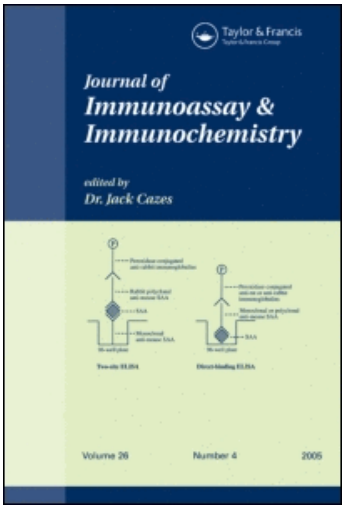

Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Influence of Stress Protein CSP 310 and Antiserum Against This Protein on Oxygen Uptake, Lipid Peroxidation, and Temperature of Winter Wheat Seedling Shoots During Cold Stress

A. V. Kolesnichenko[a]; O. I. Grabelnych[a]; V. V. Tourchaninova[a]; V. V. Zykova[a]; N. A. Koroleva[a]; T. P. Pobezhimova[a]; A. M. Korzun[a]; V. K. Voinikov[a]
[a] Siberian Institute of Plant Physiology and Biochemistry, Siberian Division of Russian Academy of Sciences, Irkutsk, Russia

Online publication date: 03 April 2003

To cite this Article Kolesnichenko, A. V. , Grabelnych, O. I. , Tourchaninova, V. V. , Zykova, V. V. , Koroleva, N. A. , Pobezhimova, T. P. , Korzun, A. M. and Voinikov, V. K.(2003) 'Influence of Stress Protein CSP 310 and Antiserum Against This Protein on Oxygen Uptake, Lipid Peroxidation, and Temperature of Winter Wheat Seedling Shoots During Cold Stress', Journal of Immunoassay and Immunochemistry, 24: 1, 41 — 55
To link to this Article: DOI: 10.1081/IAS-120018468
URL: http://dx.doi.org/10.1081/IAS-120018468

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

JOURNAL OF IMMUNOASSAY & IMMUNOCHEMISTRY
Vol. 24, No. 1, pp. 41–55, 2003

Influence of Stress Protein CSP 310 and Antiserum Against This Protein on Oxygen Uptake, Lipid Peroxidation, and Temperature of Winter Wheat Seedling Shoots During Cold Stress

A. V. Kolesnichenko,* O. I. Grabelnych, V. V. Tourchaninova, V. V. Zykova, N. A. Koroleva, T. P. Pobezhimova, A. M. Korzun, and V. K. Voinikov

Siberian Institute of Plant Physiology and Biochemistry,
Siberian Division of Russian Academy of Sciences,
Irkutsk, Russia

ABSTRACT

It is determined that infiltration of winter wheat seedling shoots by anti-CSP 310 antiserum caused a significant decrease of oxygen uptake in winter wheat shoots during short-term cold stress. On the other hand, infiltration of winter wheat seedling shoots by

*Correspondence: A. V. Kolesnichenko, Siberian Institute of Plant Physiology and Biochemistry, Siberian Division of Russian Academy of Sciences, P.O. Box 1243, Irkutsk-33, 664033, Russia; E-mail: akol@sifibr.irk.ru.

DOI: 10.1081/IAS-120018468 1532-1819 (Print); 1532-4230 (Online)

stress protein CSP 310 caused an increase of oxygen consumption. The comparison of the influence of infiltration of winter wheat shoots by CSP 310 and anti-CSP 310 antiserum on the rate of lipid peroxidation showed that, if infiltration by CSP 310 caused a decrease of conjugated diene formation, infiltration by anti-CSP 310 antiserum did not cause any significant changes in the rate of lipid peroxidation. The study of the influence of infiltration of winter wheat shoots by CSP 310 and anti-CSP 310 antiserum on the temperature of winter wheat shoots during cold stress showed that CSP 310 caused the increase of their temperature while anti-CSP 310 caused a decrease of their temperature.

INTRODUCTION

It is known that uncoupling of oxidation and phosphorylation in mitochondria, in which the so-called ''uncoupling proteins'' participate in mammals, is connected with the heat generation used for thermoregulation.[1] Some plant uncoupling proteins, such as PUMP,[2] StUCP,[3] and AtUCP[4] which are homologues of mammalian UCPs were found and characterized. All of these proteins are inner membrane integral mitochondrial proteins and are members of the mitochondrial anion carrier family.[5] Another uncoupling system in plants is connected with CN-resistant alternative oxidase (AOX) activity. The CN- and antimycin-resistant AOX, which bypasses the main cytochrome way of the respiratory chain, catalyses ubiquinol–oxygen oxido-reduction without the release of H^+ in the intramembrane mitochondrial space and, so, dissipates redox potential energy and causes the uncoupling of mitochondria.[6,7] Its activity can be inhibited by hydroxamic acids such as benzohydroxamate (BHAM).[8,9]

The plant cold stress protein CSP 310 was found to cause the uncoupling of oxidative phosphorylation of frost-resistant winter cereals seedlings. CSP 310 was isolated from winter rye[10] and its uncoupling activity was found.[11] This protein is a nuclear-encoded cytoplasmatic protein controlled by chromosomes 1 and 6 of winter wheat D-genome.[12] CSP 310 was also constitutively synthesized in 3-day-old cereal shoots but did not cause significant uncoupling.[13] Further investigation showed that, during cold stress, the constitutively synthesized form of CSP 310 changed from a form with low uncoupling activity to a form having high uncoupling activity.[14] The uncoupling activity

of CSP 310 depended on its concentration.[13] It was found that this protein affects mainly complex I of the mitochondrial respiratory chain.[15] It was shown that the mechanism of CSP 310 uncoupling action differs from the "fatty acid cycling" mechanism of plant UCP function because CSP 310 did not inhibit by addition of BSA, that is a powerful inhibitor of plant UCPs.[16,17] On the other hand, the addition of anti-CSP 310 antiserum to mitochondria isolated from stressed winter rye shoots caused the coupling of oxidation and phosphorylation.[18] So, it was supposed that CSP 310 is localized in outer mitochondrial membrane.[17]

Different plant uncoupling mitochondrial systems were found to participate in lowering of mitochondrial ROS production.[19,20] For example, it was found that the inhibition of PUMP activity in isolated potato tuber mitochondria significantly increased mitochondrial H_2O_2 generation. It was also found that substrates of UCP-like uncoupling proteins, such as linoleic acid and other free fatty acids, reduce mitochondrial H_2O_2 generation.[21,22] Unlike other plant uncoupling proteins, an addition of some concentrations of CSP 310 to isolated winter wheat mitochondria was found to induce ascorbate-dependent and NADH-dependent lipid peroxidation systems.[23] On the other hand, inhibition of CSP 310 by a specific antiserum caused an increase of the lipid peroxidation rate in isolated mitochondria.[24] However, it is necessary to remember that reaction of isolated organelles and the whole plant organism on the same treatment can be different. Therefore, it was interesting to determine what influence CSP 310 and anti-CSP 310 antisera have on peroxidation in whole winter wheat shoots during cold stress.

The main function of mammalian mitochondrial uncoupling proteins is participation in body thermoregulation by the way of thermogenesis.[25] Previously, it was shown that under cold shock ($-4^{\circ}C$) winter wheat shoots can generate heat and their temperature was above $0^{\circ}C$ for the initial 25–30 min.[26] At the same time, it was found that only a part of this difference was detected in KCN-infiltrated shoots. It was suggested that heat generation during cold shock in winter wheat shoots might be produced because of energy transfer from oxidative processes in mitochondria, and that stress protein CSP 310 can participate in this process. So, it was necessary to determine how infiltration of winter wheat shoots by CSP 310 that activate this plant uncoupling mitochondrial system and infiltration by anti-CSP 310 antiserum that inhibits this system *in organello*[24] influence the temperature of winter wheat shoots during short-term, low-temperature stress.

EXPERIMENTAL

Shoots of 3-day-old etiolated winter wheat (*Triticum aestivum L.*, winterhardy cv. Zalarinka) seedlings were used in the study. Seedlings were grown on wet filter paper in thermostat at 26°C.

Stress protein CSP 310 and anti-CSP 310 antiserum were obtained as described previously.[10] Infiltration of winter wheat shoots by stress protein CSP 310 (1 mg/mL) and anti-CSP 310 antiserum (1 mg/mL) was performed during 1 h. After infiltration, seedling shoots were washed and excess moisture was removed from the shoot surface with filter paper.

SDS-electrophoresis of plant proteins was performed in the polyacrylamide gel blocks $70 \times 80 \times 1$ mm using the Laemmli system.[27] After electrophoresis, proteins were stained by Coomassie R-250. The relative molecular weights of the proteins were determined by using a LMW kit of markers (Pharmacia, Uppsala, Sweden). Western-blotting with antibodies to CSP 310 was performed as described previously.[14]

The oxygen uptake of winter wheat shoots was recorded polarographically at 27°C using a platinum electrode of a closed type in a 1.4 mL volume cell.[28] The reaction mixture contained 50 mM KH_2PO_4, 50 mM sucrose, pH 5.2. Polarograms were used to calculate the oxygen uptake of shoot tissue.

The rate of lipid peroxidation was determined by measuring the primary products of lipid peroxidation-conjugated diene formation. Each sample contained 0.9 mL of the incubation medium and 0.1 mL total suspension. To measure the dienic conjugate contents, total lipids were extracted by hexane–isopropanol (1:1 v/v) mixture (9 mL per 1 mL of the sample) by shaking. After shaking, 1 mL H_2O was added to the mixture to stratify the hexane and isopropanol phases. Measurements of dienic conjugate contents were made in hexane phase at 233 nm on spectrophotometer "SF-46" ("LOMO", USSR).[24] The dienic conjugate contents in the sample were calculated according to 233 nm molar extinction coefficient to polyunsaturated fatty acids conjugated dienes $2.2 \times 10^5 \times M^{-1}\,sm^{-1}$.[29]

In the study of the influence of cold shock on thermogenesis samples, 3 g of seedling shoots were used. The measurement of the temperature of chilling samples was performed as described previously[26] by a copper-constantan thermocouple (wire diameter 0.1 mm) connected to the input of a high-sensitive microvoltmeter. The sensitivity of this thermocouple was 0.025°C. For the measurement, samples of seedling shoots (3 g) were tightly packed into a small container and then were subjected to a thermostat with experimental temperature (−4°C). Temperature changes were recorded as "living" for 30 min or 1 h. Then the shoots sample was

placed in hot water (95°C) for 5 min to stop all metabolic processes. Excess moisture was removed from the shoot surface with filter paper and the temperature changes were recorded immediately in the same samples as the "killed," cooled from 25°C to experimental temperature. Thus, we obtained the temperature curves following chilling with one tissue sample for "living" and for "killed" tissues, and calculated the temperature difference (ΔT^0) between "living" and "killed" seedling shoot tissues. For Figs. 4A and 5A, the means for "killed" and "living" shoot samples from different experimental samples were calculated.

All the experiments were performed in six preparations. The data obtained were analyzed statistically, i.e., arithmetic means and standard errors were determined.

RESULTS AND DISCUSSION

To determine if infiltration of winter wheat seedling shoots by stress protein CSP 310 and anti-CSP 310 antiserum influence the CSP 310 content in their tissues, soluble cytoplasmic proteins were extracted, and Western-blotting with anti-CSP 310 antiserum was performed. It was found that both of these treatments influence the content of CSP 310 subunits in winter wheat shoots (Fig. 1). Infiltration by CSP 310 during 1 h caused the increase of CSP 310 subunits content in CSP 310-infiltrated shoots. On the other hand, infiltration of winter wheat shoots by anti-CSP 310 antiserum caused the decrease of CSP 310 subunits content, and in this variant, the high-molecular-weight band of anti-CSP 310 antibodies was detected (Fig. 1).

The next set of experiments was devoted to the study of the influence of these treatments on the oxygen consumption of winter wheat shoots during cold stress. Firstly, the study of an influence of cold shock on oxygen consumption showed that it caused an increase of oxygen consumption values (Fig. 2). The infiltration of winter wheat shoots by CSP 310 caused an increase of oxygen consumption from 425.5 ± 25.1 to 533.4 ± 13.4 $n\text{Mol}\,O_2/\text{min/g}$ of tissue. These data allows us to suppose that infiltration of winter wheat shoots by CSP 310 can activate this plant mitochondrial uncoupling system. On the other hand infiltration of winter wheat shoots by anti-CSP 310 antiserum caused a decrease of oxygen consumption to 284.8 ± 14.7 $n\text{Mol}\,O_2/\text{min/g}$ of tissue. Infiltration of winter wheat shoots by non-immune antiserum did not influence the rate of winter wheat tissue oxygen consumption. So, because the effects of these treatments on winter wheat seedling shoots, oxygen consumptions were similar to their previously shown effects on isolated mitochondria[11]

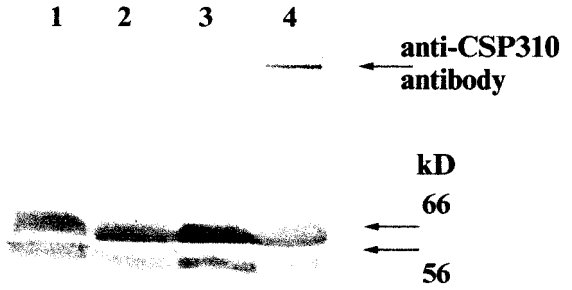

Figure 1. Western-blotting of control (1), stressed (2), infiltrated by CSP 310 (3), and infiltrated by anti-CSP 310 antiserum (4) winter wheat seedling shoots with antibodies to CSP 310.

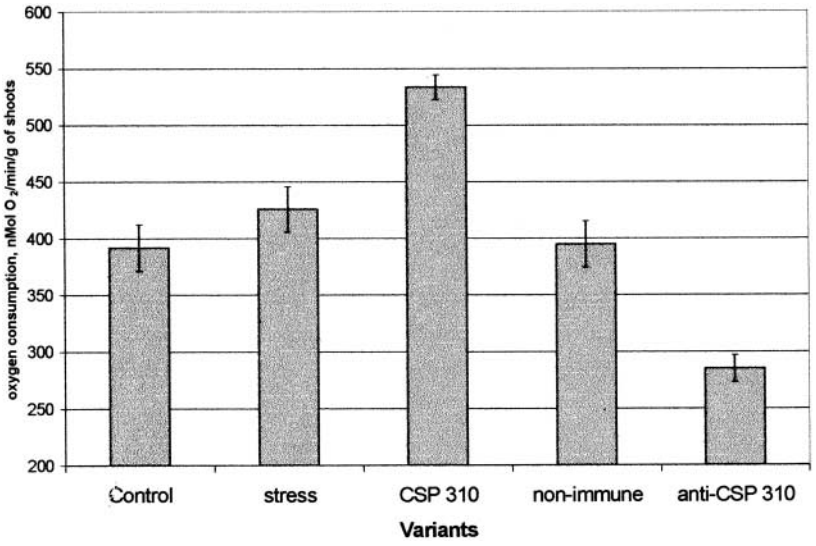

Figure 2. An influence of CSP 310, anti-CSP 310, and non-immune antiserum infiltration on oxygen consumption of winter wheat shoots during cold stress.

and both stress protein CSP 310 and anti-CSP 310 antiserum were detected in plant seedling shoots; we can conclude that both of them were transported into plant cells and associated with mitochondria. Therefore, it was suggested that they would influence the other known

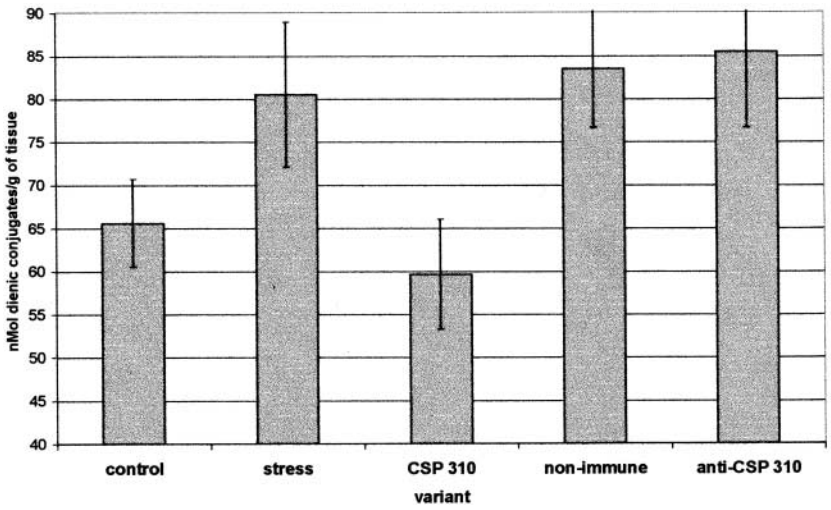

Figure 3. An influence of CSP 310, anti-CSP 310, and non-immune antiserum infiltration on lipid peroxidation in winter wheat shoots during cold stress.

functions of this uncoupling mitochondrial system, i.e., regulation of ROS formation by winter wheat mitochondria and regulation of plant body temperature.

The study of the influence of short-term, low temperature stress on conjugate diene formation in winter wheat shoot tissues showed that it caused about 20% increase in their formation (Fig. 3). These data are well correlated with data obtained by many investigators concerning an influence of cold stress on lipid peroxidation in plant tissues.[30,31] Unlike isolated mitochondria, in which CSP 310 did not have any influence on conjugate diene formation or, under some conditions, even stimulation of its formation,[23] in whole winter wheat seedling shoots, their infiltration by this protein caused about a 30% decrease of conjugate diene formation during short-term low temperature stress (Fig. 3). These values were slightly lower, but not statistically different from the value of diene conjugate contents in non-stressed winter wheat shoots (Fig. 3). The difference between CSP 310 action on isolated mitochondria and whole plant seedling tissue allows us to suppose that CSP 310 can function during low temperature stress as an antioxidant, not only by decreasing ROS formation by mitochondria. Really, preparations of CSP 310-like proteins from some cereals unlike purified CSP 310 significantly decrease the rate of ascorbate-dependent and HADH-dependent lipid peroxidation.[32] Because these preparations

consisted of significant amounts of CSP 310-like proteins with lower molecular weights, and especially independent CSP 310 subunits,[33] we can suppose that CSP 310 subunits have their own antioxidant activity and can lower lipid peroxidation during low temperature stress after winter wheat shoot infiltration by this protein and, therefore, in whole plants, CSP 310 plays a role similar to those observed in isolated mitochondria for AOX and PUMP.[21,34]

At the same time, the data obtained showed that, if inhibition of CSP 310 uncoupling activity by addition of anti-CSP 310 antibodies *in organello* caused the significant increase of diene conjugates formation in winter wheat mitochondria,[18] infiltration of winter wheat seedling shoots, both by non-immune and anti-CSP 310 antiserum did not influence the rate of diene conjugates formation (Fig. 3) unless the decrease of oxygen consumption by plant tissue infiltrated by anti-CSP 310 antiserum (Fig. 2). It is known that a plant cell has a powerful and deeply disposed system of oxidative-stress defence. It includes such mitochondrial and non-mitochondrial enzymes as superoxide dismutase, catalase, and peroxidases, as well as non-enzymatic agents, such as α-tocopherol, ascorbate, etc.[35,36] Uncoupling in plant mitochondria during low temperature stress is only one of such antioxidant mechanisms.[21,34,36] In this case, we can suppose that inhibition of CSP 310-dependent uncoupling by addition of specific antiserum to winter wheat seedling shoots mitochondria did not cause a significant increase of lipid peroxidation because of activation of other plant defence antioxidant systems.

Because the main function of mammalian uncoupling mitochondrial proteins is body temperature control during hypothermia,[1,5] we studied the influence of CSP 310-dependent uncoupling activation and inhibition on the temperature of winter wheat seedling shoots during short-term low temperature stress. During this study, we measured the temperature of winter wheat seedling shoots chilled in a thermostat from 20 to -4°C.

The data obtained showed that seedling infiltration by non-immune antiserum did not influence their temperature during low-temperature stress (Fig 4A). Temperature difference between ''non-immune antiserum infiltrated shoots'' and ''killed shoots'' did not differ from temperature difference between ''living shoots'' and ''killed shoots'' (Fig. 4B). So, infiltration of plant material by non-immune antiserum did not influence the temperature of winter wheat seedling shoots during cold stress.

Infiltration of seedling shoots by anti-CSP 310 antiserum decreases their temperature during cold stress (Fig. 5A). Temperature difference

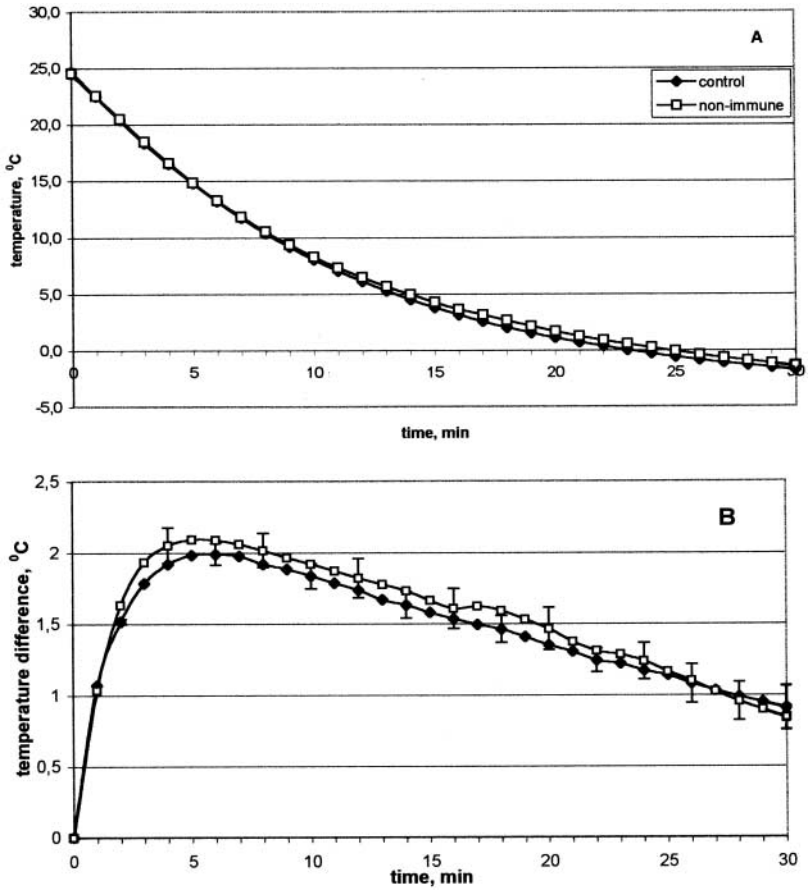

Figure 4. An influence of infiltration by water (control) and by non-immune antiserum (non-immune) on the temperature of winter wheat seedling shoots during cold stress (A) and temperature difference between infiltrated by water and killed by boiling seedling shoots (control) and infiltrated by non-immune antiserum and killed by boiling seedling shoots (non-immune) (B).

between "anti-CSP 310 antiserum infiltrated shoots" and "killed shoots" was about 0.5°C lower than temperature difference between "living shoots" and "killed shoots" (Fig. 5B). Therefore, we can conclude that inhibition of stress uncoupling protein CSP 310 activity by specific antiserum influences plant body temperature during cold stress as occurs in mammals.

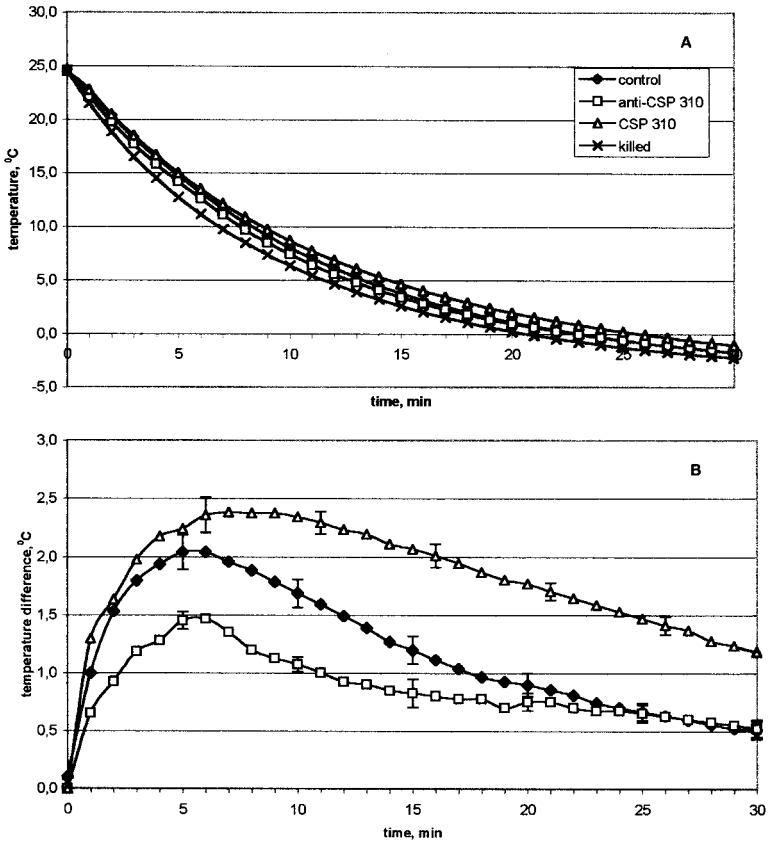

Figure 5. An influence of infiltration by water (control) by CSP 310 (CSP 310) and by anti-CSP 310 antiserum (anti-CSP 310) on the temperature of winter wheat seedling shoots during cold stress (A) and temperature difference between infiltrated by water and killed by boiling seedling shoots (control), infiltrated by CSP 310 and killed by boiling seedling shoots (CSP 310) and infiltrated by anti-CSP 310 antiserum and killed by boiling seedling shoots (anti-CSP 310) (B).

To verify this supposition, we studied the influence of activation of CSP 310 function on plant body temperature during cold stress. The data obtained showed that infiltration of seedlings by CSP 310 caused an increase of their temperature during low temperature stress (Fig. 5A). Temperature difference between "CSP 310 infiltrated shoots" and "killed shoots" was about 0.5°C higher than the temperature difference between "living shoots" and "killed shoots" (Fig. 5B). It is interesting to

Table 1. The influence of CSP 310 treatment on oxygen consumption and survival of winter wheat seedling shoots. $M \pm SD$, $n = 6$.

Variant	Oxygen consumption, nMol O_2/min/g of tissue	Surviving after cold shock (−4°C, 30 min), %
Infiltration by water	391.6 ± 20.4	72.3 ± 4.4
Infiltration by CSP 310 (1 mg/mL)	533.4 ± 10.8	91.7 ± 4.0

note that, if infiltration of seedlings by anti-CSP 310 antiserum caused the decrease of their temperature only during the first 20–25 min of cold stress, infiltration by CSP 310 caused the significant increase of seedling shoots temperature starting after the first 5 min of cold shock to the end of experiment (Fig. 5B). We can suppose that this difference between these treatments takes place because of the synthesis of CSP 310 *de novo* that occurs during cold stress[13] and lead to elimination of the inhibition effect of anti-CSP 310 antiserum. On the other hand, inhibition of the uncoupling effect of CSP 310 can activate another known plant uncoupling mitochondrial systems such as AOX and PUMP. Such interdependences between these two mitochondrial uncoupling systems were estimated previously.[37]

So, based on the data obtained, we can conclude that plant stress protein CSP 310 participates in plant defence against the increase of ROS formation during cold stress and can participate in regulation of plant body temperature during cold stress; we can suppose that activation of this defence mechanism would allow plants to survive during short-term low temperature stress. To verify this supposition, we studied the influence of winter wheat seedling shoots infiltration by CSP 310 on their survival during cold stress. The data obtained showed that this treatment caused the increase of seedling survival during cold stress from 70 to 90% (Table 1). Therefore, we can conclude that plant stress uncoupling protein CSP 310 participates in winter wheat seedlings defence against short-term low temperature stress and that activation of its function is one of the ways of increasing cold tolerance of winter wheat.

ACKNOWLEDGMENTS

This work has been performed with the support of the Russian Foundation of Basic Research (Projects 00-04-48093 and 02-04-06096).

REFERENCES

1. Ricquier, D.; Bouillaud, F. The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP, and AtUCP. Biochem. J. **2000**, *345*, 161–179.
2. Vercesi, A.E.; Martins, I.S.; Silva, M.A.P.; Leite, H.M.F.; Cuccovia, I.M.; Chaimovich, H. PUMPing plants. Nature **1995**, *375*, 24.
3. Laloi, M.; Klein, M.; Riesmeier, J.W.; Muller-Rober, B.; Fleury, Ch.; Bouillaud, F.; Ricquier, D. A plant cold-induced uncoupling protein. Nature **1997**, *389*, 135–136.
4. Maia, I.G.; Benedetti, C.E.; Leite, A.; Turcinelli, S.R.; Vercesi, A.E.; Arruda, P. AtPUMP: an *Arabidopsis* gene encoding an uncoupling mitochondrial protein. FEBS Lett. **1998**, *429*, 403–406.
5. Jezek, P.; Engstova, H.; Zackova, M.; Vercesi, A.E.; Costa, A.D.T.; Arruda, P.; Garlid, K.D. Fatty acid cycling mechanism and mitochondrial uncoupling proteins. Biochim. Biophys. Acta **1998**, *1365*, 319–327.
6. Vanlerberghe, G.C.; McIntosh, L. Alternative oxidase: from gene to function. Ann. Rev. Plant Physiol. Plant Mol. Biol. **1997**, *48*, 703–734.
7. Wagner, A.M.; Moore, A.I. Structure and function of the plant alternative oxidase: its putative role in the oxygen defence mechanism. Biosci. Rep. **1997**, *17*, 319–333.
8. Millar, A.H.; Wiskich, J.T.; Whelan, J.; Day, D.A. Organic acid activation of the alternative oxidase of plant mitochondria. FEBS Lett. **1993**, *392*, 259–262.
9. Day, D.A.; Millar, A.H.; Wiskich, J.T.; Whelan, J. Regulation of alternative oxidase activity by pyruvate in soybean mitochondria. Plant Physiol. **1994**, *106*, 1421–1427.
10. Kolesnichenko, A.V.; Borovskii, G.B.; Voinikov, V.K.; Misharin, S.I.; Antipina, A.I. Antigen from winter rye accumulated under low temperature. Russ. J. Plant Physiol. **1996**, *43*, 771–776.
11. Voinikov, V.; Pobezhimova, T.; Kolesnichenko, A.; Varakina, N.; Borovskii, G. Stress protein 310 kDa affects the energetic activity of plant mitochondria under hypothermia. J. Therm. Biol. **1998**, *23*, 1–4.
12. Voinikov, V.K.; Kolesnichenko, A.V.; Pobezhimova, T.P.; Varakina, N.N. The first and sixth chromosomes of soft winter wheat D-genome control the synthesis of 310-kDa cold-shock protein. Russ. J. Plant Physiol. **1998**, *45*, 591–594.
13. Pobezhimova, T.P.; Grabelnych, O.I.; Kolesnichenko, A.V.; Voinikov V.K. The comparison of uncoupling activity of

constituently synthesised and stress-induced forms of winter rye stress uncoupling protein CSP 310. J. Therm. Biol. **2001**, *26*, 95–101.

14. Kolesnichenko, A.V.; Zykova, V.V.; Voinikov, V.K. A comparison of the immunochemical affinity of cytoplasmic, mitochondrial and nuclear proteins of winter rye (*Secale cereale* L.) to A 310 kDa stress protein in control plants and during exposure to cold stress. J. Therm. Biol. **2000**, *25*, 203–209.
15. Grabelnych, O.I.; Pobezhimova, T.P.; Kolesnichenko, A.V.; Voinikov, V.K. Complex I of winter wheat mitochondria respiratory chain is the most sensitive to uncoupling action of plant stress-related uncoupling protein CSP 310. J. Therm. Biol. **2001**, *26*, 47–53.
16. Voinikov, V.K.; Grabelnych, O.I.; Kolesnichenko, A.V.; Pobezhimova, T.P. Cold-shock 310 kD protein uncouples oxidative phosphorylation in plant mitochondria. Russ. J. Plant Physiol. **2001**, *48*, 89–94.
17. Kolesnichenko, A.V.; Pobezhimova, T.P.; Grabelnych, O.I.; Voinikov, V.K. Stress-induced protein CSP 310: a third uncoupling system in plants. Planta **2002**, *215*, 279–286.
18. Kolesnichenko, A.V.; Grabelnych, O.I.; Sumina, O.N.; Pobezhimova, T.P.; Voinikov, V.K. An influence of antiserum against winter wheat stress uncoupling protein CSP 310 on energetic activity of some plant species mitochondria. J. Immunoassay Immunochem. **2001**, *22*, 75–83.
19. Maxwell, D.P.; Wang, Y.; McIntosh, L. The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc. Natl. Acad. Sci. USA **1999**, *96*, 8271–8276.
20. Jezek, P.; Borecky, J.; Zackova, M.; Costa, A.D.; Arruda, P. Possible basic and specific functions of plant uncoupling proteins (pUCP). Biosci. Rep. **2001**, *21*, 237–245.
21. Kowaltowski, A.J.; Costa, A.D.T.; Vercesi, A.E. Activation of the potato plant uncoupling mitochondrial protein inhibits reactive oxygen species generation by the respiratory chain. FEBS Lett. **1999**, *425*, 213–216.
22. Popov, V.N.; Simonyan, R.A.; Skulachev, V.P.; Starkov, A.A. Inhibition of the alternative oxidase stimulates H_2O_2 production in plant mitochondria. FEBS Lett. **1997**, *415*, 87–90.
23. Zykova, V.V.; Kolesnichenko, A.V.; Grabelnych, O.I.; Antipina, A.I.; Koroleva, N.A.; Vladimirova, S.V.; Pobezhimova, T.P.; Voinikov, V.K. Plant stress-related uncoupling protein CSP 310 caused lipid peroxidation in winter wheat mitochondria under chilling stress. J. Therm. Biol. **2000**, *25*, 323–327.

24. Kolesnichenko, A.V.; Grabelnych, O.I.; Tourchaninova, V.V.; Zykova, V.V.; Koroleva, N.A.; Pobezhimova, T.P.; Voinikov, V.K. An influence of stress protein CSP 310 and antiserum against this protein on lipid peroxidation in cereal mitochondria. J. Immunoassay Immunochem. **2001**, *22*, 113–126.
25. Palou, A.; Pico, C.; Bonet, M.L.; Oliver, P. The uncoupling protein, thermogenin. Int. J. Biochem. Cell Biol. **1998**, *30*, 7–11.
26. Vojnikov, V.; Korzun, A.; Pobezhimova, T.; Varakina N. Effect of cold shock on the mitochondrial activity and on the temperature of winter wheat seedlings. Biochem. Physiol. Pflanzen. **1984**, *179*, 327–330.
27. Laemmli, U.K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature **1970**, *227*, 680–685.
28. Estabrook, R.W. Mitochondrial respiratory control and the polarographic measurement of ADP:O ratio. Meth. Enzymol. **1967**, *10*, 41–47.
29. Recknagel, R.O.; Choshal, A.K. Lipoperoxidation of rat liver microsomal lipids by carbon tetrachloride. Nature **1966**, *210*, 1162–1163.
30. Parkin, K.L.; Marangoni, A.; Jackman, R.; Yada, R.; Stanley, D. Chilling Injury. A review of possible mechanisms. J. Food Biochem. **1989**, *13*, 127–153.
31. Loubaresse, M.; Paulin, A.; Dereuddre, J. Effects of freezing on membrane lipid peroxidation of rhododendron roots (Rhododendron cv. Demontague, Jean-Marie). Compt. Rend. Acad. Sci. **1991**, *313*, 453–459.
32. Kolesnichenko, A.V.; Zykova, V.V.; Grabelnych, O.I.; Koroleva, N.A.; Pobezhimova, T.P.; Konstantinov, Yu.M.; Voinikov, V.K. Influence of CSP 310 and CSP 310-like proteins from cereals on mitochondrial energetic activity and lipid peroxidation in vitro and in vivo. BMC Plant Biol. **2001**, *1*, 1–6.
33. Kolesnichenko, A.V.; Ostroumova, E.A.; Zykova, V.V.; Voinikov, V.K. The comparison of proteins with immunochemical affinity to stress protein 310 kD in cytoplasmatic proteins of winter rye, winter wheat, elymus and maize. J. Therm. Biol. **1999**, *24*, 211–215.
34. Korshunov, S.S.; Korkina, O.V.; Ruuge, E.K.; Skulachev, V.P.; Starkov, A.A. Fatty acids as natural uncouplers preventing generation of O_2^- and H_2O_2 by mitochondria in the resting state. FEBS Lett. **1999**, *435*, 215–218.
35. Møller, I.M. Plant mitochondria and oxidative stress: electron transport, NADPH turnover, and metabolism of reactive oxygen

species. Ann. Rev. Plant Physiol. Plant Mol. Biol. **2001**, *52*, 561–591.

36. Zykova, V.V.; Kolesnichenko, A.V.; Voinikov, V.K. Involvement of reactive oxygen species in plant mitochondrial response to low-temperature stress. Russ. J. Plant Physiol. **2002**, *49*, 302–310.
37. Sluse, F.E.; Almeida, A.M.; Jarmuszkiewicz, W.; Vercesi, A.E. Free fatty acids regulate the uncoupling protein and alternative oxidase activities in plant mitochondria. FEBS Lett. **1998**, *433*, 237–240.,

Received July 20, 2002
Accepted August 24, 2002
Manuscript 3077

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016