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PHOTOSYNTHETIC APPARATUS OF CHILLING-SENSITIVE PLANTS

XI. REVERSIBILITY BY LIGHT OF COLD- AND DARK-INDUCED INACTIVATION OF CYANIDE-SENSITIVE SUPEROXIDE DISMUTASE ACTIVITY IN TOMATO LEAF CHLOROPLASTS *

W.P. MICHALSKI and Z. KANIUGA **

Institute of Biochemistry, University of Warsaw, Al. Żwirki i Wigury 93, 02-089 Warsaw (Poland)

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(1) The inactivation of cyanide-sensitive, copper- and zinc-containing superoxide dismutase activity in chloroplasts following cold and dark storage of both detached leaves and growing tomato plants is accompanied by a decrease in copper and zinc content in both chloroplast preparations and butanol extracts of the enzyme. In contrast, this treatment of chloroplast preparations affects neither superoxide dismutase activity nor copper and zinc content. (2) Copper- and zinc-containing superoxide dismutase is not reactivated following the 2–3 h illumination of cold- and dark-stored detached leaves. However, prolonged illumination of growing seedlings results in the restoration of both the enzyme activity and copper and zinc content in chloroplasts. (3) The data suggest that the dissociation of copper, and probably of zinc, from the enzyme during cold and dark treatment of either detached leaves or growing plants and reincorporation of the metals following the illumination of intact plants are responsible for the reversible inactivation of chloroplast cyanide-sensitive superoxide dismutase of chilling-sensitive plants.

Introduction

The storage of detached tomato leaves at 0°C in the dark for a few days causes a marked decrease in chloroplast loosely bound manganese accompanied by inactivation of the Hill reaction activity [1] and both cyanide-insensitive [2] and -sensitive [3,4] superoxide dismutase (superoxide:superoxide oxidoreductase, EC 1.15.1.1) activities. The illumination of cold- and dark-stored detached leaves for 2 h at 8000 lx at room temperature results in restoration of the chloroplast manganese level [1], Hill reaction activity [1] and cyanide-insensitive superoxide dismutase activity [2]. Cyanide-sensitive superoxide dismutase activity is, however, not reactivated under these conditions of illumination [3,4], probably due to an irreversible loss of copper and zinc from chloroplasts during cold and dark storage of detached leaves [3].

The studies presented in this article were undertaken in order to validate this suggestion and to find conditions for the reactivation of superoxide dismutase activity inactivated by cold and dark treatment of leaves.

Materials and Methods

Plant material and its treatment. The experiments were performed using 6–8-week-old grow-

* A preliminary report of this work has appeared in abstract form [3].

** To whom correspondence should be addressed.

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol.

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ing tomato plants (*Lycopersicon esculentum*, Mill.), detached leaves and leaf chloroplasts. Leaves referred to as fresh are those used within 2 h after harvesting from tomato plants growing under greenhouse conditions. Plants and leaves were stored at 0–1°C in the dark for various periods of time (usually 1–3 days). Plants or detached leaves stored in cold and darkness followed by illumination (8000 lx at 25°C) are referred to as reactivated.

Isolation of chloroplasts and determination of photochemical activity. Isolation of chloroplasts as well as the determination of Hill reaction activity and chlorophyll content are described in the previous paper of this series [5].

Extraction of chloroplast protein and assay of superoxide dismutase activity. Extraction of chloroplast proteins containing cyanide-sensitive superoxide dismutase was performed with *n*-butanol according to the method of Asada et al. [6]. The activity of this enzyme was assayed by the method of Henry et al. [7] in which the inhibition of the rate of nitroblue tetrazolium reduction by superoxide generated in the presence of K_2O_2 and dimethyl sulphoxide in the reaction medium is measured. One unit of superoxide dismutase activity is defined as the amount of enzyme (expressed in μg protein extracted from chloroplasts) causing a 50% decrease in the rate of nitroblue tetrazolium reduction under assay conditions.

'Native' electrophoresis. Protein extracts were fractionated according to the method of Davis [8] using 7.5% polyacrylamide gel. For localization of superoxide dismutase activity on the electrophoresis gels the coupling of the photochemical generation of superoxide with the reduction of nitroblue tetrazolium was applied [9].

SDS-polyacrylamide gel electrophoresis. This was performed using the method of Weber and Osborn [10]. The protein samples for electrophoresis were suspended in 10 mM phosphate buffer, pH 7.0, containing 1% SDS and 1% 2-mercaptoethanol. The suspension was incubated for 20 min at 60°C and then insoluble residue was removed by centrifugation.

After electrophoresis performed by both methods the gels were kept overnight in 12% trichloroacetic acid and then stained for protein for 2 h at 60°C in 0.25% Coomassie brillant blue R-250.

Determination of manganese, copper and zinc contents. Metal contents in both chloroplast preparations (manganese, copper, zinc) and protein extracts (copper zinc) were determined by atomic absorption spectrophotometry using an Instrumentation Laboratory model 551 video I apparatus, following the drying and wet ashing (H_2SO_4 + HNO_3) of the samples according to the method of Elvidge and Garratt [11].

Determination of chloroplast plastocyanin content. Plastocyanin was extracted from chloroplasts by the method of Hauska et al. [12] and its content was estimated by measuring the difference (ΔA) in absorbance at 597 nm of oxidized (with ferricyanide) and reduced (with ascorbate) plastocyanin using a Cary spectrophotometer. A molar extinction coefficient of 4.9 $mM^{-1} \cdot cm^{-1}$ was used [13].

Reagents. Superoxide dismutase (erythrocuprein) from bovine erythrocytes was obtained from Miles Laboratories. Nitroblue tetrazolium, K_2O_2, 2-mercaptoethanol and *N,N,N',N'*-tetramethylethylenediamine were products of Fluka A.G. Methylenebisacrylamide was from Serva Feinbiochemia while dimethyl sulphoxide, acrylamide, and high-purity HNO_3 and H_2SO_4 were products of E. Merck. SDS was obtained from B.D.H. and Coomassie brilant blue R-250 from International Enzymes Ltd. All other reagents were of analytical grade and were provided by Polskie Odczynniki Chemiczne, Poland.

Results

Superoxide dismutase activity in chloroplast protein extracts

The observation that cyanide-sensitive superoxide dismutase activity is practically absent in protein extracts obtained from chloroplasts of cold- and dark-stored as well as stored and illuminated detached tomato leaves has now been substantiated using polyacrylamide disc gel electrophoresis of protein extracts performed by the method of Davis [8]. Before electrophoresis the active erythrocuprein was added to the protein extracts as a marker of superoxide dismutase activity. Fig. 1 presents densitograms of tomato chloroplast protein extracts obtained from fresh, cold- and dark-stored, and stored and illuminated leaves as well

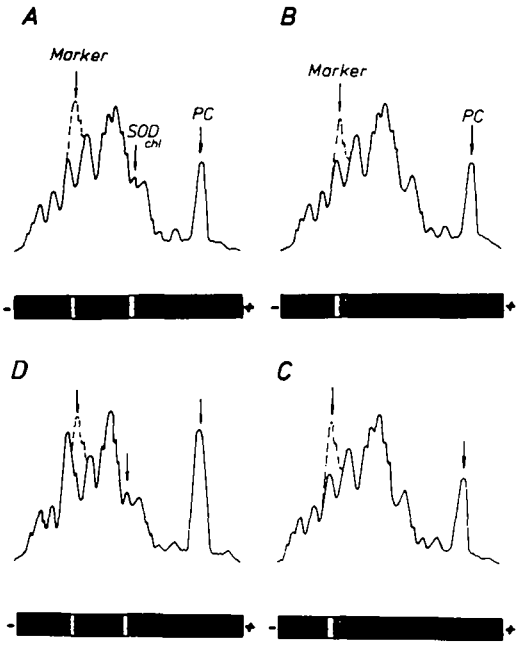

Fig. 1. 'Native' polyacrylamide disc gel electrophoresis of chloroplast protein of tomato and spinach leaves (top) and localization of chloroplast superoxide dismutase activity on gels (bottom). Protein extracts were obtained from chloroplasts isolated from fresh (A), cold- and dark-stored (B) and stored and illuminated detached tomato leaves (C) as well as fresh spinach leaves (D). The polyacrylamide gels were stained for superoxide dismutase activity (the achromatic zone) by using the procedure of Beauchamp and Fridovich [9]. The samples for electrophoresis contained about 30 μg protein. Marker, erythrocuprein; SOD chl., chloroplast superoxide dismutase; PC, plastocyanin.

as from spinach leaves. Chloroplast superoxide dismutase is more acidic than erythrocuprein but less acidic than plastocyanin, while the relative electrophoretic mobility of tomato chloroplast superoxide dismutase is the same as that of spinach (Fig. 1A and D, respectively). The protein band indicative of the superoxide dismutase activity is visible only in protein extracts from chloroplasts of fresh tomato and spinach leaves. Neither superoxide dismutase activity nor the protein band indicative of superoxide dismutase is observed on gels of protein extracts of chloroplasts from cold- and dark-stored, and stored and illuminated tomato leaves (Fig. 1B and C). There is, however, no extra band for modified superoxide dismutase for preparations from both cold- and dark-stored as well as stored and illuminated leaves. Thus, the data of Fig. 1 indicate that the cyanide-sensitive superoxide dismutase is inactivated due to cold and dark storage of detached tomato leaves and their illumination does not reactivate this enzyme. In contrast, in spinach chloroplasts, superoxide dismutase is not affected by cold and dark treatment of leaves.

SDS-polyacrylamide gel electrophoresis of chloroplast protein

The lack of the protein band indicative of superoxide dismutase in chloroplast extracts following cold and dark storage of tomato leaves before or after illumination (cf. Fig. 1) may result from either changes in the relative electrophoretic mobility of protein, degradation of the enzyme molecule or its dissociation into subunits. In order to check these possibilities, SDS-polyacrylamide gel electrophoresis of chloroplast protein extracts was performed. Although the pattern obtained for protein extracts from tomato and spinach chloroplasts shows some differences, it is essentially the same for fresh, cold- and dark-stored, as well as stored and illuminated leaves (densitograms not shown). Thus, no changes are found in the protein fractions containing superoxide dismutase with a molecular weight of about 16000 which could be expected for a protein fraction containing superoxide dismutase subunits. Furthermore, the constant proportion of individual protein fractions, including the subunits of superoxide dismutase, seems to exclude the proteolytic degradation of this enzyme following cold and dark storage of tomato leaves.

Metal content in chloroplast preparations and protein extracts

Chloroplasts isolated from leaves of higher plants contain cyanide-sensitive superoxide dismutase [6,14,15], an enzyme containing two atoms of copper and two atoms of zinc per molecule [6]. Removal of copper from the enzyme results in complete inhibition of its activity [16–18] and in the case of bovine superoxide dismutase, a change in the relative electrophoretic mobility [19,20]. Plastocyanin is also a copper-containing protein in chloroplasts [21,22]. Since a large (up to 50%) depletion of chloroplast manganese content occurs

TABLE I

EFFECT OF COLD AND DARK STORAGE AND ILLUMINATION OF TOMATO AND SPINACH LEAVES ON THE EXTENT OF CHANGES IN CHLOROPLAST COPPER, ZINC AND MANGANESE CONTENT AS WELL AS SUPEROXIDE DISMUTASE AND HILL REACTION ACTIVITIES

Detached leaves were stored at 0°C in the dark for 3 days and then illuminated (8000 lx) at 25°C for 2 h. Protein extracts were obtained as described in Materials and Methods. Metal contents are expressed either in ngatom/mg chloroplast chlorophyll or ngatom/mg protein of chloroplast protein extracts. Values of a representative experiment, as well as the range of values (in parentheses) found in the indicated number of experiments (also in parentheses) performed, are included.

Source of chloroplasts	Preparation	Hill reaction activity (μmol) DCIP reduced/h per mg Chl)	Superoxide dismutase activity (Units/mg protein)	Metal content		
				Cu	Zn	Mn
Tomato						
Fresh leaves	Chloroplasts	80.0		16.68 (14.48–18.10) (8)	36.10 (33.34–41.14) (8)	25.5 (25.5–27.3) (8)
Stored leaves		5.0		8.66 (7.08–8.66) (9)	25.70 (22.94–28.14) (9)	12.7 (10.9–14.6) (9)
Stored and illuminated leaves		77.3		9.33 (7.08–10.86 (8)	29.37 (27.68–29.83) (8)	23.7 (23.7–25.5) (8)
Fresh leaves	Protein extracts		9.3	22.19 (20.15–24.87) (8)	38.24 (33.96–43.44) (8)	–
Stored leaves			0.1	12.28 (10.54–12.28) (8)	28.30 (28.00–31.05) (8)	–
Stored and illuminated leaves			0.6	12.59 (12.59–14.95) (7)	31.36 (31.05–31.81) (7)	–
Spinach						
Fresh leaves	Chloroplasts	141.5		27.38 (26.76–28.49) (6)	86.88 (81.98–90.70) (6)	34.6 (32.8–38.2) (6)
Stored leaves		136.2		26.91 (25.18–28.64) (6)	85.04 (80.00–88.86) (6)	36.4 (32.8–36.4) (6)
Stored and illuminated leaves		135.7		26.60 (25.18–28.01) (6)	86.11 (81.83–91.00) (5)	34.6 (32.8–38.2) (5)
Fresh leaves	Protein extracts		22.9	51.62 (47.21–55.08) (6)	94.52 (90.24–97.89) (6)	–
Stored leaves			20.2	52.41 (48.16–56.81) (7)	94.37 (89.32–100.34) (7)	–
Stored and illuminated leaves			22.2	51.15 (48.00–56.19) (6)	92.54 (91.62–96.82) (6)	–

following the cold and dark storage of tomato leaves [1], we decided to check the content of both copper and zinc in chloroplasts and chloroplast protein extracts containing superoxide dismutase. As shown in Table I, the loss of cyanide-sensitive superoxide dismutase activity following the cold and dark storage of tomato leaves is accompanied by a marked decrease in copper and zinc content (by about 50 and 25% of the control, respectively) in both chloroplast preparations and protein extracts. In contrast to both the restoration of the manganese level and the Hill reaction activity following 2 h illumination of leaves, neither copper and zinc content nor chloroplast superoxide dismutase activity is restored by light. On the other hand, both cold and dark storage and illumination of leaves of spinach—a chilling-resistant plant—affect neither the metal content nor superoxide dismutase activity (Table I).

Dialysis of chloroplast preparations from fresh leaves against redistilled water for 24 or 48 h results only in a small decrease in copper and zinc content (by about 10 and 20% of the control, respectively). Thus, it may be concluded that the main portion of copper depleted from chloroplasts during the cold and dark treatment of leaves represents the metal bound to the chloroplast proteins.

Since there are no changes in copper content calculated from the plastocyanin concentration (Table II), it may be excluded as a component responsible for the observed loss of copper from chloroplasts following the cold and dark storage of tomato leaves.

TABLE II

PLASTOCYANIN CONTENT IN CHLOROPLASTS ISOLATED FROM FRESH, COLD- AND DARK-STORED AS WELL AS STORED AND ILLUMINATED TOMATO LEAVES

Chloroplast were isolated from detached leaves and treated as described in Materials and Methods. Copper content was calculated from the plastocyanin concentration.

Expt. No.	Source of chloroplasts	Plastocyanin content	
		nmol/mg Chl	ngatom Cu/mg Chl
1	Fresh leaves	2.53	5.10
	Stored leaves	2.42	4.88
	Stored and illuminated leaves	2.52	5.07
2	Fresh leaves	2.88	5.81
	Stored leaves	2.77	5.59
	Stored and illuminated leaves	2.89	5.82

Reversibility of chloroplast superoxide dismutase activity by illumination of cold- and dark-stored tomato seedlings

The lack of restoration of both copper and zinc levels and superoxide dismutase activity in chloroplasts following illumination of detached leaves (Fig. 1 and Table I) led us to search for conditions under which changes in these two parameters could be reversible, similarly to the Hill reaction activity and manganese content [1]. Since detached leaves have wilted even in high humidity as early as after 3–4 h of illumination, for further experiments growing young tomato plants were used, which respond to cold and dark-treatment and illumination similarly to detached leaves [5].

Cold and dark storage for 3 days of growing tomato plants results in the abolition of both Hill reaction and superoxide dismutase activities. The former activity is reactivated after 1–3 h of illumination at 25°C while the latter increases only up to 30% of the control following 5–7 hours exposure to light. However, when cold- and dark-stored tomato seedlings were illuminated for 12 h, then kept for 12 h in the dark and again exposed to light for 3 h, both superoxide dismutase activity and metal content were completely restored, even in plants stored in the cold and darkness for 3 days in which all these parameters exhibited the greatest changes (Fig. 2). These data are in agreement with the suggestion that the mechanism of cold- and dark-induced inactivation of superoxide dismutase activity depends on depletion of the enzyme metal ions and their migration outside the chloroplasts, while illumination of leaves results in the reincorporation of these metals into apoenzyme, similarly to what was observed for manganese with respect to the restoration of Hill reaction activity [1].

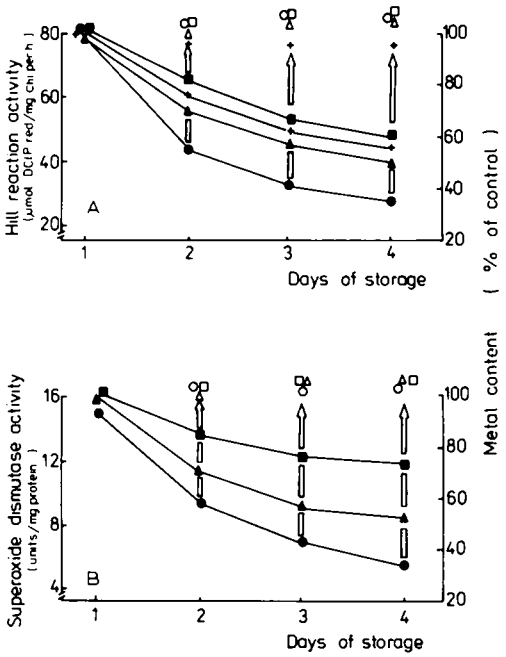

Fig. 2. Reversibility of superoxide dismutase (bottom; ●, ○) and Hill reaction (top; ●, ○) activities as well as metal content in chloroplasts (A) and protein extracts (B) of cold- and dark-stored growing tomato plants following their prolonged illumination at 25°C. The plants were stored for 1, 2 and 3 days and then illuminated for 12 h, kept in the dark during the night and again illuminated 8000 lx for 3 h. This treatment is indicated by the open arrows. The elevated values of both superoxide dismutase and Hill reaction activities as well as the metal content are indicated by open symbols. Control values of chloroplast manganese (+), copper (▲, △) and zinc (■, □) contents were 20.3, 17.15, and 34.33 ngatom/mg Chl, respectively, whereas the initial level of copper and zinc of chloroplast protein extracts were 26.38 and 43.35 ngatom/mg protein, respectively. Data represent one of three independent experiments.

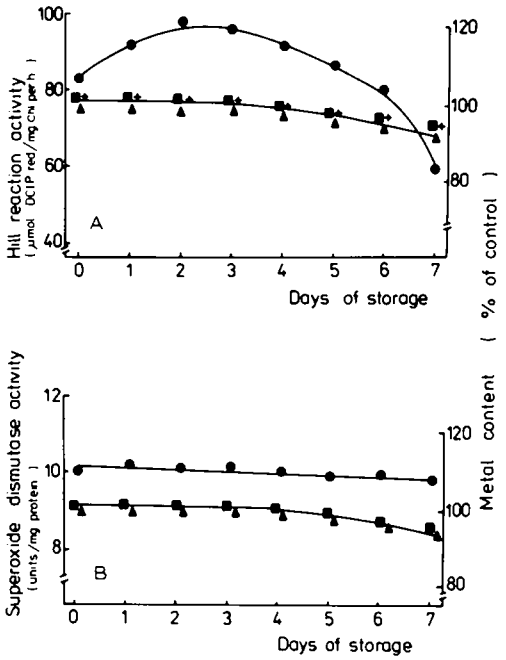

Fig. 3. The stability of superoxide dismutase (●) (A) and Hill reaction (●) (B) activities and metal content in tomato leaf chloroplasts (A) and protein extracts (B) during their cold and dark storage. The chloroplast preparation (about 6 mg Chl/ml) was kept at 2–3°C in the dark for the time indicated, then recentrifuged at 1000× *g* for 10 min and the pellet was analyzed for both metal content and Hill reaction activity (top) as well as being used for protein extraction and determination of superoxide dismutase activity and copper and zinc content (bottom). Control values of chloroplast manganese (+), copper (▲) and zinc (■) contents were 22.2, 15.26 and 37.17 ngatom/mg chlorophyll, respectively, whereas the initial levels of copper and zinc in protein extracts were 22.37 and 42.21 ngatom/mg protein, respectively. Data represent one of four independent experiments.

Stability of superoxide dismutase activity and metal content in cold- and dark-stored chloroplast preparations

In order to check the stability of superoxide dismutase in cold- and dark-stored chloroplast preparations, the experiments presented in Fig. 3 were carried out. As can be seen, both superoxide dismutase and Hill reaction activities as well as metal content were stable for several days under these conditions. Where was also no change in metal content. A slight enhancement of Hill reaction activity observed after 2 days of storage probably results from the uncoupling process.

Discussion

Inactivation of chloroplast superoxide dismutase following the cold and dark storage of leaves appears to be a very interesting phenomenon not only as a factor controlling the photoperoxidation (cf. Ref. 4) but also with respect to the mechanism by which this enzyme loses its activity. In contrast to the restoration of Hill reaction activity [5] and levels of several chloroplast com-

ponents such as protein and ATP [23], free fatty acids [24] and galactolipids [25], superoxide dismutase activity is not reactivated following a short time of illumination (2 h) of cold- and dark-stored detached leaves (Table I). Thus, studying the mechanism by which superoxide dismutase is inactivated during cold and dark treatment of tomato leaves three main possibilities which should be considered. Firstly, inactivation of superoxide dismutase may be due to proteolytic degradation of the enzyme. However, as shown previously [26] and in the present paper using SDS-polyacrylamide gel electrophoresis, chloroplast proteins are relatively stable during cold and dark storage of tomato leaves. Secondly, the possibility that during cold and dark treatment of leaves the dissociation of superoxide dismutase takes place, resulting in the loss of its activity, may be excluded for two reasons: (i) it is well known that superoxide dismutase from various sources is relatively resistant to dissociation for which either a high concentration of urea [17,27,28] or SDS plus mercaptoethanol is required [29]; (ii) the dissociation of the enzyme into subunits by urea does not destroy its catalytic activity [17,27] nor the ability to form catalytically active hybrids [28].

Thirdly, the finding that cold and dark storage of leaves results in the loss of about 50% of chloroplast manganese accompanied by almost complete inactivation of the Hill reaction activity [1] led us to consider the possibility that depletion of copper and/or zinc from superoxide dismutase could be responsible for the inactivation of this enzyme. In fact, it was found that cold and dark storage of leaves causes a decrease in total copper and zinc content in chloroplast preparations by about 50 and 25% of the control, respectively (Table I). The same pattern of changes was observed in the chloroplast protein extracts following their dialysis against 20 mM phosphate buffer, pH 7.8. In contrast to restoration of manganese content [1], the illumination of cold- and dark-stored detached leaves does not increase the level of copper in both chloroplast preparations and protein extracts while zinc content increases only by 10% of the control value. On the other hand, dialysis of chloroplast preparations from fresh leaves against distilled water removes copper and zinc by an extent of 10 and 20%, respectively, indicating that this portion of metal is either free or loosely bound.

The function of copper in superoxide dismutase is associated with the catalytic activity while zinc plays a structural role [17,18]. Removal of both metals from the enzyme by dialysis and gel filtration [18], in the presence of either EDTA at pH 3.8 [19,20] or neutral cyanide [20], results in a loss of catalytic activity. There are two proteins in chloroplasts known to contain copper: plastocyanin [21,22] and superoxide dismutase [6]. The total copper content in chloroplasts from various plants is 2–5-times higher than that in plastocyanin [21,22,30]. However, similarly to Photosystem I activity [25], the copper content in plastocyanin is relatively stable and does not change during the cold and dark storage of tomato leaves (cf. Table II).

The coincidence between a large decrease in copper content in both chloroplast preparations and chloroplast protein extracts (Table I), as well as the loss of superoxide dismutase activity not detectable by quantitative (Table I) and electrophoretic assay (Fig. 1), seems to suggest that inactivation of this enzyme following the cold and dark storage of leaves is due to removal of copper from the enzyme. In contrast to bovine superoxide dismutase [20], there is no extra band for tomato apoenzyme at an altered position in gels (Fig. 1). Moreover, the copper added to the protein extracts does not reconstitute superoxide dismutase activity, probably due to nonspecific binding of copper to some proteins present in excess, to chloroplast superoxide dismutase in butanol protein extracts.

Finally, there are some interesting phenomena characteristic only of tomato chloroplasts, a chilling-sensitive plant: (i) Cold and dark storage of tomato chloroplasts affects neither manganese, copper and zinc content nor superoxide dismutase and Hill reaction activities (Fig. 3). (ii) Superoxide dismutase activity as well as copper and zinc levels are restored only in cold- and dark-stored growing tomato plants and upon much longer times of illumination (Fig. 2). Thus, the question as to why the dissociation of copper from superoxide dismutase and the reactivation of the enzyme by reincorporation of this metal take place only in intact cells and growing illuminated plants, respectively, remains to be elucidated.

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References

1 Kaniuga, Z., Ząbek, J. and Sochanowicz, B. (1978) Planta (Berl.) 144, 49–56
2 Kaniuga, Z., Ząbek, J. and Michalski, W.P. (1979) Planta (Berl.) 145, 145–150
3 Kaniuga, Z. and Michalski, W.P. (1980) First European Bioenergetics Conference, Urbino, Short Reports, pp. 399–400, Patrone Editore, Bologna
4 Michalski, W.P. and Kaniuga, Z. (1981) Biochim. Biophys. Acta 637, 159–167
5 Kaniuga, Z., Sochanowicz, B., Ząbek, J. and Krzystyniak, K. (1978) Planta (Berl.) 140, 121–128
6 Asada, K., Urano, M. and Takahashi, M. (1973) Eur. J. Biochem. 36, 257–366
7 Henry, L.A.E., Halliwell, B. and Hall, D.O. (1975) FEBS Lett. 66, 303–306
8 Davis, B.J. (1964) Ann. N.Y. Acad. Sci. 121, 404–427
9 Beauchamp, C. and Fridovich, I. (1871) Anal. Biochem. 44, 276–287
10 Weber, K. and Osborn, M. (1969) J. Biol. Chem. 244, 4406–4412
11 Elvidge, D.A. and Garratt, D.C. (1954) Analyst 79, 146–149
12 Hauska, G.A., McCarthy, R.E., Berzborn, R.J. and Racker, E. (1971) J. Biol. Chem. 246, 3524–3531
13 Katoh, S. (1971) Methods Enzymol. 23, 408–413
14 Elstner, E.F. and Heupel, A. (1975) Planta (Berl.) 123, 145–154
15 Jackson, C., Dench, J., Moore, A.L., Halliwell, B., Foyer, C.H. and Hall, D.O. (1978) Eur. J. Biochem. 91, 339–344
16 McCord, J.M. and Fridivich, I. (1969) J. Biol. Chem. 244, 6042–6055
17 Forman, H.J. and Fridovich, I. (1973) J. Biol. Chem. 248, 2645–2649
18 Fridovich, I. (1974) Adv. Enzymol. 41, 35–97
19 Rotilio, G., Rigo, A., Viglino, P. and Calabrese, L. (1977) in Superoxide and Superoxide Dismutases (Michelson, A.M., McCord, J.M. and Fridovich, I., eds.), pp. 207–214, Academic Press, New York
20 Rigo, A., Viglino, P., Calabrese, L., Cocco, D. and Rotilio, G. (1977) Biochem. J. 161, 27–30
21 Katoh, S., Suga, I., Shiraton, I. and Takamiya, A. (1961) Arch. Biochem. Biophys. 94, 136–141
22 Plesnicar, M. and Bendall, D.S. (1970) Biochim. Biophys. Acta 216, 192–199
23 Sochanowicz, B. and Kaniuga, Z. (1979) Planta (Berl.) 144, 153–159
24 Kaniuga, Z. and Michalski, W.P. (1978) Planta (Berl.) 140, 129–136
25 Michalski, W.P. and Kaniuga, Z. (1980) Biochim. Biophys. Acta 589, 84–99
26 Sochanowicz, B. and Kaniuga, Z. (1979) Plant (Berl.) 145, 137–143
27 Bannister, J.W., Anastasi, A. and Bannister, W.H. (1978) Biochem. Biophys. Res. Commun. 30, 469–472
28 Marmocchi, F., Venardi, G., Bossa, F., Rigo, A. and Rotilio, G. (1978) FEBS Lett. 94, 109–111
29 Beauchamp, C. and Fridovich, I. (1973) Biochim. Biophys. Acta 317, 50–64
30 Baszyński, T., Ruszkowska, M., Król, M., Tukendorf, A. and Wolińska, D. (1978) Z. Pflanzenphysiol. 89, 207–216