Changes in Translatable RNA Population during Hardening of Chlorella ellipsoidea C-27

Joh, Toshio
Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University

Yoshimoto, Makoto
Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University

Honjoh, Ken-ichi
Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University | Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University

Miyamoto, Takahisa
Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University

他

https://doi.org/10.5109/24017

出版情報：九州大学大学院農学研究院紀要. 37 (3/4), pp.257-263, 1993-03. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 37 (3•4), 257-263 (1993)

Changes in Translatable RNA Population during Hardening of *Chlorella ellipsoidea C-27*

Toshio Joh, Makoto Yoshimoto, Ken-ichi Honjoh, Takahisa Miyamoto and Shoji Hatano

Laboratory of Food Hygienic Chemistry, Faculty of Agriculture, Kyushu University 46-09, Fukuoka 812, Japan

(Received October 30, 1992)

The effects of hardening on gene expression in *Chlorella ellipsoidea C-27* were investigated. *In vitro* translation products of translatable poly(A)$^+$RNA prepared from unhardened and hardened *Chlorella* cells were separated by SDS-polyacrylamide gel electrophoresis and two-dimensional polyacrylamide gel electrophoresis, and visualized by fluorography. A time course study showed that changes in profile of poly(A)$^+$RNA occur from the 1st hour of hardening. A comparison of *in vitro* translation products from unhardened and 24-h hardened cells indicated that 13 polypeptides newly appear and 10 polypeptides increase in abundance during hardening. The results suggest that expression of specific genes is involved in the development of frost hardiness.

INTRODUCTION

Some plant species develop freezing tolerance when exposed to low nonfreezing temperature (Graham and Patterson, 1982; Guy, 1990). It is known that during this cold acclimation many physiological and biochemical changes occur in plant cells: alterations in lipid composition, increases of sugar and soluble protein content, and changes in enzyme activities (Graham and Patterson, 1982; Uemura and Yoshida, 1984; Calderon and Pontis, 1985; Lynch and Steponkus, 1987; Guy, 1990). Weiser (1970) has first suggested that the biochemical, biophysical, and physiological changes that occur in plant cells during cold acclimation involve the altered gene expression. This suggestion is now supported by evidences that cold acclimation-specific changes in mRNA and protein profiles are identified in a wide variety of higher plants (Guy, 1990; Thomashow, 1990). However, the functions of the cold-induced proteins and the molecular mechanisms regulating their changes are still unclear.

We previously have showed that hardened cells of *Chlorella ellipsoidea C-27* are able to survive slow freezing to -196°C (Hatano et *al.*, 1976a), and the involvement of RNA and protein syntheses and lipid changes in the hardening process of the algal cells suggests the similarity of their hardening mechanism to that of higher plants (Hatano et *al.*, 1976b; Hatano, 1978; Hatano et *al.*, 1981, 1982). We also recently indicated alteration in profiles of in *vivo* soluble proteins during hardening of the strain C-27 (Yoshimoto et al., 1991). In order to further investigate the molecular genetic basis of frost hardiness, we analyzed *in vitro* translation products of poly(A)$^+$RNA from unhardened and hardened cells.

This paper demonstrates that changes in gene expression at transcriptional level

are involved in the development of frost hardiness of *Chlorella ellipsoidea* C-27.

MATERIALS AND METHODS

Plant materials

Chlorella ellipsoidea IAM C-27 was grown synchronously in the MC medium of Watanabe (1960), at 25°C, under 10 klux, with 1.3% CO_2 in air, at a concentration of about 1.0×10^{10} cells per liter, under a 16-h light/B-h dark regime, as described previously (Hatano *et al.*, 1976a). Inasmuch as the cells were hardened most at the L_2 stage (an intermediate stage in the ripening phase of the cell cycle) (Hatano *et al.*, 1976a), L_2 cells were used here.

Hardening

L_2 cells synchronized at 25°C were directly hardened at 3°C. During hardening, the culture was aerated with 1.3% CO, in air and kept in the light (10 klux). Portions of the cell culture were withdrawn at intervals and centrifuged at 1,000 X g for 5 min at 3°C. After washing once with distilled water, cells were suspended in RNA extraction buffer (0.1 M Tris-HCl, pH 9.0, 0.1 M NaCl, 10 mM EDTA, and 1% SDS) at a concentration of about 1.5X 10^9 cells/ml. The cell suspension was frozen in liquid nitrogen and stored at -80°C.

Isolation of poly(A)$^+$RNA

The frozen suspension was thawed at 4°C and to this an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v/v) and 0.2 volumes of 2-mercaptoethanol were added. The mixture was homogenized with glass beads of 0.5 mm in diameter in a reciprocal shaker (Vibrogen-Zellmühle; Edmund Biihler Co., Ttibingen, F.R.G.), operated at 4,500 rpm at 4°C for 12 min. The homogenate was freed from the beads by passage through a sintered-glass funnel and the filtrate was centrifuged at 3,500X g for 10 min at 4°C. The upper aqueous phase containing total nucleic acids was washed twice with an equal volume of phenol/chloroform/isoamyl alcohol to remove proteins and cell debris completely. Total nucleic acids were precipitated with 0.05 volumes of 5 M NaCl and 2.5 volumes of ethanol overnight at -20°C, washed with cold 70% ethanol and then dissolved in TE buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA). Total RNA was precipitated overnight at 4°C by the addition of 5 M LiCl to a final concentration of 2 M. Poly(A)$^+$RNA was purified from the RNA by the use of an oligo(dT)-cellulose (Sigma, St. Louis, MO, U.S.A) as described by Maniatis et *al.* (1982).

Analysis of *in vitro* translation products

Poly(A)$^+$RNA (0.5 to 1.0 μg) was translated by using a wheat germ extract *in vitro* translation kit (Boehringer Mannheim Yamanouchi, Tokyo, Japan) in the presence of 80 mM CH_3COOK, 2 mM $Mg(CH_3COO)_2$, and 2.2 MBq of [^{35}S]methionine (37 TBq/mmol; Hungarian Academy of Sciences, Budapest, Hungary). The translation products were separated by the SDS-polyacrylamide gel electrophoresis (SDS-PAGE) procedure of Laemmli (1970) and by the two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) procedure of O'Farrell(l975). Following electrophoresis, the gel

was fixed, treated with EN³HANCE (NEN, Boston, MA, U.S.A), dried and then exposed to Kodak XAR-5 film with an intensifying screen at -80°C.

RESULTS

We previously reported changes in pattern of *in vivo* proteins during hardening in C. *ellipsoidea C-27* (Yoshimoto et al., 1991). To confirm that these changes occur at transcriptional level, *in vitro* translation products of poly(A)⁺RNA from hardened cells for various periods up to 24 h were analyzed. Figure 1 shows the results of SDS-PAGE of the *in vitro* translated proteins. Polypeptides of 51, 47, 30, 26, 23, 18, and 15 kDa apparently increased during hardening (indicated by arrowheads on the right side), while polypeptides of 28 and 16 kDa disappeared (arrowheads on the left side). Especially 47 kDa polypeptide seemed to increase in a two-step process, that is, this polypeptide once increased at the 1st hour of hardening, but decreased at and after the 2nd hour of hardening, and then increased again after the 6th hour of hardening. Changes in the profiles of the translation products occurred from the 1st hour of hardening, suggesting that response of gene expression to low temperature is relatively sensitive.

Furthermore, in *vitro* translation products from unhardened and 24-h hardened cells were analyzed in detail by 2D-PAGE. In Fig. 2, alphabetized and numbered spots

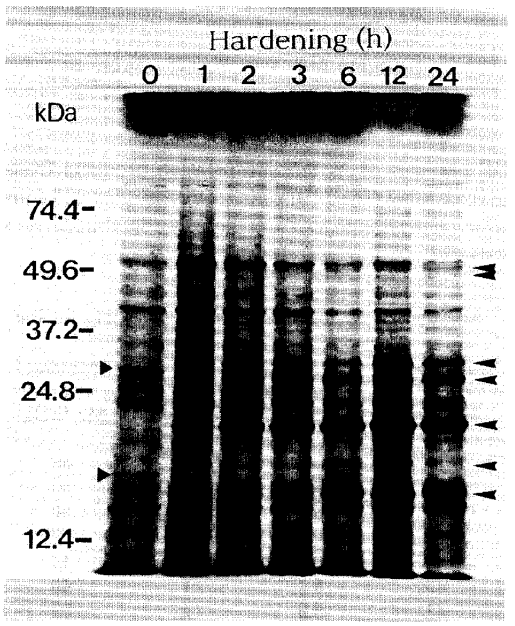

Fig. 1. Fluorograph of *in vitro* translation products from poly(A)⁺RNA of hardened cells for various periods. The [^{35}S] methionine-labeled translated proteins of 2×10^5 cpm were loaded in each lane and separated by 12.5% polyacrylamide gel containing 0.1% (w/v) SDS. Arrowheads on the right and left sides of the electrophoregraph indicate bands of increasing and decreasing translated polypeptides during hardening, respectively.

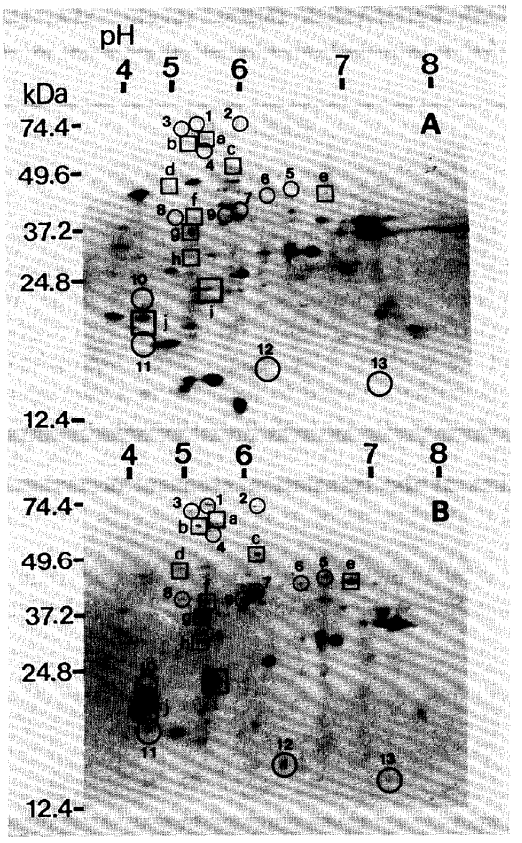

Fig. 2. 2D-PAGE patterns of *in vitro* translation products from poly(A)+RNA of unhardened (A) and 24-h hardened (B) cells. Open circles and squares indicate spots of polypeptides which newly appeared and increased in abundance at the 24th hour of hardening, respectively.

Table 1. Analysis of *in* vitro translation products during hardening.

Induced			Increased		
spot*	Mr (kDa)	PI	spot*	Mr (kDa)	PI
1	74	5.3	a	65	5.3
2	73	5.9	b	64	5.2
3	71	5.1	c	50	5.9
4	62	5.3	d	47	5.0
5	45	6.6	e	44	6.8
6	43	6.4	f	40	5.3
7	41	5.9	g	37	5.3
8	40	5.0	h	30	5.3
9	40	5.8	i	23	5.4
10	22	4.3	j	21	4.3
11	18	4.3			
12	15	6.2			
13	14	7.3			

*Spots are corresponding to Fig. 2.

indicate polypeptides that newly appeared and increased in abundance during hardening, respectively. The results showed that at least *13* polypeptides newly appear and 10 polypeptides increase in abundance and some polypeptides decrease or disappear during hardening. The relative molecular masses and the isoelectric points of polypeptides that newly appeared and increased by a 24-h hardening were summarized in Table **1.** Of these, 4 polypeptides (spots e, i, j, and 7) increased markedly by a 24-h hardening. These results suggest that at least 23 translatable poly(A)$^+$RNA species are induced' at the 24th hour of hardening and the proteins encoded by them are involved in the hardening process.

DISCUSSION

It is known that expression of specific genes is involved in the development of frost hardiness in higher plants and proteins encoded by these genes play an important role in the hardening process (Guy, 1990; Thomashow, 1990). We previously reported alterations in profiles of *in vivo* soluble and membrane proteins during hardening in C. *ellipsoidea* C-27 (Yoshimoto et al., 1991). In order to confirm that these alterations in the *Chlorella* are involved in gene expression in the same way as higher plants, poly(A)$^+$RNA were prepared from hardened cells for various periods up to *24* h and the *in vitro* translation products were analyzed. As expected, changes in profiles of translation products during hardening were observed; 23 polypeptides newly appeared or increased in abundance at the 24th hour of hardening (Figure 2 and Table 1), suggesting that the development of frost hardiness in the *Chlorella* is also accompanied by expression of specific genes.

As compared the number of *in vivo* newly appeared or increased polypeptides during hardening with that of *in vitro,* there were 19 polypeptides *in vivo* as against 23 *in vitro* (Yoshimoto *et al.,* 1991). As the molecular mass of the changed polypeptides ranged between 74 and 14 kDa *in vitro* as against between 141 and 27 kDa in *vivo* (Yoshimoto *et al.,* 1991), *in vivo* changed polypeptides are larger than them of *in vitro.* Normally, in *in vitro* translation experiments proteins of high molecular mass seem to be troublesome to be synthesized and in other reports about *in vitro* translation proteins of high molecular mass can not be detected abundantly, either (Danyluk and Sarhan, 1990; Koga-Ban *et al.,* 1991; Lee *et al.,* 1991). Consequently, the difference in range of the molecular mass of the changed polypeptides between *in vitro* and *in vivo* may come from the problem of *in vitro* translation system itself. Also, in comparison of the molecular mass and the isoelectric point of the changed polypeptides between both, any polypeptide does not agree except one polypeptide (spot h in Figure 2 and Table 1). Inside cells, some polypeptides translated from poly(A)$^+$RNA are processed to become mature proteins. Therefore these disagreements are presumed to be attributable to post-translational modification of protein that occurs only *in vivo*.

Although proteins of high molecular mass could not be synthesized abundantly, 23 polypeptides newly appeared or increased in abundance during hardening in the *Chlorella,* showing that its development of frost hardiness involves expression of specific genes in the same as higher plants. Guy (1990) and Thomashow (1990) summarized many reports about changes in synthesis and accumulation of proteins

during cold acclimation in plants. According to them, 16 polypeptides reported in tomato are the most numerous in the number of increased proteins during cold acclimation in one plant. As compared with that, the number of newly appeared or increased proteins in the *Chlorella*, 23 polypeptides, seems to be very abundant. Although, of course, all of these polypeptides would not be involved in the development of frost hardiness, the fact that in spite of unicellular plant (but maybe because of unicellular plant) such many changes in protein synthesis occur during hardening shows that a mechanism of the development of frost hardiness in the *Chlorella* may be somewhat different from that in higher plants.

ACKNOWLEDGEMENTS

The present research was supported by the Egashira Foundation for Promotion of the Hotel and the Restaurant Industry. We are grateful to Mr. Jun Funabashi and Mr. Yasumichi Mizota for technical support.

REFERENCES

Calderon, P. and Pontis, H. G. 1985 Increase of sucrose synthase activity in wheat plants after a chilling shock. Plant *Science,* 42: 173-176

Danyluk, J. and Sarhan, F. 1990 Differntial mRNA transcription during the induction of freezing tolerance in spring and winter wheat. *Plant Cell Physiol., 31: 609-619*

Graham, D. and Patterson; D. 1982 Responses of plants to low, nonfreezing temperatures: proteins, metabolism, and acclimation. *Ann, Rev, Plant Physiol., 33: 347-372*

Guy, C. L. 1990 Cold acclimation and freezing tolerance: role of protein metabolism. *Ann. Rev. Plant Physiol. Plant Mol. Biol., 41: 187-223*

Hatano, S. 1978 Studies on frost hardiness in *Chlorella ellipsoidea:* effects of antimetabolites, surfactants, hormones and sugars on the hardening process in the light and dark. In Plant cold Hardiness and Freezing Stress. Edited by Li, P. H. and Sakai, A. Academic Press, New York, pp. 175-196

Hatano, S., Kabata, K. and Sadakane, H. 1981 Transition of lipid synthesis from chloroplasts to a cytoplasmic system during hardening in *Chlorella ellipsoidea. Plant Physiol., 67: 216-220*

Hatano, S., Kabata, K., Yoshimoto, M. and Sadakane, H. 1982 Accumulation of free fatty acids during hardening of *Chlorella ellipsoidea. Plant Physiol., 70:* 1173–1177

Hatano, S., Sadakane, H., Tutumi, M. and Watanabe, T. 1976a Studies on frost hardiness in *Chlorella ellipsoidea* I. Development of frost hardiness of *Chlorella ellipsoidea* in synchronous culture. *Plant Cell Physiol.,* **17:** 451-457

Hatano, S., Sadakane, H., Tutumi, M. and Watanabe, T 1976b Studies on frost hardiness in *Chlorella ellipsoidea* II. Effects of inhibitors of RNA and protein synthesis and surfactants on the process of hardening. *Plant Cell Physiol., 17: 643-651*

Koga-Ban, Y., Abe, M. and Kitagawa, Y. 1991 Alteration in gene expression during cold treatment of rice plant. *Plant Cell Physiol., 32:* 901–905

Laemmli, U. K. 1970 Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227: 680–685

Lee, S. P., Chen, T. H. H. and Fuchigami, L. H. 1991 Changes in the translatable RNA population during abscisic acid induced freezing tolerance in bromegrass suspension culture. *Plant Cell Physiol., 32: 45-56*

Lynch, D. V. and Steponkus, P. L. 1987 Plasma membrane lipid alterations associated with cold

acclimation of winter rye seedlings (*Secale cereale* L. cv Puma). *Plant Physiol., 83: 761-767*

Maniatis, T., Fritsch, E. F. and Sambrook, J. 1982 *Molecular Cloning: a Laboratory Manual.* Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

O'Farrell, P. H. 1975 High resolution two-dimensional electrophoresis of proteins. J. *Biol. Chem.,* **19:** 4007-4021

Thomashow, M. F. 1990 Molecular genetics of cold acclimation in higher plants. *Adv. Genet., 28:* 99-131

Uemura, M. and Yoshida, S. 1984 Involvement of plasma membrane alterations in cold acclimation of winter rye seedlings (*Secale cereale* L. cv Puma). *Plant Physiol., 75: 818-826*

Watanabe, A. 1960 List of algal strains in collection at the Institute of Applied Microbiology, University of Tokyo. *J. Gen. Appl. Microbiol., 6: 283-292*

Weiser, C. J. 1970 Cold resistance and injury in woody plants. Science,, **169:** 1269-1278

Yoshimoto, M., Okamura, H., Joh, T., Miyamoto, T. and Hatano, S. 1991 Changes in soluble and membrane proteins of *Chlorella ellipsoidea* during early time of hardening. *J. Fac. Agr., Kyushu Univ., 36* (1, *2):* 69–77