S8-022

Development of chlorophyll bleaching in cucumber leaves after light-chilling stress: Irreversible nature of PSI photoinhibition

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Keywords: chilling injury, visible symptom, PSI photoinhibition, degradation, chlorophyll bleachin

Introduction

It has been known that the tropical and subtropical plants exhibit a distinct physiological damage when they are exposed to low temperatures. Almost 30 years ago, Lyons pointed out that the chilling injury of plants exhibits several distinct characteristics (Lyons 1973). First, there exists a threshold temperature below which the symptoms of chilling injury appear. Secondly, the damage to photosynthesis due to chilling injury is irreversible. Third characteristic for the chilling injury is that visible symptoms are developed only after the plants were returned to normal temperature, not just after the chilling treatment. Among these characteristics, the existence of threshold temperature for the damage can be explained by the existence of the threshold temperature for the photoinhibition of PSI (Sonoike 1996). PSI, which had been believed to be tolerant to various kinds of environmental stresses, is shown to be photoinhibited when chilling sensitive plants were exposed to chilling temperature under moderate light (Sonoike and Terashima 1994, Terashima et al. 1994). The temperature dependence of the damage to PSI shows the threshold temperature at 10˚C (Terashima et al. 1994), which explains the temperature dependence in the decrease of photosynthesis due to chilling injury (Sonoike 1998). The aim of this study is to elucidate the cause of other two characteristics of the chilling injury. It was concluded that the development of visible symptom in the chilled leaves after warming-up the temperature is due to the degradation of photoinhibited PSI complex along with its chlorophyll.

Materials and methods

Cucumber (*Cucumis sativus* L. cv. Nanshin) plants were grown hydroponically (Terashima et al. 1991) at 30˚C under conditions of 14 h of light (190 µmol m^{-2} s^{-1}) and 10 h of darkness. Attached leaves were chilled for 5 h by placing them on water at 4˚C under the growth light (190 µmol m^{-2} s^{-1}). The relative content of chlorophyll per unit leaf area was determined non-destructively using chlorophyll meter (SPAD-502, Minolta, Tokyo, Japan). The absorption change around 830 nm due to P-700 oxidation was determined *in vivo* using a pulse-modulated system (PAM 101/102, Walz, Effeltrich, Germany) (Schreiber et al. 1988). Thylakoid membranes were isolated from light-chilled and untreated leaves as described in Terashima et al. (1991). In order to determine the concentration of photo-oxidizable P-700, light minus dark difference absorption changes at 701 nm were measured using a spectrophotometer (model 356, Hitachi, Tokyo, Japan) (Terashima et al. 1994, Sonoike 1995). Chlorophyll concentration was determined after extraction with 80% acetone according to Porra et al. (1989).

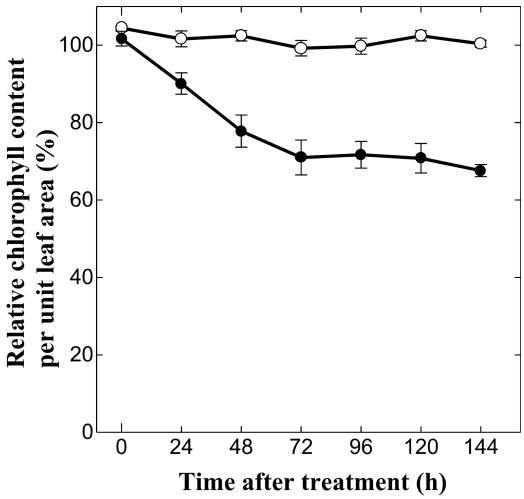
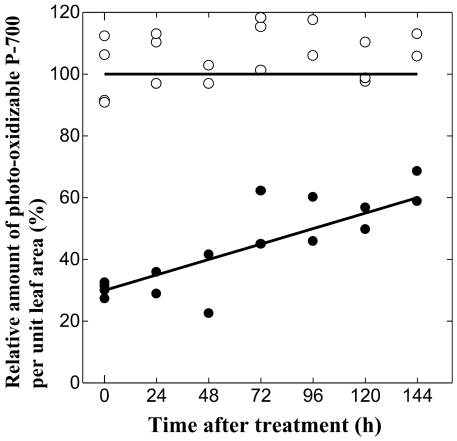
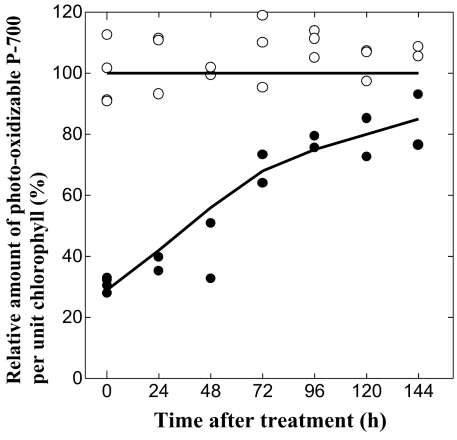


Fig.1 Time-course changes of chlorophyll content determined *in vivo*
Chlorophyll content per unit leaf area was determined with chlorophyll meter. Open circles, untreated leaves; closed circles, the leaves chilled under growth light. Bars show the standard deviation.

Results

When cucumber leaves were treated at 4˚C under light at 190 µmol m^{-2} s^{-1} for 5 h, chlorophyll content did not change just after the treatment (Fig.1). After the chilled cucumber plants were returned to growth condition (30˚C, 190 µmol m^{-2} s^{-1}), the leaves started to bleach. After 72 h following the chilling treatment, chlorophyll content per unit leaf area decreased by 30% (Fig.1). The decreased level of chlorophyll did not change during 72 h – 144 h following the chilling treatment. The bleaching was observed only when plants were transferred to the growth condition. Chlorophyll content was not affected by the treatment at 4˚C for 24 h under growth light (data not shown). Although the chlorophyll content of the chilled leaves did not change just after the chilling treatment as shown in Fig.1, the functional PSI content per unit leaf area determined in isolated thylakoid membranes decreased by 70% (Fig.2A). The level recovered gradually in 6 days (0-144 h) following the chilling treatment, but the amount of photooxidizable P-700 was still 50% of the control level even after 144 h following the chilling treatment (Fig.2A). If the amount of photo-oxidizable P-700 was expressed as unit chlorophyll basis, however, the amount of photo-oxidizable P-700 recovered to 90% of control level in 144 h following the chilling treatment (Fig.2B). The result suggests that PSI complex which lost its function was degraded along with its chlorophyll. This is also supported by the close relationship between the amount of chlorophyll and the amount of





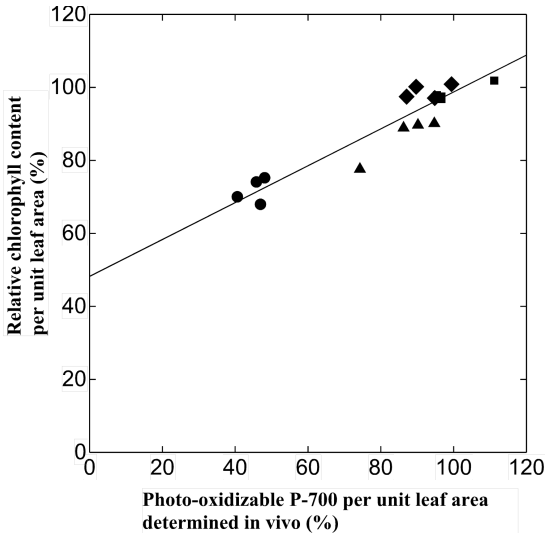


Fig.3 Correlation between chlorophyll content and P-700
Relative chlorophyll content respect to photo-oxidizable P-700 (functional PSI) was plotted after 96 h following the chilling treatment at 4˚C under growth light (190 μmol m^{-2} s^{-1}) (circles); at 4˚C under weak light (95 μmol m^{-2} s^{-1}) (triangles); at 12˚C under strong light (480 μmol m^{-2} s^{-1}) (squares), or at 30˚C under growth light (diamonds). The regression line for the symbols was drawn by least square method.

functional PSI at 96 h after the treatment, where the decrease of chlorophyll had already stopped (Fig.3).

Discussion

In this study, we investigated the relationship between PSI photoinhibition and bleaching of chlorophyll after chilling stress. The chlorophyll content did not change while 70% of PSI function was lost just after the chilling treatment. At this point, there is no correlation between PSI photoinhibition and bleaching of chlorophyll. However, the chlorophyll amounts subsequently decreased to the level of decreased PSI function in next 4 days (Fig.3). We can conclude that the bleaching of the light-chilled leaves is brought about by the degradation of photoinhibited, non-functional PSI complex along with its chlorophyll. The apparent recovery of photo-oxidizable P-700 in unit chlorophyll basis (Fig.2B) should indicate that the degradation of damaged PSI is almost complete. Many reports on chilling injury indicated that the symptoms of the chilling damage are more obvious or gradually appear after when the plant is returned to optimal growth temperatures (Lyons 1973, Smillie et al. 1987, Murata et al. 1992, Whitaker 1995, Wu and Browse 1995). The present results demonstrated that such bleaching of chlorophyll in chilled leaves may be due to the degradation of PSI. In addition, the bleaching of chlorophyll was detected only after the chilled leaves were returned to the normal temperature. The result can be explained by the involvement of enzymatic process in the degradation of PSI as suggested by previous experiment using protease inhibitors (Sonoike et al. 1997). PSI photoinhibition is virtually irreversible and the rate of the recovery is quite slow (Fig.2A). The degradation of damaged PSI and accompanying bleaching of chlorophyll must be necessary since energy absorption by the pigments in damaged reaction center is dangerous for plants through the production of active species of oxygen.

Acknowledgments

We are grateful to the Molecular Genetics Research Laboratory, The University of Tokyo for allowing us to use the facilities. This research was supported in part by Grant-in-Aid for Scientific Research (B) (11440233) from the Japan Society for the Promotion of Science and Grant-in-Aid for Scientific Research on Priority Area (C) Genome Biology (12206029) from the Ministry of Education, Science, Sports and Culture.

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