuga et al., 1978) and the present papers suggest that in contrast to aged isolated chloroplasts in which light-induced lipid peroxidation seems to be related to photoinactivation or to photodestruction of chloroplasts (Heath and Packer, 1968a; Takahama and Nishimura, 1975) the light-induced peroxidation in the cold-treated detached leaves removes inhibitory fatty acids and thereby results in the restoration of Hill reaction activity. This process appears to be supported also by the removal of fatty acids due to light-stimulated synthesis of chloroplast lipids and by an increase of the manganese level following illumination of cold-stored leaves (manuscript in preparation).

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