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PHOTOSYNTHETIC APPARATUS OF CHILLING-SENSITIVE PLANTS

X. RELATIONSHIP BETWEEN SUPEROXIDE DISMUTASE ACTIVITY AND PHOTOPEROXIDATION OF CHLOROPLAST LIPIDS *

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(1) The rate of photoperoxidation of chloroplast lipids, as measured by malondialdehyde formation following the illumination of either leaves or chloroplast preparations, is found to be approx. 2-fold higher in chloroplasts from both cold- and dark-stored as well as stored and illuminated tomato leaves than in those from fresh leaves. (2) Enhanced lipid photoperoxidation can also be observed in chloroplasts from fresh leaves treated with cyanide as well as in superoxide dismutase-depleted chloroplasts following washing with Tris or Hepes. (3) Cyanide-sensitive superoxide dismutase activity is not detected in chloroplasts isolated from cold- and dark-stored leaves. Their illumination does not reactivate the enzyme activity. (4) On the basis of these observations, it is concluded that inactivation of chloroplast cyanide-sensitive superoxide dismutase due to cold and dark treatment of leaves, rather than diminished electron transport, is responsible for accelerated chloroplast lipid photoperoxidation.

Introduction

Photorperoxidation of chloroplast lipids was previously studied in fresh [1–4], aged [5] and Tris-washed chloroplasts [3]. It has been postulated that enhanced photoperoxidation, as measured by the formation of malondialdehyde, could be due to the inhibition of electron transport which affects both the level of superoxide anion in chloroplasts [6] and of singlet molecular oxygen [3]. These two molecular species have been considered to be involved in lipid photoperoxidation [3,4]. It appears, however, that their level depends also upon the activity of cyanide-sensitive superoxide dismutase (superoxide: superoxide oxidoreductase, EC 1.15.1.1.) which was found to be present in both spinach [7–9] and tomato [10] chloroplasts.

Recently, it was found that the cold and dark treatment of leaves of chilling-sensitive plants not only results in diminution of the electron transport in chloroplasts before the site of electron donation by diphenylcarbazide [11], but also stimulates photoperoxidation of chloroplast lipids [12] and decreases the total superoxide dismutase activity [10]. These observations suggest that some correlation may exist between superoxide dismutase activity and photoperoxidation of chloroplast lipids.

The data presented in this paper indicate that enhanced lipid photoperoxidation in chloroplasts of cold- and dark-stored tomato leaves is due to irreversible inactivation of cyanide-sensitive superoxide dismutase.

Materials and Methods

Plant material and treatment of leaves. Leaves of tomato (*Lycopersicon esculentum* Mill. var. Eurocross and Revermoon) were grown under greenhouse conditions used for commercial purposes. Leaves of

* A preliminary report of this work has appeared in abstract form [13].

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Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid; DCIP, dichlorophenolindophenol.

spinach (*Spinacia oleracea* L. var. Matador) were purchased in the local market.

Fresh leaves of both plant species were stored at 0°C in the dark for 3 days and than illuminated (8000 lx) for 2 h at 25°C (cf. Ref. 11).

Isolation of chloroplasts, determination of photochemical activities and washing of chloroplasts with buffers. The isolation of chloroplasts and the determination of both the Hill reaction activity and chlorophyll content were as previously described [11]. The electron-transport activity from either water or hydroxylamine to methyl viologen was assayed routinely as the O_2 uptake resulting from the reoxidation of reduced methyl viologen. A membrane-covered (Clark-type) oxygen electrode was used for the O_2 assay. Tris washing of chloroplast preparations (0.1 mg Chl/ml) was performed with 0.8 M Tris-HCl buffer, pH 8.0, for 10 min at 4°C as described by Yamashita and Butler [14]. Hepes washing was done in the same way using 0.8 M Hepes buffer, pH 8.0.

Determination of lipid photoperoxidation. Photoperoxidation of lipids was followed by the thiobarbituric acid method [15]. For determination of both chloroplast malondialdehyde content and the rate of malondialdehyde formation, an extinction coefficient of 155 $mM^{-1} \cdot cm^{-1}$ at 532 nm, corrected for non-specific absorbance at 600 nm, was applied [1]. The amount of malondialdehyde was determined in chloroplast preparations obtained from the illuminated leaves, usually about 1.5 h after the end of illumination. The rate of malondialdehyde formation was assayed in chloroplasts following their illumination at 25°C (20 000 lx) for 5, 10, 15 and 20 min in a reaction medium containing: 0.4 M sucrose, 50 mM Hepes buffer (pH 7.0) and 20 mM NaCl with continuous stirring. The rate of malondialdehyde formation expressed in nmol/min was calculated per mg of chlorophyll.

Assay of superoxide dismutase activity. Erythrocuprein (a cyanide-sensitive superoxide dismutase from bovine erythrocytes) activity was assayed at pH 7.8 by the standard method of McCord and Fridovich [16] in which the inhibition of cytochrome *c* reduction in the xanthine-xanthine oxidase system was followed.

The activity of chloroplast superoxide dismutase was measured as the inhibition of nitrite formation from hydroxylamine [8,17], using either the oxidation of xanthine by xanthine oxidase [16] or the reoxidation of reduced methyl viologen [8] for superoxide generation.

Superoxide dismutase activity in chloroplast protein extracts was assayed according to the method of Henry et al. [18] by the inhibition of the rate of nitroblue tetrazolium reduction by superoxide generated in the presence of $K_2^+O_2^-$ and dimethyl sulphoxide in the reaction medium. In this method, one unit of superoxide dismutase activity is defined as the amount of enzyme (expressed in μg of protein extracted from chloroplasts) causing a 50% decrease of the rate of nitroblue tetrazolium reduction under assay conditions.

Extraction of chloroplast protein. Proteins were extracted from chloroplast preparations by treatment with *n*-butanol for 90 min at 4°C with continuous stirring and then gently centrifuged as described by Asada et al. [7]. The water phase was collected and dialyzed overnight against 20 mM phosphate buffer, pH 7.8.

Determination of manganese. The samples, dried at 120°C, were treated with a mixture of concentrated sulphuric and nitric acids [19]. Manganese in chloroplast preparations was determined by atomic absorption spectrometry using an Instrumentation Laboratory model 551 Video I spectrometer. Determination was also carried out in ashed chloroplast samples by a complexometric method using pyridyl-2-azonaphthol (cf. Ref. 20).

Enzymes. Superoxide dismutase (erythrocuprein) from bovine erythrocytes was obtained from Miles Laboratories (U.S.A.) and its activity (12 300 U/mg of protein) was estimated using the method described above [16]. Xanthine oxidase was prepared by the method of Klenov and Emberland [21] from bovine cream.

Reagents. Thiobarbituric acid was purchased from Koch-Light Ltd. Tris and Hepes buffers were obtained from Sigma; methyl viologen and nitroblue tetrazolium were from Serva, while $K_2^+O_2^-$ was from ICN Pharmaceuticals Inc. (U.S.A.). Dimethyl sulphoxide and high-purity nitric and sulphuric acids (Suprapur) were products of E. Merck. All other reagents were of analytical grade and were provided by P.O.Ch., Poland.

Results

Photoperoxidation in leaves

It is known that the illumination of isolated spinach chloroplasts induces both peroxidation of unsaturated fatty acids and formation of malondialdehyde, a product of the decomposition of fatty acid peroxides [1–4]. This is accompanied by a loss of photosynthetic electron-transport activity. In contrast, illumination of leaves of chilling-sensitive plants following their storage at 0°C in the dark causes restoration of photosynthetic electron-transport activity together with photoperoxidation of unsaturated fatty acids accumulated in chloroplasts during cold and dark treatment of leaves [12].

Fig. 1A indicates that illumination of fresh tomato leaves for 2 h induces neither photoperoxidation of chloroplast lipids nor accumulation of malondialdehyde. On the other hand, illumination of cold- and dark-stored leaves results in a rapid increase of malondialdehyde content in chloroplasts (Fig. 1B). The stimulation of malondialdehyde formation in chloroplasts from cold- and dark-stored leaves in which the electron transport is blocked between water and the photochemical reaction center, Photosystem II [11], resembles the stimulation of malondialdehyde formation in both Tris-washed and aged chloroplasts [3].

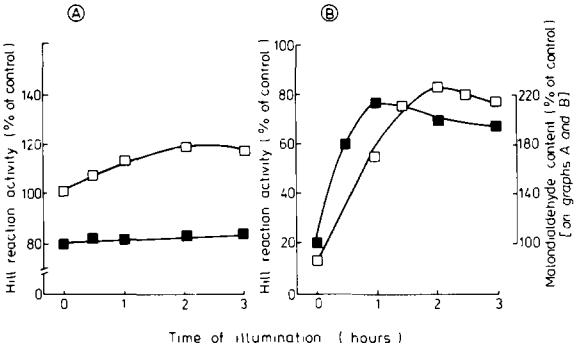

Fig. 1. The effect of illumination of fresh, cold- and dark-stored tomato leaves on the Hill reaction activity and malondialdehyde content in chloroplasts. (A) Fresh leaves were illuminated (8000 lx) at 25°C for the time indicated. (B) Fresh leaves were stored for 3 days at 0°C in the dark and were then illuminated as described in A. Hill reaction activity (□———□) and initial level of malondialdehyde content (●———●) of the control chloroplasts were 77.0 μmol DCIP reduced/h per mg Chl and 120.0 nmol/mg Chl, respectively.

Photoperoxidation in the presence of diminished electron transport

Since both cold and dark treatment of tomato leaves [11] and Tris washing of spinach chloroplasts inactivate electron transport [3,11] and stimulate photoperoxidation of chloroplast lipids, we have studied the rate of malondialdehyde formation in chloroplasts isolated from cold- and dark-stored tomato leaves and in Tris-washed chloroplasts obtained from fresh tomato leaves. As can be seen in Table I, the rate of malondialdehyde formation in chloroplasts isolated from cold- and dark-stored as well as stored and illuminated leaves is approx. 2-fold higher than that in chloroplasts isolated from fresh leaves. These differences appear to be independent of the electron transport as manifested by the Hill reaction activity. In Tris-washed chloroplasts (Table I), the rate of photoperoxidation is also very fast, though the Hill reaction activity does not exceed 20% of the control. These results suggest that some other factor(s), rather than diminished electron transport, may be responsible for the enhancement of photoperoxidation of chloroplast lipids following both cold and dark treatment of leaves of chilling-sensitive plants as well as Tris washing of chloroplasts.

TABLE I

THE RATE OF LIPID PHOTOPEROXIDATION IN RELATION TO THE HILL REACTION ACTIVITY IN CONTROL AND TRIS-WASHED CHLOROPLASTS

Source of chloroplasts	Photoperoxidation (nmol malondialdehyde produced/min per mg Chl)	Hill reaction activity (μmol DCIP reduced/h per mg Chl)
Control		
Fresh leaves	1.92	76.0
Cold- and dark-stored leaves	3.90	24.3
Stored and illuminated leaves	4.30	70.4
Tris-washed		
Fresh leaves	4.56	14.4
Cold- and dark-stored leaves	4.48	10.3
Stored and illuminated leaves	4.40	12.6

Photoperoxidation in Tris- and Hepes-washed chloroplasts

While inactivation of the Hill reaction activity following cold and dark treatment of leaves of chilling-sensitive plants is mainly due to depletion of large amounts of chloroplast manganese [20], Tris washing of chloroplasts not only removes loosely bound manganese [20,22], but also, as shown recently [10], regulates chloroplast superoxide dismutase activity, which may be responsible for the extent of chloroplast lipid photoperoxidation. The data of Table II indicate that: (i) despite different extents of depletion of manganese content and inactivation of the Hill reaction, both Tris and Hepes washing of chloroplasts of fresh leaves stimulate malondialdehyde formation by about 100%, even in the absence of cyanide in the chloroplast suspension, while in the control preparation malondialdehyde formation is stimulated by about 150% in the presence of cyanide. (ii) In chloroplast preparations isolated from cold- and dark-stored as well as stored and illuminated leaves, malondialdehyde formation (in the presence of cyanide) is faster than in the control by about 100 and 150%, respectively, and increases following Tris and Hepes washing of chloroplast preparations. These results suggest that depletion and/or inactivation of superoxide dismutase is responsible for enhanced photoperoxidation of chloroplast lipids in both cold- and dark-stored leaves and buffer-washed chloroplasts, as well as following the inhibition of superoxide dismutase activity caused by the addition of cyanide.

Effect of exogenous superoxide dismutase on photoperoxidation

Cyanide-sensitive superoxide dismutase was found to be present in spinach [7–9] and tomato [10] chloroplasts. Since the properties of spinach chloroplast superoxide dismutase appear to be similar to those of erythrocuprein [7], we used the latter for the experiment presented in Table III. The exogenous superoxide dismutase (erythrocuprein) inhibits light-induced malondialdehyde formation by about 50% of the control when added in a large excess to chloro-

TABLE II

THE RATE OF MALONDIALDEHYDE FORMATION IN TRIS- AND HEPES-WASHED CHLOROPLASTS FROM FRESH, COLD- AND DARK-STORED, AND STORED AND ILLUMINATED TOMATO LEAVES IN RELATION TO MANGANESE CONTENT

Cold- and darked stored leaves: leaves were stored at 0°C in the dark for 3 days. Stored and illuminated leaves: leaves were illuminated (8000 lx) for 2 h at 25°C following cold and dark storage for 3 days. Chloroplasts (0.1 mg Chl/ml reaction medium) were illuminated (20 000 lx) for 20 min in the presence of 10^{-3} M cyanide where indicated. Every 5 min of incubation period the samples were withdrawn for measurements of malondialdehyde production. Both manganese content and Hill reaction activity of the control (unwashed chloroplasts from fresh leaves) were 1.09 μg/mg Chl and 67.0 μmol DCIP reduced/h per mg Chl, respectively. The presence of cyanide did not affect the Hill reaction activity.

Source of chloroplasts	Buffer used for washing	Hill reaction activity (% of control)	Loss of Mn content (%)	Rate of malondialdehyde formation (nmol/min per mg Chl)	
				−KCN	+KCN
Fresh leaves	None	100	0	2.05	4.65
	Hepes	81	18	4.05	5.45
	Tris	15	62	4.35	5.00
Cold- and dark-stored leaves	None	19	55	3.80	5.15
	Hepes	13	59	4.45	4.95
	Tris	8	66	4.65	5.60
Stored and illuminated leaves	None	76	17	4.50	4.90
	Hepes	65	35	4.95	5.35
	Tris	10	61	5.25	5.00

TABLE III

INHIBITION OF MALONDIALDEHYDE FORMATION IN ILLUMINATED CHLOROPLASTS BY EXOGENOUS SUPEROXIDE DISMUTASE (ERYTHROCUPREIN)

The chloroplasts were isolated from tomato leaves as described in Materials and Methods. Superoxide dismutase was free of proteolytic activity. The specific activity of this enzyme was 12 300 standard units/mg of protein. The basic reaction mixture contained chloroplasts equivalent to 0.1 mg Chl/ml. Chloroplasts were preincubated with superoxide dismutase for 15 min in the dark and were then illuminated as described in Materials and Methods and in the legend to Table I.

Additions	Rate of malondialdehyde formation (nmol/min per mg Chl)		
	Fresh leaves	Cold- and dark-stored leaves	Stored and illuminated leaves
None	2.75	3.95	4.65
Superoxide dismutase:			
50 μg/mg Chl	2.45	3.55	3.95
500 μg/mg Chl	1.85	1.70	2.10
500 μg/mg Chl (heated at 80°C, 5 min)	2.70	3.90	4.65

plast preparations from fresh, cold- and dark-stored, and stored and illuminated leaves. Thus, the increased rate of malondialdehyde formation in chloroplasts from cold- and dark-stored as well as stored and illuminated leaves presented in Tables II and III seems to suggest that the photoperoxidation of chloroplast lipids may result from decreased superoxide dismutase activity.

Superoxide dismutase in isolated chloroplasts

It was previously found [10] that the total superoxide dismutase activity in chloroplasts is greatly diminished following cold and dark storage of both leaves and intact tomato plants. In the present study, superoxide dismutase activity was measured in the presence of cyanide to differentiate cyanide-sensitive and -insensitive activity.

Changes in both total and cyanide-sensitive superoxide dismutase activities in chloroplasts following cold and dark treatment of leaves and their illumination as well as in washed chloroplast preparations are shown in Table IV. The total superoxide dismutase activity is diminished in chloroplasts from both cold- and dark-stored and stored and illuminated leaves to about one-third of that of the control. Tris washing of chloroplasts is more effective than the Hepes washing in inactivation (or depletion) of superoxide dismutase activity. This is probably due to more effective depletion of chloroplast loosely bound manganese by Tris washing (cf. Table II). Furthermore, there is no essential difference between cyanide-sensitive and -insensitive superoxide dismutase activity when either cold and dark treatment of leaves or buffer washing of chloroplasts is applied. These observations suggest that both treatments destroy or inactivate the chloroplast cyanide-sensitive superoxide dismutase activity and are probably responsible for an increased rate of malondialdehyde production (cf. Table II).

TABLE IV

THE RELATIVE SUPEROXIDE DISMUTASE ACTIVITY AND ITS SENSITIVITY TO CYANIDE FOLLOWING TRIS OR HEPES WASHING OF CHLOROPLASTS FROM FRESH, COLD- AND DARK-STORED, AND STORED AND ILLUMINATED TOMATO LEAVES

The conditions of Tris or Hepes washing and the isolation of chloroplasts are described in Materials and Methods. After washing with buffers, chloroplasts were fragmented by homogenization in an isotonic solution using a Potter-Elvejhem Teflon homogenizer. Superoxide dismutase activity is expressed as its ability to inhibit nitrite formation from hydroxylamine in the xanthine-xanthine oxidase O_2^--generating system according to the method of Elstner and Heupel [17], in the presence of 10^{-4} M EDTA to bind Mn^{2+}. The assay was carried out in the dark. The reaction mixture contained chloroplast fragments equivalent to 50 μg Chl/ml and 10^{-3} M KCN where indicated.

Source of chloroplasts	Buffer used for washing	Superoxide dismutase activity (% inhibition)	
		−KCN	+KCN
Fresh leaves	None	88	30
	Hepes	34	35
	Tris	11	12
Cold- and dark-stored leaves	None	23	25
	Hepes	31	32
	Tris	10	9
Stored and illuminated leaves	None	36	38
	Hepes	37	34
	Tris	9	10

Superoxide dismutase activity in chloroplasts with electron transport restored by hydroxylamine

Since the cold and dark treatment of tomato leaves induces inhibition of the Hill reaction activity at a similar site and to a similar extent as does Tris washing of chloroplasts [11], we applied hydroxylamine for restoration of diminished electron transport, which as shown by Asada et al. [6], appears to be responsible for the univalent reduction of oxygen (in the presence of methyl viologen) with the formation of superoxide anion. Thus, the determination of superoxide dismutase activity in chloroplasts from fresh, cold- and dark-stored, and stored and illuminated leaves was based on the inhibition of nitrite formation due to photosynthetic hydroxylamine oxidation in the presence of methyl viologen [8,23]. When a very low concentration of hydroxylamine is used (10–20 μmol/mg Chl), there is no inhibition of DCIP reduction in chloroplasts from either fresh or stored and illuminated leaves, while electron transport in chloroplasts from cold- and dark-stored leaves is completely restored (Table V). Similarly, complete restoration of photosynthetic electron transport is obtained in chloroplasts from cold- and dark-stored leaves when both the oxidation of hydroxylamine and the reduction of methyl viologen (Table V) are followed by oxygen uptake in the presence of cyanide to inhibit catalase and superoxide dismutase activities.

As can be seen in Table VI, in chloroplasts from fresh leaves, nitrite formation in the absence of cyanide is greatly inhibited due to the reaction of superoxide dismutase. Conversely, in chloroplasts treated with cyanide or isolated from cold- and dark-stored leaves, superoxide dismutase is inactivated and therefore nitrite formation is almost 3-fold higher than in the control. Illumination of cold- and dark-stored leaves does not change this pattern and the ratio of nitrite formation in the presence to that in the absence of cyanide is the same (about 1.2) and much lower than that of the control. These results confirm the suggestion that cold and dark storage of

TABLE VI

THE RELATIVE SUPEROXIDE DISMUTASE ACTIVITY IN CHLOROPLASTS FROM FRESH, COLD- AND DARK-STORED, AND STORED AND ILLUMINATED TOMATO LEAVES

The amount of nitrite formed was determined by the method described by Elstner and Heupel [8,17] after the illumination (20 000 lx) of chloroplasts for 10 min at 25°C in the presence of methyl viologen (100 μM) and hydroxylamine (20 μmol/mg Chl). 10^{-3} M cyanide was added where indicated. The reaction mixture also contained 20 mM Hepes buffer, pH 7.4, 25 mM NaCl, 3 mM $MgCl_2$ and chloroplasts equivalent to 100 μg Chl/ml.

Source of chloroplasts	Nitrite formation (nmol/mg Chl)		$[NO_2^-]_{+KCN}/[NO_2^-]_{-KCN}$
	−KCN	+KCN	
Fresh leaves	0.95	2.70	2.84
Cold- and dark-stored leaves	2.50	2.90	1.18
Stored and illuminated leaves	2.10	2.55	1.21

TABLE V

RESTORATION BY HYDROXYLAMINE OF BOTH DCIP AND METHYL VIOLOGEN REDUCTION IN CHLOROPLASTS FROM COLD- AND DARK-STORED TOMATO LEAVES

The reaction mixture contained 20 μmol hydroxylamine/mg Chl and methyl viologen (100 μM) where indicated. The O_2 uptake was assayed polarographically in the presence of 10^{-2} M cyanide.

Source of chloroplasts	DCIP reduction (μmol DCIP reduced/h per mg Chl)		Methyl viologen (μmol O_2/h per mg Chl)	
	$-NH_2OH$	$+NH_2OH$	$-NH_2OH$	$+NH_2OH$
Fresh leaves	64.5	71.0	61.0	73.8
Cold- and dark-stored leaves	66.5	67.5	8.1	69.0
Stored and illuminated leaves	55.6	68.9	63.1	79.0

tomato leaves induces irreversible inactivation of cyanide-sensitive superoxide dismutase activity which upon illumination of the leaves is not restored.

Superoxide dismutase in chloroplast protein extracts

In order to check the presence of superoxide dismutase in chloroplasts from fresh, cold- and dark-stored, and stored and illuminated tomato leaves, the assay was performed using protein extracts obtained by the treatment of chloroplasts with *n*-butanol according to the method of Asada et al. [7]. It was found that superoxide dismutase activity in protein extracts of tomato and spinach leaves is equal to about 9 and 22 U/mg of protein, respectively (Table VII). The superoxide dismutase activities of these two extracts were completely sensitive to cyanide. On the other hand, superoxide dismutase activity was practically absent in extracts obtained from chloroplasts of cold- and dark-stored as well as stored and illuminated tomato leaves. Superoxide dismutase activity in spinach chloroplast extracts was not affected under these conditions.

Discussion

The physiological role of the active molecular oxygen species, O_2^- and $^1O_2$, has been discussed for various biological systems [3,4]. Takahama and Nishimura [3,4] have postulated that singlet molecular oxygen is a lipid photoperodixation-initiating agent in spinach chloroplasts and that the mechanism of its production can operate via O_2^- oxidation. It was suggested that only part of the superoxide generated in chloroplasts is accessible to be scavenged by superoxide dismutase. On the other hand, exogenous superoxide dismutase appears to react with O_2^- only in the aqueous phase in which singlet molecular oxygen is not stable [24]. The superoxide anion formation responsible for singlet molecular oxygen production occurs within chloroplast membranes and

TABLE VII

CYANIDE-SENSITIVE SUPEROXIDE DISMUTASE ACTIVITY IN CHLOROPLAST PROTEIN EXTRACTS OF FRESH, COLD- AND DARK-STORED, AND STORED AND ILLUMINATED TOMATO AND SPINACH LEAVES

Hill reaction activity is expressed in μmol DCIP reduced/h per mg Chl. Chloroplasts were isolated from leaves treated as described in Materials and Methods.

Expt. No.	Source of chloroplasts	Tomato		Spinach	
		Hill reaction activity	Superoxide dismutase activity (U/mg protein)	Hill reaction activity	Superoxide dismutase activity (U/mg protein)
1.	Fresh leaves	85.0	10.3	108.0	24.5
	Cold- and dark-stored leaves	5.3	0.6	96.0	19.8
	Stored and illuminated leaves	81.4	0.0	98.5	21.3
2.	Fresh leaves	58.0	8.4	111.5	24.6
	Cold- and dark-stored leaves	4.6	0.3	126.2	23.8
	Stored and illuminated leaves	50.1	0.1	127.0	22.9
3.	Fresh leaves	68.0	8.6	120.0	21.3
	Cold- and dark-stored leaves	11.0	0.0	110.3	19.8
	Stored and illuminated leaves	70.0	0.0	126.5	21.9

is inaccessible to the enzyme. Allen [25] found, however, that superoxides are produced in illuminated chloroplasts by the oxidation of reduced ferredoxin, i.e., outside the thylakoid membrane. Since some of the cyanide-sensitive superoxide dismutase is bound to the thylakoid membrane and most of it appears to be located in the stroma [9], this enzyme may be considered as an effective endogenous inhibitor of photoperoxidation. This suggestion is supported by the following observations. (i) The treatment of chloroplasts with either hypertonic buffers (Tris and Hepes) or cyanide (Table II) causes removal or inactivation of the chloroplast superoxide dismutase and enhanced photoperoxidation (Tables II and II). (ii) Moreover, the inhibitory effect of cyanide on superoxide dismutase activity (Tables IV and VI) seems to indicate that a copper-zinc enzyme is present in chloroplasts [7–9].

The data presented in this paper indicate that enhanced lipid photoperoxidation in chloroplasts following cold and dark treatment of tomato leaves does not result from diminished electron-transport activity (Table I), but from inactivation of superoxide dismutase. Moreover, in contrast to the results of Takahama and Nishimura [4], when exogenous superoxide dismutase (erythrocuprein) is used in excess and preincubation is carried out in the dark before exposure of the chloroplasts to light, inhibition of lipid peroxidation as high as 50% of the control is observed (Table III). Similar inhibition of lipid photoperoxidation by exogenous superoxide dismutase in pea chloroplasts was found by Merzliak et al. [26].

The inactivation of chloroplast superoxide dismutase following cold and dark storage of leaves (Tables IV, VI and VII) appears to be a very interesting phenomenon, not only as a factor controlling photoperoxidation, but also with respect to the mechanism by which this enzyme loses its activity. In contrast to the restoration of the Hill reaction activity [11] and levels of several chloroplast components such as protein and ATP [27], free fatty acids [12] and galactolipids [28], superoxide dismutase activity is not reactivated following illumination of cold- and dark-stored leaves (Tables IV, VI and VII). Our preliminary experiments [29] concerning changes of copper content in chloroplasts seem to indicate that inactivation of superoxide dismutase activity due to cold and dark storage of tomato leaves results from a depletion of copper and/or zinc from the enzyme. A similar mechanism was previously observed with respect to inactivation of the Hill reaction activity due to a loss of about 50% of chloroplast manganese [20].

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