EFFECT OF LIGHT ON LIPID PEROXIDATION IN CHLOROPLASTS

Robert L. Heath and Lester Packer

Department of Physiology, University of California, Berkeley, California

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During an investigation of possible mechanisms involved in chloroplast deterioration, evidence was sought for lipid peroxidation since this reaction is frequently associated with membrane damage. The high concentrations of unsaturated fatty acids, particularly linolenic acid, in chloroplast lipids (1) suggests their potential susceptibility to rapid cyclic peroxidation reactions. Although chlorophyll is known in chemical systems to catalyze lipid peroxidation (2), the occurrence of this reaction in chloroplasts has not been demonstrated.

Lipid peroxidation has been found in mitochondria (3) and microsomes (4) where a slow reaction associated with deterioration and lysis has been described by Tappel and Zalkin. In mitochondria, Schneider, et al., (5) have also shown that the process is closely associated with swelling and membrane disruption, and eventual disintegration of mitochondria accompany production of lipid peroxide products under certain metabolic conditions. In microsomes an enzymatic peroxidation of lipids linked to electron transport in the NADPH oxidase system has recently been described by Hockstein et al.(6). It now appears that a similar situation may exist in chloroplasts since the formation of malonaldehyde, a lipid peroxidation decomposition product, is remarkably increased upon illumination of spinach chloroplasts.

METHODS

Chloroplasts were isolated from spinach leaves in NaCl (175 mM) Tris-HCl (50 mM, pH 8) and stored for periods up to one hour at 0° C in the dark. The experiments with chloroplasts were performed in tubes kept at 25° C either in the light (1800 ft-candles) or dark for specified time intervals. Assays were then performed to test for the occurrence of malonaldehyde, a decomposition product of polyunsaturated fatty acid hydroperoxides (7,8) by the 2-thiobarbituric acid (TBA) reaction. The procedure involved addition of 2.0 ml of 0.5% TBA (in 20% trichloroacetic acid) to an equal volume of sample. This mixture was incubated for 25 minutes at 95° C, rapidly cooled to room temperature, and absorbancy

determined at 532 and 600 mμ. The amounts of malonaldehyde formed (in the total reaction mixture) were calculated using an extinction coefficient of 155 mM^{-1} Cm^{-1} at 532 mμ (9) corrected for non-specific absorbance at 600 mμ. Low concentrations of NaCl, which are present in reaction mixtures due to dilution of chloroplasts, do not affect TBA values.

RESULTS

The kinetics of malonaldehyde formation in spinach chloroplasts are shown in figure 1. An initial level of malonaldehyde, detected at zero time, is maintained nearly constant in the dark control during the eight-hour time course of the experiment. However, upon illumination, the initial level of malonaldehyde in chloroplasts is significantly increased following a brief induction period. The time course of the TBA reaction indicates a near-linear phase of malonaldehyde formation (22 mμ moles/mg chlorophyll/hr.) between 2-5 hours, after which the reaction terminates. Hence, this experiment provides evidence that endogenous unsaturated lipid components of spinach chloroplasts decompose to form malonaldehyde by a light-stimulated process. It is not known yet whether the effect of illumination is upon induction of lipid peroxidation in chloroplasts or upon the subsequent steps involved in decomposition to form malonaldehyde.

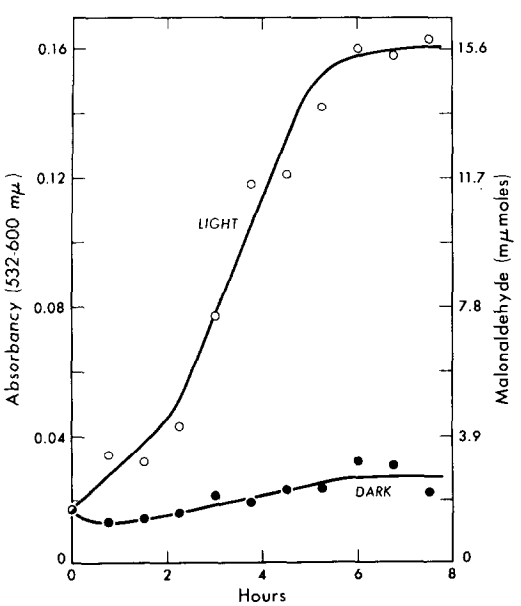

Figure 1. Effect of Illumination on the Occurrence of the TBA Reaction in Spinach Chloroplasts. The reaction mixture (15 ml) contained chloroplasts (10 μg chlorophyll/ml), NaCl (35 mM), and Tris-HCl buffer (20 mM at pH 8).

Some other factors which affect peroxidation of endogenous chloroplast lipids were further studied as shown in Tables I and II. The results of Table I show that the amounts of malonaldehyde formed in chloroplasts is dependent upon chlorophyll concentration. Malonaldehyde formation, after the induction phase, appears nearly linear with chlorophyll concentrations up to 30 μg/ml in illuminated chloroplasts. This extended time study of the TBA reaction, of up to 28 hours, shows that chloroplasts maintained in the dark maintain nearly the same initial level of malonaldehyde. Usually no change in the light-stimulated level of malonaldehyde occurs after termination of the reaction (cf. figure 1).

TABLE I

DEPENDENCE OF LIGHT-STIMULATED MALONALDEHYDE FORMATION ON CHLOROPLAST CONCENTRATION

The reaction mixture (15 ml) contained chloroplasts as indicated, NaCl (2 mM) and Tris (20 mM, pH 8). Other conditions as in Methods.

Chlorophyll μg/ml	Illumination	mμ moles Malonaldehyde Formed					
		0	4	7	21	24	28
10	+	5.3	9.8	13.8	18.2	12.4	12.9
	-	3.0	3.7	1.7	1.5	1.2	1.3
20	+	2.7	11.5	25.2	24.0	24.1	23.4
	-	2.6	5.4	2.4	2.4	3.6	2.3
30	+	3.3	17.3	29.4	34.2	34.1	34.6
	-	2.5	4.6	4.4	2.7	3.4	3.2
40	+	3.6	19.2	27.3	36.5	36.5	37.2
	-	2.8	4.6	2.9	2.6	3.8	3.6

The addition of partially-peroxidized linolenic acid to the reaction mixture increases the TBA reaction, and again this effect is found to be light stimulated. Table II shows that the formation of malonaldehyde occurs in the two controls since chloroplasts, or linolenic acid alone, cause significant formation. When chloroplasts are incubated together with linolenic acid, there is a two-fold increase of malonaldehyde formed after 4 hours in samples receiving illumination. In the dark condition, chloroplasts actually cause inhibition of the malonaldehyde formation by linolenic acid.

TABLE II

DEPENDENCE OF LIGHT-STIMULATED MALONALDEHYDE FORMATION ON AN EXOGENOUS FATTY ACID

The reaction mixture (15 ml) contained chloroplasts (chlorophyll: 10 μg/ml) where indicated, NaCl (35 mM), Tris-HCl (20 mM, pH 8), and linolenic acid where indicated (5 mM). Net formation of malonaldehyde was calculated by subtracting total amounts formed in controls (chloroplast only and linolenic acid only) from values obtained in the experimental tubes (chloroplast + linolenic acid).

Conditions	Illumination	mμ moles Malonaldehyde Formed / Hours	
		0	4
Chloroplasts	+	1.3	8.7
	-	1.1	2.2
Linolenic acid	+	16.8	35.2
	-	15.5	28.4
Chloroplasts + Linolenic acid	+	25.9	59.4
	-	19.5	5.3
Net Formation (calculated)	+	7.8	15.5
	-	3.0	-25.3

DISCUSSION

Although it now appears that the formation of malonaldehyde is enhanced by illumination of chloroplasts in vitro, it remains to be clarified if the effect of light is upon formation or breakdown of hydroperoxides. A decrease in the functional reaction of chloroplasts in the presence of free fatty acids, reported by McCarty and Jagendorf (10), could be related to the light-stimulated malonaldehyde formation reported here because the TBA reaction in illuminated chloroplasts is accelerated (Table II) by free unsaturated fatty acids.

Swelling produced by formation of free fatty acids in mitochondria is well known from the studies of Lehninger and Remmert (11) Chloroplasts also show a deteriorative type of swelling change (12) that requires light (or electron transfer) similar to high-amplitude mitochondrial swelling (5). However, it is not known yet if fatty acids are involved in chloroplast swelling. Nevertheless, factors affecting release of fatty acids in chloroplasts may modify the action of light on deteriorative process such as swelling and lipid peroxidation.

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