Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism"	Vol. 32	Fasc. 3	(139)-(142)	18. 12. 1992

Does Glutathione play a Role in Freezing Tolerance of Plants?

By

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Key words: H_2S fumigation, frost hardening, low temperature acclimation, thiols.

Summary

STUIVER C. E. E., DE KOK L. J. & KUIPER P. J. C. 1992. Does glutathione play a role in freezing tolerance of plants? - Phyton (Horn, Austria) 32 (3): (139)-(142).

During low temperature hardening enhanced levels of glutathione (GSH) are generally observed in plant shoots and are often related to the development of freezing tolerance. The present communication shows that there is no direct relation between an increased GSH content and freezing tolerance of leaves, and little supporting evidence for the function of GSH as a protectant against freezing-induced injury.

Introduction

The physiological background of freezing tolerance of plant tissue is still largely unknown and subject for discussion (STEPONKUS 1984, GUY 1990, ALBERDI & CORCUERA 1991). Irreversible denaturation of proteins due to freezing-induced disulfide bond formation has been suggested as one of the causes of frost injury in plants (LEVITT 1962, 1980). Indeed, during freezing a loss of both soluble proteins and soluble protein sulfhydryl was found in spinach leaf discs, which could directly be related to the development of freezing injury and was not further enhanced after thawing (STUIVER & al. 1988). However, it remains questionable whether the loss of protein sulfhydryl is the primary cause or the consequence of protein denaturation.

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GSH and freezing tolerance

LEVITT 1962, 1980 suggested that an antioxidant as GSH may play a role in the protection of proteins against freezing-induced denaturation. Indeed, high levels of GSH, accompanied with high activities of glutathione reductase are observed in plants during winter and upon frost hardening (ESTERBAUER & GRILL 1978, DE KOK & OOSTERHUIS 1983, GUY & CARTER 1984, GUY & al. 1984, SMITH & al. 1989, 1990). However, both the roles of GSH and glutathione reductase in freezing tolerance are questionable, since an enhanced GSH content itself did not guarantee a higher freezing tolerance (DE KOK & al. 1981, GUY & al. 1984). Furthermore, the significance of glutathione reductase, an NADPH-dependent enzyme which reduces oxidized glutathione, has still to be proved, because its apparent activity at freezing temperatures has not yet been measured (SMITH & al. 1990). In addition, it is dubious whether GSH directly protects proteins against freezing-induced denaturation: the observed loss of soluble proteins and soluble protein sulfhydryl during freezing was not preceded by or accompanied with a substantial loss of GSH (STUIVER & al. 1988). Loss of GSH would be expected if GSH functions as an antioxidant under freezing conditions, when the activity of glutathione reductase is negligible. GSH was only lost after the subsequent thawing.

GSH content at low temperature

To our opinion more critical experiments were needed in order to obtain insight into any specific role of GSH in freezing tolerance. Wheat and spinach were exposed to low temperature (3 and 5° C, respectively) and the relation between the GSH content and the freezing tolerance of the leaves was further studied. Low temperature exposure resulted in a rapid and substantial enhanced content of water-soluble non-protein sulfhydryl compounds in the leaves (Fig. 1, Table 1). In wheat, the enhancement in sulfhydryl content reached a maximum value after 3 days of exposure to 3° C, and the content remained unaltered upon a further low temperature exposure (Fig. 1). A similar low temperature-induced enhancement of sulfhydryl compounds was observed in spinach leaf tissue (Table 1). Upon a short-term exposure of plants to 10 and 5° C the sulfhydryl content in the leaves enhanced rapidly. The increase in sulfhydryl content was highest at 5° C (Table 1). In both wheat (Fig. 1) and spinach (Table 1) leaves GSH accounted for up to 90 % of the total sulfhydryl content, whereas cysteine was the other major sulfhydryl compound present (STUIVER & al. 1988, BUWALDA & STUIVER, unpublished results). This rapid increase in sulfhydryl (GSH) content during low temperature exposure was not accompanied with a similar rapid increase in freezing tolerance of the tissue: the freezing tolerance of wheat leaves developed gradually upon low temperature exposure and was substantial after 2 weeks (Fig. 1). There was a 6° C increase in freezing tolerance after 6 weeks of low temperature exposure.

H_2S fumigation at low temperature

In general, H_2S fumigation resulted in a rapid and substantial accumulation of water-soluble non-protein sulfhydryl compounds in shoots due to enhanced lev-

els of GSH and cysteine (DE KOK 1989, 1990). In wheat shoots the low temperature-induced increase in sulfhydryl (GSH) content could dramatically be enhanced by supplementary H_2S fumigation. Similar to the observations of DE KOK & al. 1981 and GUY & al. 1984 a strongly enhanced GSH content itself did not increase freezing tolerance of the tissue. On the contrary, an H_2S-induced accumulation of sulfhydryl (GSH) in wheat shoots during low temperature was accompanied with a strongly depressed development of freezing tolerance (STUIVER & al. 1992). Besides, fumigation of wheat with H_2S at 20° C for 2 weeks also resulted in an accumulation of sulfhydryl compounds in the leaves. Here, the freezing tolerance of the tissue was similar to that of non-fumigated plants.

Conclusions

It is unlikely that GSH plays any direct role in the protection of plants against freezing injury. An enhanced content of GSH at low temperature may rather be due to an imbalance between the rate of assimilatory sulfur reduction and protein synthesis under these conditions. Besides, the transport of GSH from the shoots to the roots may be strongly reduced at low temperature. The latter could also explain the dramatic increase in sulfhydryl content upon a combination of H_2S fumigation and low temperature exposure.

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Table 1. Effect of short-term low temperature exposure of spinach on the content of water-soluble non-protein sulfhydryl compounds in the leaves. Spinach plants were grown and exposed to different temperatures as described by STUIVER & al. 1988, 1992. The sulfhydryl content was determined according to DE KOK & al. 1988 and represents the mean of 3 measurements with 2 plants in each (± SD).

Treatment	SH content in spinach leaves (μmol g FW^{-1})	
	1st pair of leaves	2nd pair of leaves
Initial value	0.23 ± 0.03	0.33 ± 0.05
24 h, 20 °C	0.25 ± 0.04	0.32 ± 0.07
48 h, 20 °C	0.21 ± 0.02	0.37 ± 0.02
24 h, 10 °C	0.28 ± 0.03	0.52 ± 0.07
48 h, 10 °C	0.30 ± 0.02	0.48 ± 0.08
24 h, 5 °C	0.51 ± 0.05	0.90 ± 0.07
48 h, 5 °C	0.95 ± 0.08	1.50 ± 0.10

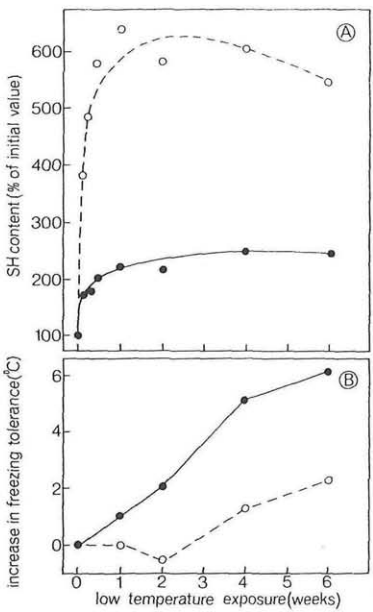

Fig. 1. Effect of prolonged low temperature exposure (3° C) and H_2S fumigation (0.25 µl l^{-1}) on the content of total water-soluble non-protein sulfhydryl compounds and freezing tolerance of winter wheat leaves. Data are derived from STUIVER & al. 1992. Closed symbols, 0 µl l^{-1} H_2S; open symbols, 0.25 µl l^{-1} H_2S. (A), sulfhydryl content, expressed as % of the initial value (0.24 µmol g FW^{-1}); (B), increase in freezing tolerance, expressed as the increase in °C of LT_{50} (temperature at which 50 % of the total electrolytes had leaked out of the tissue after thawing; initial LT_{50} was -11 °C).