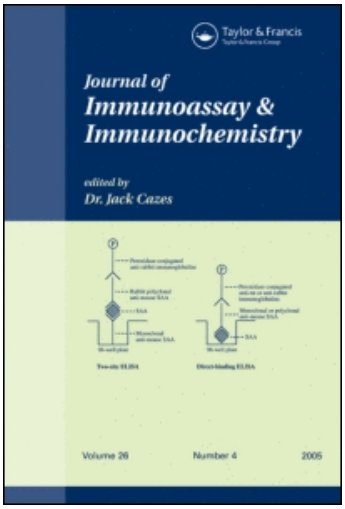

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AN INFLUENCE OF ANTISERUM AGAINST WINTER WHEAT STRESS UNCOUPLING PROTEIN, CSP 310, ON ENERGETIC ACTIVITY OF SOME PLANT SPECIES MITOCHONDRIA

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ABSTRACT

It is determined that addition of an anti-CSP 310 antiserum to isolated mitochondria of cereals (winter rye, winter wheat, and maize) caused an increasing of mitochondrial respiratory control. In a similar manner, addition of this antiserum to isolated pea mitochondria did not cause this effect. It is shown that coupling effect of antiserum is not dependent upon the presence of bovine serum albumin in mitochondria incubation media. Therefore, these results show that the mechanism of oxidation and phosphorylation uncoupling, and participation of immunochemically related CSP 310 proteins that can be precipitated by anti-CSP 310 antiserum is specific for cereals.

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INTRODUCTION

The process of plant adaptation to unfavourable temperatures is provided by synthesis of different stress proteins. [1, 2, 3] One of the important functions of these proteins is to protect the organism from overcooling during the first moments of the hypothermic action, by means of thermogenesis.[4] This defense mechanism, which is connected with the decreasing of coupling of oxidation and phosphorylation in mitochondria, is widespread among mammals. Currently, three uncoupling proteins (UCPs) are known in mammalian species.[5]

Previously, it was shown that hypothermia caused to frost-resistant winter wheat yielded significant changes in its respiratory activity – increasing of state 4 respiration and decreasing of respiratory control. These changes were observed only for frost-resistant winter wheat, but not in frost–sensitive winter wheat cultivar.[6] Currently, there are known two plant uncoupling proteins – PUMP [7] and StUCP.[8] All of these mammalian and plant uncoupling proteins are inner membrane integral mitochondrial proteins and are members of the mitochondrial anion carrier family.[5]

On the other hand, plant cold stress protein CSP 310, which was isolated from winter rye[9] and was found to have uncoupling activity,[10] was found to be a nuclear encoding cytoplasmatic protein.[11] The CSP 310 presence in winter wheat mitochondria was shown[10] but it was shown that uncoupling activity is a feature of cytoplasmatic CSP 310 from stressed plants.[10]

On the other hand, it is not known whether the uncoupling action of this stress protein determined by in vitro experiments exists in vivo or not. Because of strong induction of this stress protein during cold stress and rapid start – after 5 minutes of incubation – of CSP 310 uncoupling action, we suppose that, during the procedure of isolation of plant mitochondria, this stress protein is associated with plant mitochondria because of chilling of plant material and that addition of anti – CSP 310 antiserum to incubated in vitro mitochondria would precipitate this uncoupling protein and eliminate the uncoupling activity of CSP 310 associated with mitochondria during the procedure of its isolation.

So, the aim of current work is to examine if there is any presence of uncoupling activity of CSP 310, isolated from different plant species, mitochondria that can be eliminated by addition of anti-CSP 310 anti-serum in the incubation media.

EXPERIMENTAL

Three-day-old etiolated shoots of winter wheat (Triticum aestivum L, cv. Zalarinka), winter rye (Secale cereale L. cv. Dymka), and maize (Zea maise L., cv. VIR 36), as well as six-day-old etiolated shoots of pea (Pisum sativum L.) germinated on moist paper at 260°C, were used in this work.

Mitochondria were extracted from winter wheat, winter rye, maize, and pea shoots by differential centrifugation.[12] Isolated mitochondria were resuspended in the following medium: 20 mM MOPS-KOH buffer (pH 7.4), 300 mM sucrose, 10 mM KCl, 5 mM EDTA, 1 mM $MgCl_2$, 4 mM ATP, 6 mM ADP, 10 mM malate, and 10 mM glutamate.

Mitochondrial respiratory activity was recorded polarographically at 270 C using platinum closed type electrode with a 1.4 mL volume cell. The incubation media contained 125 mM KCl, 18 mM KH_2PO_4, 1 mM $MgCl_2$, and 5 mM EDTA, pH 7.4. 10 mM Malate, in the presence of 10 mM glutamate, was used as an oxidation substrate. Antiserums were dissolved in incubation media and were added to the polarographic cell to mitochondria in 1 cycle of phosphorylation (1 mg antiserum per 3 mg of mitochondrial protein) after transition of mitochondria from a phosphorylating to a non-phosphorylating state.

Polarograms were used to calculate the rates of phosphorylative respiration (state 3), non-phosphorylative respiration (state 4), respiration control by Chance-Williams, and the ADP:O ratio.[13] All experiments were made in three biological replications. Concentrations of mitochondria protein and CSP 310 were analysed by the Lowry method.[14] The data obtained were analysed statistically, i.e., arithmetic means and standard errors were determined.

For isolation of CSP 310, three days old, stressed at –10°C for 1 h, etiolated shoots of winter rye were used. The isolation and purification of winter wheat CSP 310 and obtaining of anti-CSP 310 antiserum were performed in according with the previously described method.[9] Non-immune antiserum was obtained from non-immunised rabbits.

RESULTS AND DISCUSSION

Freshly isolated from winter wheat, winter rye, maize, and pea mitochondria were used for determining their energetic activities. The highest respiratory control was observed for maize mitochondria. The lowest respiratory control and the highest degree of uncoupling of oxidation and phosphorylation were observed in winter rye. An addition of 0.8 mg of non-immune antiserum to 0.7 mg of these mitochondria failed to result in any

significant changes in their activities. Rate of non-phosphorylative respiration and respiration control by Chance-Williams did not change after addition of non-immune antiserum into the polarographic cell (Table 1).

The addition of 0.8 mg of anti-CSP 310 antiserum to the polarographic cell, containing 0.7 mg of state 4 mitochondria, caused significant decreasing of non-phosphorylative respiration and increasing of respiratory control in cereals (Table 1). Especially high increasing of respiratory control was observed for maize mitochondria. In contrast, the addition of anti-CSP 310 antiserum to pea mitochondria failed to result in any changes in respiratory control (Table 1).

Previously, the presence of CSP 310 and proteins immunochemically related to it at some cereal species was shown.[15] In that work, it was shown that concentration of CSP 310 differs with the species investigated. The highest concentration of CSP 310 was detected for winter rye cytoplasmic proteins. In winter wheat, CSP 310 was detected at a lower concentration and, in maize cytoplasmic proteins, only proteins immunochemically related to CSP 310 were detected. In connection with these results, we can suppose that the added to mitochondria amounts of anti-CSP 310 antibodies can precipitate practically all CSP 310 from maize mitochondria. Consequently, the rate of its non-phosphorylative respiration decreased maximally and its respiratory control increased to a maximum (Table 1). With winter wheat mitochondria, the added amount of antibodies can precipitate only a part of existing CSP 310, so the effect of its addition was lower (Table 1). With winter rye, which maintains the highest concentration of CSP 310, added antibodies precipitate only a few parts of this protein, so the effect was the lowest (Table 1).

Because of the absence of influence of anti-CSP 310 antibodies on pea mitochondria (Table 1), we conclude that there are no CSP 310-like proteins with uncoupling activity in pea shoots.

To determine if the coupling effect of anti-CSP 310 antiserum depends on the amounts of added anti-CSP 310 antibodies, we performed experiments with addition to the incubated polarograph cell containing winter rye mitochondria, of varying amounts of anti-CSP 310 antiserum. The data obtained shows that the coupling effect of antiserum increased with increasing added amounts of antiserum (Fig. 1). The highest coupling effect was observed at the addition of 4.8 mg of anti-CSP 310 antiserum to 'incubated at state 4' mitochondria (Fig. 1). The stronger effect of increasing anti-CSP 310 antiserum concentrations shows that we observed the precipitation of CSP 310 that is associated with incubated mitochondria and which caused uncoupling of their oxidation and phosphorylation.

It is known that all of "classical" mammalian and plant uncoupling proteins belong to the mitochondrial anion carrier family.[5] Therefore,

Table 1. An Influence of Anti-CSP 310 Antiserum on the Rate of Non-phosphorylative Respiration and Respiratory Control at Mitochondria of Some Plant Species. 10 mM Malate in the Presence of 10 mM Glutamate Was Used as an Oxidazing Substrate $M \pm m, n = 6$

Species	The rate of phosphorylative respiration, nmol O_2/min/mg protein	The rate of non-phosphorylative respiration, nmol O_2/min/mg protein			Respiratory control		
		Without any addition	With addition of non-immune antiserum	With addition of anti-CSP 310	Without any addition	With addition of non-immune antiserum	With addition of anti-CSP 310
Maize	86.62 ± 6.33	19.25 ± 2.91	18.84 ± 2.05	12.37 ± 2.05	4.50 ± 0.23	4.59 ± 0.20	7.00 ± 0.29
Rye	81.43 ± 4.07	32.04 ± 3.55	30.24 ± 3.71	22.69 ± 2.88	2.54 ± 0.13	2.69 ± 0.13	3.59 ± 0.21
Wheat	102.12 ± 8.98	26.64 ± 2.34	26.64 ± 2.14	21.47 ± 2.14	3.83 ± 0.18	3.83 ± 0.16	4.76 ± 0.25
Pea	71.41 ± 3.57	19.56 ± 2.78	19.45 ± 2.25	19.56 ± 2.05	3.65 ± 0.15	3.67 ± 0.18	3.65 ± 0.16

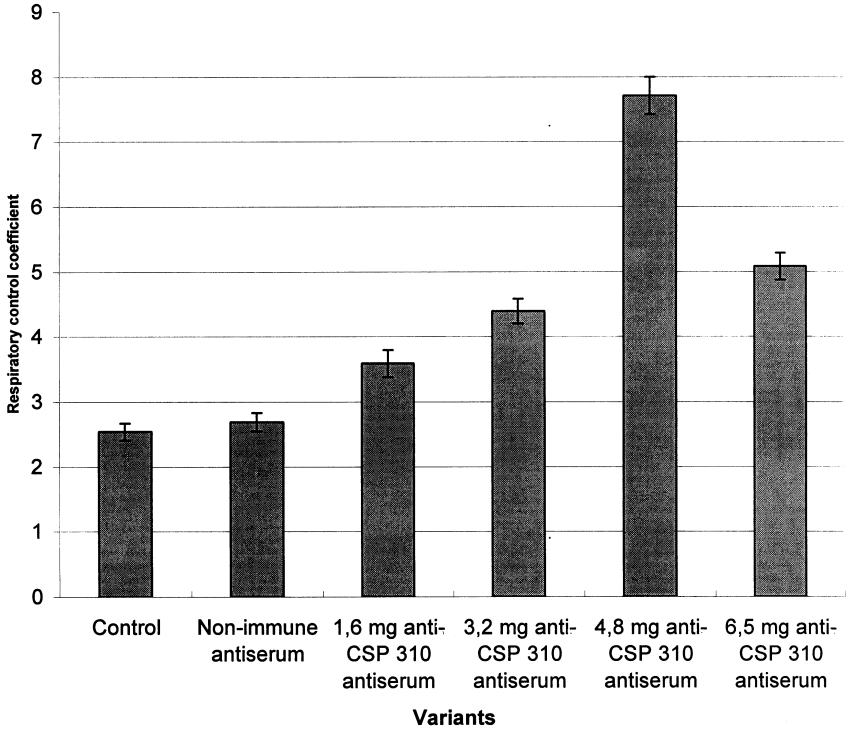

Figure 1. An influence of different amounts of anti-CSP 310 antiserum added to the polarographic cell on the respiratory control of winter rye mitochondria. 10 mM malate in the presence of 10 mM glutamate was used as an oxidizing substrate. M + m, n = 6.

their activity in vitro depends on the presence of free fatty acids in mitochondria incubation media.[16] Bovine serum albumin (BSA), which binds free fatty acids, is known to be an inhibitor of "classical" plant uncoupling proteins.[16] If CSP 310 is functional as other known uncoupling proteins, it would be expected that an addition of BSA to the incubation medium will eliminate free fatty acids from the incubation medum, and eliminate the uncoupling effect of CSP 310. Therefore, in such cases, an addition of BSA will decrease the coupling effect of addition of anti-CSP 310 antiserum.

To investigate this question, we performed an experiment with consecutive addition to incubated mitochondria BSA (in concentration, that which inhibits uncoupling activity of known plant uncoupling proteins) and anti-CSP 310 antiserum in the most effective concentrations. The data

Table 2. An Influence of BSA on the Effect of Anti-CSP 310 Antiserum on Winter Rye Mitochondria. 10 mM Malate in the Presence of 10 mM Glutamate Was Used as an Oxidazing Substrate. $M \pm m, n = 6$

The rate of phosphorylative respiration, nmol O_2/min/mg protein	The rate of non-phosphorylative respiration, nmol O_2/min/mg protein			Respiratory control		
	Without any addition	With addition of 4 mg BSA	With addition of 4 mg BSA and 4.8 mg anti-CSP 310	Without any addition	With addition of 4 mg BSA	With addition of 4 mg BSA and 4.8 mg anti-CSP 310
81.43 ± 4.07	32.04 ± 3.55	16.89 ± 2.91	9.22 ± 1.89	2.54 ± 0.13	4.82 ± 0.21	8.83 ± 0.19

obtained show that the coupling effect of anti-CSP 310 antiserum addition is not dependent upon the presence of BSA in the incubation medium (Table 2). This result shows that the uncoupling action of CSP 310 did not depend on the presence of free fatty acids in the incubation medium. So, we can suppose that the mechanism of the uncoupling action of CSP 310 on mitochondria did not deal with the pure protonophoric activity as do other known "classical" uncoupling proteins.[16]

So, the data obtained show the existence in cereals mitochondria of a stress protein CSP 310 uncoupling effect that can be eliminated by addition of anti-CSP 310 antiserum. They show that the mechanism of CSP 310 uncoupling action did not depend on the presence of free fatty acids in incubation media.

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REFERENCES

1. Houde, M.; Danyluk, J.; Laliberte, J.F.; Passart, E.; Dhundsa, R.S.; Sarhan, F. Cloning, Characterisation and Expression of cDNA Encoding a 50 kD Protein Specifically Induced by Cold Acclimation in Wheat. Plant Physiol. **1992**, *99*, 1381-1387.

2. Griffith, M.; Antikainen, M.; Hon, W.C.; Pihakaski Maunsbach, K.; Yu, X.M.; Chun, J.U.; Yang, D.S.C. Antifreeze Proteins in Winter Rye. Physiol. Plantarum **1997**, *100*, 327-332.
3. Limin, A.E.; Danyluk, J.; Chauvin, L.P.; Fowler, D.B.; Sarhan, F. Chromosome Mapping of Low-Temperature Induced Wcs120 Family Genes and Regulation of Cold-Tolerance Expression in Wheat. Molec. Gen. Genet. **1997**, *253*, 720-727.
4. Skulachev, V.P. *Energetics of Biomembranes*, Nauka: Moscow, 1989.
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. Biochem. Biophys. Acta.1998, *1365*, 319-327.
6. Voinikov, V.K.; Pobezhimova, T.P.; Varakina, N.N.; Zhirov, E.G. The Effect of Single Chromosomes of Frost-Resistant Winter Wheat on Frost Resistance and Mitochondrial Activity of Plants Under Hypothermia. Genetica **1987**, *23*, 287-294.
7. 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.
8. 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.
9. Kolesnichenko, A.V.; Borovskii, G.B.; Voinikov, V.K.; Misharin, K.I.; Antipina, A.I. Antigen From Winter Rye Accumulated Under Low Temperature. Russ. J. Plant Physiol. **1996**, *43*, 771-776.
10. Voinikov, V.; Pobezhimova, T.; Kolesnichenko, A.V.; Varakina, N.; Borovskii, G. Stress Protein 310 kD Affects the Energetic Activity of Plant Mitochondria Under Hypothermia. J. Therm. Biol. **1998**, *23*, 1-4.
11. Voinikov, V.K.; Kolesnichenko, A.V.; Pobezhimova, T.P.; Varakina, N. The First and Sixth Chromosomes of Soft Winter Wheat D-Genome Control the Synthesis of 310-kD Cold-Shock Protein. Russ. J. Plant Physiol. **1998**, *45*, 591-594.
12. Douce, R. *Mitochondria in Higher Plants: Structure, Functions and Biogenesis*. Academic Press: New York, 1985.
13. Estabrook, R.W. Mitochondrial Respiratory Control and the Polarographic Measurement of ADP:O Ratio. Meth. Enzimol. **1967**, *10*, 41-47.
14. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein Measurement with the Folin Phenol Reagent. J. Biol. Chem. **1957**, *193*, 265-2750.
15. 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.
16. Jarmuszkiewicz, W.; Miyasaka, A.A.; Sluse-Goffart, C.M.; Sluse, F.E.; Vercesi, A. Linoleic Acid-Induced Activity of Plant Uncoupling Mitochondrial Protein in Purified Tomato Fruit Mitochondria During Resting, Phosphorylating, and Progressively Uncoupled Respiration. J. Biol. Chem. **1998**, *273*, 34882-34886.

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