Galactolipase activity and free fatty acid levels in chloroplasts

Novel approach to characteristics of chilling sensitivity of plants

Zbigniew Kaniuga and Joanna Gemel

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

Received 3 April 1984

Both galactolipase activity and free fatty acid content are much higher in chloroplasts of chilling-sensitive plants (bean, broad bean, tomato and maize) than in those of chilling-resistant ones (spinach, pea and lettuce). However, monogalactosyldiacylglycerol and digalactosyldiacylglycerol content in chloroplasts of these two groups of plants relates neither to free fatty acid levels nor galactolipase activity. It seems that chloroplast galactolipase activity and free fatty acid level may be useful biochemical parameters discriminating chilling-sensitive from chilling-resistant plants.

Galactolipase activity *Free fatty acid* *Chloroplast* *Chilling sensitivity* *Galactolipid*

1. INTRODUCTION

In studies on the mechanism of chilling injury in plants a number of physiological, physical and biochemical methods are usefully applied. In most of them the changes of membrane structure, function and properties in response to low temperature are measured (cf. [1,2]).

Studying cold treatment of leaves of CS plants we have observed loss of Hill reaction activity [3] accompanied by degradation of chloroplast galactolipids [4], accumulation of FFA [5] and release of manganese from chloroplasts. The latter results in inhibition of O_2 evolution. FFA-induced release of loosely bound Mn from chloroplasts during cold treatment of leaves was also observed following digestion of chloroplasts with bean-leaf galactolipase and dark storage of tomato plants at room temperature ([7] and in preparation). In contrast, cold treatment of spinach leaves affected neither chloroplast FFA level [5], Mn content nor Hill reaction activity [6,7].

All these observations prompted us to compare the FFA level, galactolipase activity and galactolipid content in chloroplasts of both CS and CR plants. The results seem to suggest that galactolipase activity and FFA level in chloroplasts may be a useful biochemical index discriminating CS from CR plants.

2. MATERIALS AND METHODS

Leaves of spinach (*Spinacia oleracea* var. Matador), lettuce (*Lactuca sativa* var. Amplus) were purchased in the local market. Tomato leaves (*Lyopersicon esculentum* var. Norton) were harvested from plants grown under greenhouse conditions used for commercial purposes. Cabbage leaves (*Brassica oleracea* var. Ditmarska) were harvested from seedlings of about 15 cm height growing in a greenhouse. Leaves of bean (*Phaseolus vulgaris* var. Piękny Jaś and var.

Paper XIII in the series: Photosynthetic Apparatus of Chilling-Sensitive Plants

Abbreviations: CS, chilling-sensitive; CR, chilling-resistant; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; FFA, free fatty acid; Chl, chlorophyll

Published by Elsevier Science Publishers B.V.
00145793/84/$3.00

Juliszka), broad bean (*Vicia faba* var. Windsor), pea (*Pisum sativum* var. Rarytas), *Pisum arvense*, and maize (*Zea mays* var. Karłowa) were harvested usually between 14 and 18 days after planting seeds. Conditions of both seed germination and growing of the plants have been described in [3].

Chloroplasts were isolated as in [8] using Tris buffer instead of Hepes and omitting bovine serum albumin from the isolating medium.

Galactolipids: MGDG and DGDG were extracted with hot isopropanol from lyophilized chloroplasts, followed by column chromatography on silica gel (100–200 mesh) and purification by thin-layer chromatography [9]. The amount of MGDG and DGDG was calculated on the basis of galactose determination by the phenol method [10].

Galactolipase (lipid acyl hydrolase (galactolipase), cf. [12]) preparations were isolated from chloroplast preparations as in [11] omitting the inactivation step of protein inhibitor since we did not observe its presence.

Galactolipase activity was estimated following incubation for 15 min at 30°C of lyophilized spinach chloroplasts used as substrate of the enzyme (cf. [11]). No detergent was added since unsaturated FFAs at low concentrations are the most effective surface-active agent [13]. The reaction mixture for enzyme activity measurement contained 20 mM phosphate buffer (pH 7.0), 2 μg enzyme preparation and substrate, equivalent to 100 μg chlorophyll. Incubation was terminated by addition of 0.1 N HCl in 96% ethanol. The activity of the preparation was expressed as μmol FFA liberated/min per mg protein.

Chlorophyll and protein content were estimated as in [14] and [15], respectively. The content of FFA was determined using the rhodamine 6G method in [16].

3. RESULTS

Fig.1 shows the time course of FFA accumulation in chloroplasts during cold treatment of tomato, bean and spinach leaves. The data indicate that the FFA level in chloroplasts of CR plants (spinach) is much lower than that of CS plants (tomato, bean). Similar differences were found in the chloroplast FFA content of fresh leaves of these two groups of plants (fig.2).

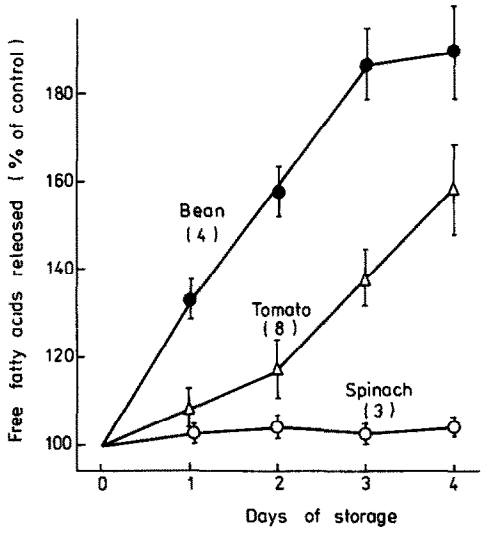

Fig.1. FFA accumulation in chloroplasts during cold treatment of bean, tomato and spinach leaves. Leaves were stored at 0–4°C as in [3]. Points with vertical lines represent the means ± SE for the number of experiments shown in parentheses.

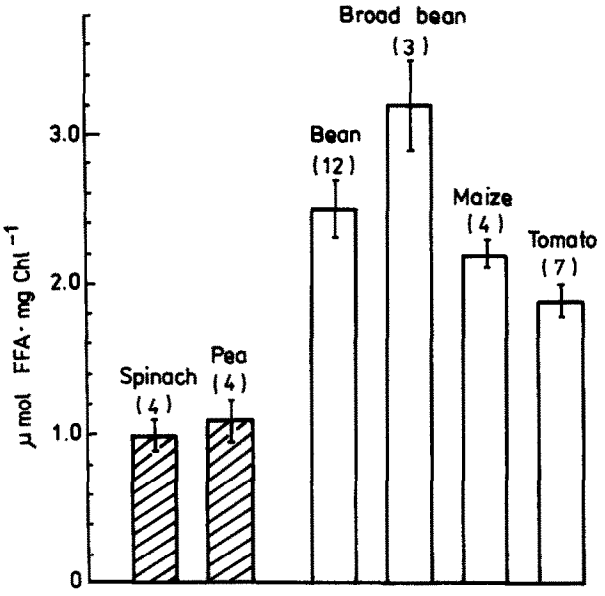

Fig.2. FFA levels in chloroplasts of chilling-resistant (hatched bars) and chilling-sensitive (open bars) plants. Bars with vertical lines represent means ± SE for the number of experiments shown in parentheses.

Table 1

Galactolipase activity in chloroplasts of chilling-resistant and chilling-sensitive plants

Plant	Galactolipase activity (μmol FFA · min^{-1} · mg protein^{-1})		
Chilling-resistant			
Spinach	0	0	0.05
Cabbage	0	0	
Lettuce	0	0	
Pisum arvense	0.33	0.33	
Pea (var. Rarytas)	0.67	0.83	0.83
Chilling-sensitive			
Maize	1.50	1.67	
Bean (var. Juliszka)	2.33	2.83	
Tomato	2.50	3.20	
Broad bean	2.80	3.50	
Bean (var. Piękny Jaś)	3.00	3.10	3.80

Individual values are for different experiments

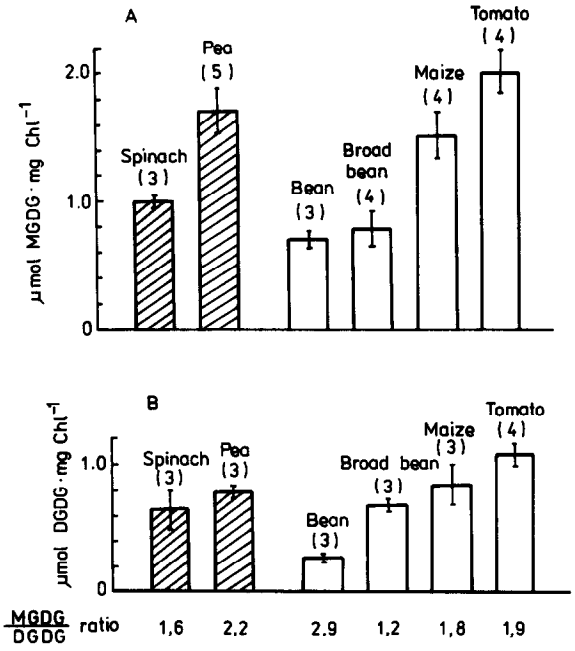

Fig.3. MGDG (top) and DGDG (bottom) levels in chloroplasts of chilling-resistant (hatched bars) and chilling-sensitive (open bars) plants. Bars with vertical lines represent means ± SE for the number of experiments shown in parentheses.

Galactolipase activity in chloroplasts of CS plants is much higher than in CR plants (table 1). The data also indicate a close relation between the enzyme activity and FFA level in chloroplasts of both groups of plants.

Surprisingly, there is no relation between MGDG and DGDG content in chloroplasts of CS and CR plants (fig.3) as well as between galactolipid content and galactolipase activity in all plants studied. The MGDG/DGDG ratio is approx. 2 in agreement with the value reported for many plants [17].

4. DISCUSSION

Attempts to correlate the fatty acid composition of chloroplast polar lipids of tomato, bean, pea [18] and of some *Passiflora* species (which varied in their resistance to chilling injury) [19] and the degree of their unsaturation with chilling sensitivity of these plants did not succeed. Similarly, CR plants did not contain more unsaturated fatty acid than CS ones [20], whereas in tomato chloroplasts chill-hardening led to an increase in unsaturation of phospholipids while that of the glycolipids was not greatly altered [21]. On the other hand, CR cultivars of alfalfa contained a greater percentage of polyunsaturated fatty acid in the chloroplast membrane and a greater double bond index than the CS cultivars [22].

Determination of Arrhenius activation energy (E_a) for photoreduction of $NADP^+$ [23] or 2,6-dichlorophenolindophenol [24] by chloroplasts isolated from CS and CR plants is another test to examine the effect of low temperature on chloroplast function and particularly the membrane-related response. While discontinuity of the Arrhenius plot was at first observed for chloroplasts of CS plants and a constant slope for CR plants, a deviation from this correlation was, however, found in [24]. It was therefore concluded that the presence or lack of discontinuity in the Arrhenius plot does not necessarily correlate with chilling sensitivity.

Galactolipase activity and FFA level in chloroplasts have yet to be studied in relation to chilling of plants [1,2,25]. In thylakoid membranes MGDG and DGDG are the major polar lipids while sulpholipid, phosphatidylcholine and phosphatidylglycerol content is 4-fold lower than that

of galactolipids [26]. The lipid acyl hydrolase from *Phaseolus* sp. leaves and probably from other plants has a wide substrate specificity [12] which is approximately proportional to the quantity of each polar lipid present in the thylakoid membrane [26]. Thus it may be assumed that the major fraction of FFA released by lipid acyl hydrolase is of galactolipid origin. In addition chilling temperature (0–12°C) does not seem to be a limiting factor for the hydrolysis of chloroplast lipids by lipid acyl hydrolase, even of animal origin [29].

A simple characteristic of chilling sensitivity seems to be a decline of photoreductive activity in isolated chloroplasts, i.e., decrease of oxygen evolution or Hill reaction activity [3,27,28] as well as a change in chloroplast fluorescence [28]. Similarly, differences in FFA levels and galactolipase activity in chloroplasts of CS and CR plants, as indicated here, may provide a useful index for discrimination between CS and CR plants.

ACKNOWLEDGEMENTS

We wish to thank Mrs Joanna Cieśla and Mrs Barbara Zabłocka for assistance in some experiments. The technical assistance of Mr W. Łasiński is acknowledged. This study was partially supported by the Department of Plant Physiology, Polish Academy of Sciences (project MR II/7).

REFERENCES

[1] Lyons, J.M. (1973) Annu. Rev. Plant Physiol. 24, 445–466.
[2] Lyons, J.M., Raison, J.K. and Steponkus, P.L. (1979) in: Low Temperature Stress in Crop Plants. The Role of the Membrane (Lyons, J.M. et al. eds) pp.1–24, Academic Press, New York.
[3] Kaniuga, Z., Sochanowicz, B., Ząbek, J. and Krystyniak, K. (1978) Planta 140, 121–128.
[4] Michalski, W.P. and Kaniuga, Z. (1980) Biochim. Biophys. Acta 589, 84–99.
[5] Kaniuga, Z. and Michalski, W.P. (1978) Planta 140, 129–136.
[6] Kaniuga, Z., Ząbek, J. and Sochanowicz, B. (1978) Planta 144, 49–56.
[7] Kaniuga, Z., Gemel, J. and Madzio, B. (1982) 2nd Eur. Bioenerg. Conf. Lyon, Abstr. 489–490.
[8] Lynn, W. and Brown, R. (1967) J. Biol. Chem. 242, 412–417.
[9] Kates, M. (1972) in: Techniques in Biochemistry and Molecular Biology (Work, T.S. and Work, E. eds) pp.393–469, Elsevier, Amsterdam, New York.
[10] Roughan, P.G. and Batt, R.D. (1968) Anal. Biochem. 22, 74–88.
[11] Anderson, M.M., McCarthy, R.E. and Zimmer, A. (1974) Plant Physiol. 53, 699–704.
[12] Galliard, T. (1980) in: The Biochemistry of Plants, vol.4, Lipids: Structure and Function (Stumpf, P.K. ed) pp.85–116, Academic Press, New York.
[13] Galliard, T. (1971) Eur. J. Biochem. 22, 90–98.
[14] Arnon, D. (1949) Plant Physiol. 24, 1–15.
[15] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[16] Anderson, M.M. and McCarty, R.E. (1972) Anal. Biochem. 45, 260–270.
[17] Douce, R. and Joyard, J. (1980) in: The Biochemistry of Plants, vol.4, Lipids: Structure and Function (Stumpf, P.K. ed) pp.321–362, Academic Press, New York.
[18] Bishop, D.G., Kenrick, J.R., Bayston, J.H., Macpherson, A.S., Johns, S.R. and Willing, R.I. (1979) in: Low Temperature Stress in Crop Plants. The Role of the Membrane (Lyons, J.M. et al. eds) pp.375–389, Academic Press, New York.
[19] Patterson, B.D., Kenrick, J. and Raison, J.K. (1978) Phytochemistry 17, 1089–1092.
[20] Wilson, J.M. and Crawford, M.M. (1974) J. Exp. Bot. 25, 121–131.
[21] Rosinger, C.H., Wilson, J.M. and Kerr, M.W. (1982) J. Exp. Bot. 33, 321–331.
[22] Peoples, T.R., Koch, D.W. and Smith, S.C. (1978) Plant Physiol. 67, 472–473.
[23] Shneyour, A., Raison, J.K. and Smillie, R.M. (1973) Biochim. Biophys. Acta 292, 152–161.
[24] Nolan, W.G. and Smillie, R.M. (1977) Plant Physiol. 59, 1141–1145.
[25] Graham, D. and Patterson, B.D. (1982) Annu. Rev. Plant Physiol. 33, 347–372.
[26] Rawyler, A. and Siegenthaler, P.-A. (1981) Biochim. Biophys. Acta 638, 30–39.
[27] Smillie, R.M. and Nott, R. (1979) Plant Physiol. 63, 796–801.
[28] Smillie, R.M. (1979) in: Low Temperature Stress in Crop Plants. The Role of the Membrane (Lyons, J.M. et al. eds) pp.187–202, Academic Press, New York.
[29] Rawyler, A. and Siegenthaler, P.-A. (1981) Biochim. Biophys. Acta 635, 348–358.