Activity of alternative oxidase and plant uncoupling mitochondrial protein in potato tubers stored at low temperature or submitted to artificial aging

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Received: 21/05/2004, Accepted: 08/07/2004

Potato tubers (*Solanum tuberosum* L. cv. Binje) were stored at 18 and 4ºC in order to verify changes in mitochondrial metabolism, especially in the activities of the alternative oxidase (AOX) and plant uncoupling mitochondria protein (PUMP). Tubers were also sliced and submitted to artificial aging. The oxygen consumption rate (OCR), respiratory control (RC) and ADP/O ratio for mitochondria obtained after storage at 18ºC were not altered, regardless of the time of storage. Considerable increment in OCR, for both respiratory states 3 and 4, was observed after storing the tubers for five and ten days at 4ºC; and it was accomplished by reductions in RC and ADP/O. The AOX activity was evident after five days at 4ºC, and it was enhanced after ten days of storage at that temperature. No significant changes in PUMP activities were observed after one day at 18ºC or at 4ºC, neither after artificial aging. However, increased swelling of potato mitochondria was observed in the presence of valinomycin and linoleic acid when the tubers were kept at 4ºC for five and ten days. Besides the changes in AOX and PUMP activities, slight alterations in ascorbate peroxidase (APX) and catalase (CAT) activities were observed after storing the tubers at 4°C for 5 days. The activities of these enzymes were higher after storage at 4°C for 10 days. The results suggest the combined effect of low temperature and time of exposure in promoting AOX activity, probably as a protective mechanism against cell damage in response to mitochondrial oxidative stress.
Key words: *Solanum tuberosum* L., mitochondrial swelling, oxidative stress, respiration.

Atividades da oxidase alternativa e da proteína desacopladora de mitocôndrias de plantas em tubérculos de batata armazenados a baixas temperaturas ou submetidos ao envelhecimento artificial: Tubérculos de batata (*Solanum tuberosum* L. cv. Binje) foram armazenados a 18 e a 4ºC, ou envelhecidos artificialmente, com o objetivo de verificar possíveis alterações no metabolismo mitocondrial, sobretudo nas atividades da oxidase alternativa (AOX) e da proteína desacopladora de mitocôndrias de plantas (PUMP). Nenhuma alteração se observou, em qualquer tempo, nas taxas de consumo de oxigênio (OCR), controle respiratório (RC) e razão ADP/O de mitocôndrias obtidas após armazenamento a 18ºC. Já após cinco e dez dias a 4ºC, verificou-se considerável incremento em OCR, tanto no estado respiratório 3 quanto no 4, o que resultou em reduções em RC e na razão ADP/O. A atividade da AOX foi evidente após cinco dias a 4ºC, sendo maior após dez dias nessa temperatura. Houve pequenas alterações na atividade da PUMP depois de um dia de exposição a 18ºC ou a 4ºC, bem como após o envelhecimento artificial de fatias dos tubérculos. Passados cinco e dez dias de exposição a 4ºC, foi possível notar um incremento no inchamento mitocondrial, em presença de valinomicina e ácido linoléico. Apesar das alterações nas atividades da AOX e PUMP, notaram-se apenas pequenos incrementos nas atividades da peroxidase do ascorbato (APX) e catalase (CAT), maiores após cinco e dez dias de exposição a 4°C. Os resultados revelam o efeito combinado da baixa temperatura e do tempo de armazenamento na promoção de incrementos na atividade da AOX, evidenciando um possível papel protetor dessa proteína contra estresse oxidativo mitocondrial, mediado por baixa temperatura.
Palavras-chave: *Solanum tuberosum* L., estresse oxidativo, inchamento mitocondrial, respiração.

INTRODUCTION

Plants can present two distinct proteins capable of causing mitochondrial uncoupling: the alternative oxidase (AOX) and the plant uncoupling mitochondrial protein (PUMP). AOX has been characterized as a 34 kDa protein located on the matrix surface of the inner mitochondrial membrane (Ducet and Gauvrit, 1977). It was initially purified from the thermogenic appendix of *Arum maculatum, Arum italicum* and *Sauromatum guttatum* (Elthon and McIntosh, 1986, 1987). AOX can catalyse the oxidation of ubiquinol and the reduction of oxygen to water. When this protein is active oxidative phosphorylation is lowered because two sites of proton ejection to the inter-membrane space are aborted (Siedow and Berthold, 1986; Wagner and Krab, 1995; Siedow and Umbach, 2000). The AOX activity can be inhibited by hydroxamic acids such as salicylhydroxamic acid (SHAM) (Schonbaum et al., 1971) and stimulated by organic acids like pyruvate (Millar et al., 1996). Apart from the thermogenic activity in spadices of plants from Araceae, the physiological role of AOX in other tissues has not been elucidated. Several independent studies have proposed that this enzyme can prevent the production of reactive oxygen species (ROS) in response to a variety of stresses (Wagner, 1995; Millar and Day, 1996, 1997; Maxwell et al., 1999).

PUMP was first isolated from potato tuber mitochondria and was characterized as a 32 kDa protein (Vercesi et al., 1995; Laloi et al., 1997). This protein is similar to the animal uncoupling protein (UcP) and can transport protons from the inter-membrane region to the mitochondrial matrix, resulting in lower oxidative phosphorylation. This protein is stimulated by linoleic acid and inhibited by bovine serum albumin (BSA) and by ATP, GTP, GDP and ADP (Jezek et al., 1996; Vercesi et al., 1998). The role of PUMP is also unclear, but it has been proposed that it has a supplementary function when AOX is inhibited or absent (Sluse et al., 1998; Almeida et al., 1999; Jarmuszkiewicz et al., 2000).

Several investigations revealed no AOX activity in potato tubers stored at room temperature (28°C). However, cyanide-insensitive respiration attributed to AOX has been observed after slicing and artificially aging tubers by incubation under continuous aeration in a 100 µM $CaSO_4$ solution (Dizengremel and Lance, 1976; Ducet and Gauvrit, 1977). Similar AOX induction was observed after exposure of *Plantago lanceolata* or *Zea mays* seedlings to low temperature (13°C) (Smakman and Hofstra, 1982; Elthon et al., 1986) or by incubating *Nicotiana tabacum* cell suspensions and callus cultures at 18°C (Vanlerberghe and McIntosh, 1992). On the other hand, moderate PUMP gene expression (stUCP) was observed in potato tubers stored at room temperature, and high gene expression was detected in roots, stems, flowers and fruits (Laloi et al., 1997). The stUCP gene expression in potato leaves was detected after exposure at 4°C, and transcript accumulation increase with time of exposure (Laloi et al., 1997).

AOX and PUMP show physiological similarities in many aspects. Both proteins are energy-dissipating systems, which decrease the efficiency of oxidative phosphorylation (Siedow and Berthold, 1986; Vercesi et al., 1995; Wagner and Krab, 1995) and apparently are related to protection of mitochondria against oxidative stress (Purvis, 1997; Kowaltowski et al., 1998). Furthermore, AOX and PUMP seems to be stimulated by low temperature and for this reason a thermogenic function has been proposed for them (Smakman and Hofstra, 1982; Elthon et al., 1986; Vanlerberghe and McIntosh, 1992; Laloi et al., 1997; Nantes et al., 1999). Hence, many groups interested in different aspects of mitochondrial metabolism have carried out studies focusing on the activities of AOX and PUMP. In mitochondria from the fruits of *Lycopersicon esculentum* the AOX protein level decreased with fruit ripening (from the green to the red stage). In contrast, the PUMP levels decreased from the yellow stage on, suggesting a differential role of these proteins on the post-harvest ripening process (Jezek et al., 1996; Almeida et al., 1999). On the other hand, the mRNA levels of both the AOX and PUMP proteins increased in a similar pattern during mango fruit ripening, suggesting the gene expressions of both proteins are under similar control in this species (Considine et al., 2001).

In this work, the respiratory metabolism of potato tuber mitochondria was studied in order to investigate the possibility that AOX and PUMP activities are simultaneously stimulated under low temperature. For this purpose, potato tubers were incubated at low temperatures (18 and 4°C) and then the isolated mitochondria were evaluated for oxygen consumption rate, respiratory control, ADP/O ratio and the activities of AOX and PUMP.

MATERIAL AND METHODS

Potato tubers (*Solanum tuberosum* L. cv. Binje) obtained in a local market were stored in the dark at 28 ± 2°C (control) or at 4 ± 2°C for one, five or ten days in a Diurnal Growth Chamber (Forma Sci. Inc./Mod. 3740, Ohio, USA). Another lot of potato tubers was sliced and artificially aged according to Dizengremel and Lance (1976).

Partially purified mitochondria were obtained from potato tubers by differential centrifugation (Lance, 1971). Mitochondrial protein was determined as recommended by Fanger (1987). Oxygen consumption was measured with a Clark oxygen electrode (Yellow Springs Inst. Co., Yellow Springs, OH, USA). Reactions, conducted at 25°C, were started by adding an aliquot of the mitochondrial suspension to 3 mL of the respiration buffer medium containing 300 $mmol.L^{-1}$ mannitol, 20 $mmol.L^{-1}$ KCl; 2 $mmol.L^{-1}$ $MgCl_2$, 2 $mmol.L^{-1}$ KH_2PO_4, 100 $\mu mol.L^{-1}$ ATP, 0.1% BSA, 10 $mmol.L^{-1}$ HEPES (pH 7.2) and 5 $\mu mol.L^{-1}$ rotenone. Succinate (10 $mmol.L^{-1}$) was used as exogenous substrate. Antimycin-A (5 $\mu g.mg^{-1}$ protein) and SHAM (1 $mmol.L^{-1}$) were used as inhibitors of the cytochrome oxidase and of the AOX pathways, respectively.

Western blots were based on Umbach and Siedow (1993). Samples of total mitochondrial protein (50 µg) were prepared in a denaturating buffer and separated by SDS-PAGE (Laemmli, 1970) carried out in a Bio-Rad Mini-PROTEAN II cell. The proteins were blotted onto a nitrocellulose membrane, and probed with a 1/100 (v/v) dilution of a monoclonal antibody raised against *Sauromatum guttatum* AOX (Elthon and McIntosh, 1986). The visualization of the bands of interest was obtained with *p*-nitrotetrazolium-blue and bromo-chloro-indolyl phosphate solution (NBT/BCIP system, GIBCO/BRL).

Indirect activity of PUMP was determined by the swelling method (Jezek et al., 1996) in which PUMP activity is considered to be proportional to the increase of mitochondrial volume. Variations of mitochondrial volume were monitored by determining changes in absorbance at 520 nm during 5 minutes at 25°C. Traces were started by addition of 0.5 mg mitochondrial protein in 3 mL of a reaction medium containing 150 $mmol.L^{-1}$ KCl plus 2 $mmol.L^{-1}$ HEPES (pH 7.2) and swellings were initiated by addition of valinomycin (0.06 $\mu mol.L^{-1}$).

Catalase (CAT, EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11) activities were determined in a partially purified mitochondrial extract. An extract was prepared containing 5 mg of mitochondrial protein in 1 mL of 50 $mmol.L^{-1}$ potassium phosphate buffer (pH 7.8) and 0.01% Triton X-100. CAT activity was assayed in a reaction mixture containing 50 $mmol.L^{-1}$ potassium phosphate buffer (pH 7.0), 12.5 $mmol.L^{-1}$ H_2O_2 and 20 µL of the mitochondrial extract in a 3 mL final volume. Activity was determined at 30°C by following the decomposition of H_2O_2 at 240 nm (Havir and McHale, 1987). APX activity was determined in a 1 mL reaction mixture containing 50 $mmol.L^{-1}$ potassium phosphate buffer (pH 7.6); 0.22 $mmol.L^{-1}$ ascorbate; 0.3 $mmol.L^{-1}$ H_2O_2 and 10 µL of the mitochondrial extract. Activity was determined at 25°C by following the oxidation of ascorbate at 290 nm (Hossain and Asada, 1984).

RESULTS AND DISCUSSION

Similar oxygen consumption rates (OCR), respiratory control (RC) and ADP/O ratios were obtained for potato mitochondria isolated from tubers stored at 28°C, 18°C (data not shown) or 4°C for one day (table 1). Storage at 4°C for longer periods (five and ten days) as well as artificial aging of sliced potato tubers, caused an increase of OCR values for both respiratory states 3 and 4, and simultaneous decrease of RC and ADP/O values (table 1). These results suggest that respiration uncoupling, probably related to AOX, was activated or induced by low temperature (Ducet and Gauvrit

Table 1. Effects of low temperature and artificial aging on the respiratory metabolism of potato tuber mitochondria. Oxygen uptake rate (O_2 uptake), respiratory control rate and ADP/O ratio were determined in mitochondria isolated from potato tubers stored at 28°C, from aged slices (Aged), and from tubers stored at 4°C, for one, five and ten days. Traces were started by addition of 0.5 mg mitochondrial protein (mit) in a reaction medium containing 10 $mmol.L^{-1}$ succinate as respiratory substrate. All traces were performed in the presence of 100 $\mu mol.L^{-1}$ ATP, 0.1% BSA and 5 $\mu mol.L^{-1}$ rotenone. The O_2 uptake in state 3 was determined after addition of 100 $\mu mol.L^{-1}$ ADP. Values are the mean ± SE of three replicates, from three different mitochondrial preparations.

Treatments	O_2 uptake (nmol $O_2.min^{-1}.mg^{-1}$ protein)		Respiratory control rate	ADP/O
	State 3	State 4		
28°C	147 ± 6.4	34 ± 1.7	4.3 ± 0.2	1.8 ± 0.1
Aged	154 ± 8.6	96 ± 4.9	1.5 ± 0.2	1.2 ± 0.1
4°C/1 day	146 ± 5.8	32 ± 1.2	4.6 ± 0.3	1.8 ± 0.1
4°C/5 days	166 ± 7.2	66 ± 0.7	2.5 ± 0.3	1.5 ± 0.2
4°C/10 days	217 ± 18.4	117 ± 12.7	1.8 ± 0.1	1.4 ± 0.1

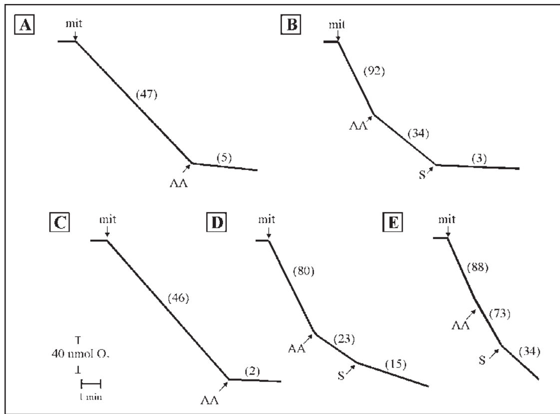

Figure 1. AOX activity of potato tuber mitochondria submitted to low temperature and artificial aging. Mitochondria were partially purified from tubers stored at 28°C (A), from aged slices (B), and from tubers stored at 4°C for one (C), five (D) and ten (E) days. Traces were started by addition of 0.5 mg mitochondrial protein (mit) in a reaction medium containing 10 $mmol.L^{-1}$ succinate as respiratory substrate. All traces were performed in presence of 100 $\mu mol.L^{-1}$ ATP, 0.1% BSA and 5 $\mu mol.L^{-1}$ rotenone. Numbers in parenthesis correspond to the oxygen uptake rate (nmol de O_2 min^{-1} mg^{-1} protein), and arrows indicate additions of 5 $\mu g.mg^{-1}$ protein Antimycin-A (AA) and 1 $mmol.L^{-1}$ SHAM (S). The traces are representative of three replicates, from three different mitochondrial preparations.

1977; Dizengremel et al., 1982). Indeed, addition of AA and SHAM during oxygen consumption assays led to an enhanced activity of AOX, especially after storing potato tubers for five and ten days at 4ºC (figures 1D and 1E), as well as after artificially aging potato slices (figure 1B). On the contrary, no AOX activity was detected for mitochondria obtained from tubers kept at 28ºC or at 4ºC for one day (figures 1A and 1C, respectively). These results are in agreement with those of Ducet and Gauvrit (1977) and Dizengremel et al. (1982), and indicate that in potato tuber mitochondria, AOX activity seems to be stimulated in a time dependent manner at 4ºC.

From the oxygen consumption traces, estimates were made of the activity of cytochrome oxidase and the AOX pathways. About 95% of the total respiration of tubers stored at 28°C and at 4°C for one day can be attributed to the cytochrome oxidase pathway. The remaining 5% was due to the AOX pathway (oxygen consumption in the presence of AA) (figure 2). On the other hand, cytochrome oxidase respiration decreased after storing potato tubers for five and ten days at 4°C. In these cases, 16 and 38%, respectively, of the total respiration was due to the AOX pathway (figure 2).

In aged potato slices mitochondria the AOX pathway accounted for 37% of the total respiration. Furthermore, the AOX pathway and the residual respiration showed an equivalent participation of total respiration when potato tubers were stored for ten days at 4°C (37 and 43%, respectively) (figure 2). Enhanced residual respiration rates are rather uncommon. For this reason, higher concentrations of AA and SHAM were tested (traces not shown). When AA concentration was raised to 20 $\mu g.mg^{-1}$ protein no changes in the cytochrome oxidase pathway were observed (figure 3). On the other hand, oxygen consumption was strongly reduced by 4 $mmol.L^{-1}$ SHAM, leading to reduced residual respiration (figure 3). Thus the AOX pathway in fact accounted for 75% of total respiration, a high value for an alternative respiratory pathway. Increased respiration rates attributed to the AOX pathway were also observed 24 h after transferring *Plantago lanceolata* seedlings from 21°C to 13°C. In such a situation AOX activity accounted for 60% of the total respiration (Smakman and Hofstra, 1982). The AOX pathway was also increased to 84% of total respiration, when *Zea mays* seedlings were cultivated at 13°C during 14 days (Elthon et al., 1986). In these cases, it was hypothesized that the high AOX activity was the result of *de novo* synthesis of this protein as a response to low temperatures.

In this work, immunoblot analysis using a monoclonal antibody against *Sauromatum guttatum* AOX showed bands in the 35 kDa region in all treatments, except in the control (figure 4). The most intense bands were observed when the tubers were stored for ten days at 4ºC. This may explain, at least in part, the increased AOX activity observed in the

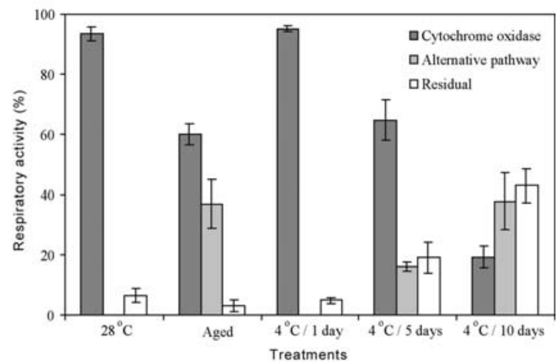

Figure 2. Respiratory activity of mitochondria obtained from potato tubers stored at 28°C, aged slices (Aged), and from tubers stored at 4°C for one, five and ten days. Values are the means ± SE of three replicates, from three different mitochondrial preparations.

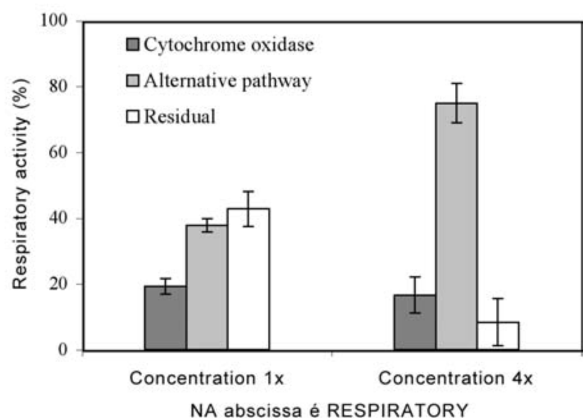

Figure 3. Effects of increased concentration of respiratory inhibitors on the cytochrome oxidase and AOX activities in mitochondria from potato tubers stored at 4°C for ten days. The concentrations of respiratory inhibitors used were: 5 μg.mg^{-1} protein Antimycin-A and 1 mmol.L^{-1} SHAM (concentration 1x) and 20 μg.mg^{-1} protein Antimycin-A and 4 mmol.L^{-1} SHAM (concentration 4x). Values are the means ± SE of three replicates, from three different mitochondrial preparations.

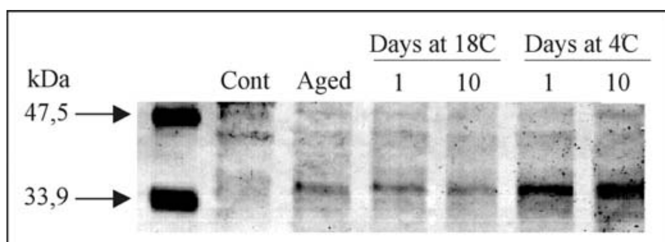

Figure 4. Effects of low temperature and artificial aging on AOX accumulation in potato tuber mitochondria. Mitochondria were isolated from tubers stored at 28°C, from aged slices (Aged), and from tubers stored at 18ºC or 4°C, for 1 and 10 days. Immunoblot analysis was performed using a monoclonal antiserum against *Sauromatum gutatum* AOX. The amount of protein loaded in each slot was 50 μg.

oxygen consumption assays (figures 1E and 2), indicating that there is a direct relationship between low temperature and AOX accumulation and activity. Comparing the oxygen consumption rates with the results of the immunoblot analysis, it is possible to infer that storage of tubers at 4°C for a short time (1 day) led to AOX accumulation, however it was not sufficient to promote its activation. In addition, after a long exposure at 4°C (10 days), increased accumulation in AOX levels was paralleled with increased activity of AA resistant respiration. It is interesting to observe that AOX is a dimer, which is activated by disruption of disulfide-bonds between the monomers (Umbach and Siedow, 1993; Day et al., 1995).

The role of AOX is well established in tissues like the spadix from the Araceae, where it is responsible for heat production during floral development (Raskin et al., 1989). However, the general role for AOX in non-thermogenic tissues is still under discussion. This alternative pathway could be important in the response of plants to a variety of stresses. It has also been proposed that, in stress conditions, AOX activity is able to prevent the potential over-reduction of the ubiquinone pool and, consequently, reduce the generation of reactive oxygen species (ROS) such as superoxide anions and hydroxyl radicals (Wagner and Krab, 1995; Moller, 2001).

The results related to mitochondria isolated from the artificially aged slices indicated enhanced AOX activity (figure 2) and higher levels in AOX content (figure 4). It is interesting to note that, in this case, artificial aging was obtained by maintaining the slices in contact with a solution of 0.1 mmol.L^{-1} $CaSO_4$, under continuous and forced aeration. The enhanced oxygen concentration and forced Ca^{2+} uptake may trigger oxidative stress (Cadenas and Boveris, 1980; Cadenas, 1997).

Mitochondrial swelling was stimulated by valinomycin and linoleic acid and it was faster and more intense in mitochondria isolated from tubers stored for five and ten days at 4°C (figures 5C and 5D) than in tubers stored for one day at 4°C or kept at 28°C (figures 5A and 5B). Moreover, BSA was more effective to inhibit swelling in mitochondria isolated from potatoes stored at 28°C and at 4°C for one day (figures 5A and 5B).

Mitochondrial permeability and swelling can be enhanced by the activity of special transporters such as the "plant inner membrane anion channel" (PIMAC) (Beavis and Vercesi, 1992), the K^+ channel (Pastore et al., 1999) and the "plant mitochondrial uncoupling protein" (PUMP) (Vercesi et al., 1995). As specific inhibitors of the PIMAC and K^+ channel activities were not included in the reaction medium, all these channels must have contributed to the mitochondrial swelling observed (figure 5). However, it was noted that swelling was stimulated by linoleic acid and inhibited by BSA, indicating a major contribution of the PUMP, especially after longer periods under low temperatures, when BSA was less effective in inhibiting swelling (figures 5C and 5D). From the swelling traces it is possible to infer that PUMP activity was slightly stimulated by low temperatures, in a time-dependent manner, and this may have been caused by activation of pre-existing PUMP or by *de novo* synthesis of this protein. Indeed, cold-

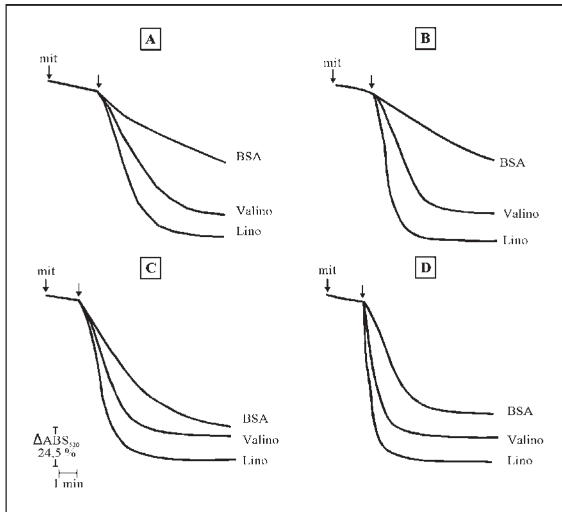

Figure 5. Swelling of mitochondria isolated from potato tubers stored at 28°C (A), and at 4°C for one (B), five (C) and ten (D) days. Traces were started by addition of 0.25 mg mitochondrial protein (mit), and swelling was started by addition of 0.06 μmol.L^{-1} valinomycin (Valino traces); 0.06 μmol.L^{-1} valinomycin plus 0.5 mg.mL^{-1} BSA (BSA traces); or 16 μmol.L^{-1} linoleic acid (Lino). Addition of valinomycin or linoleic acid to the reaction medium is indicated by the arrow. The traces are representative of three replicates, from three different mitochondrial preparations.

induction of PUMP genes has been shown for many species. In potato, Laloi et al. (1997) observed induction of *StUCP* gene expression by cold treatment, especially in flowers. High accumulation of *AtPUMP* transcripts was also observed in *Arabidopis thaliana* submitted to low temperatures (Maia et al., 1998). Two UCP genes (*SfUCPa* and *SfUCPb*) were found to be induced by low temperature treatments and were probably related to thermogenesis in the spadix of skunk cabbage (Ito, 1999), where heat production was attributed to the action of AOX only. Furthermore, increased activity of PUMP and its accumulation was obtained following natural aging or low temperature storage of potato tubers (Nantes et al., 1999).

The present results indicate that simultaneous AOX and PUMP activities were stimulated by storage of potato tubers at 4°C, for five and ten days (figures 1 and 5). For both proteins, the increased activities could be the result of protein activity stimulation or *de novo* synthesis, or both. It is possible that these two proteins have an important thermogenic role when tubers are kept at low temperature. In mammals, this role is specifically related to the uncoupling protein 1 (UCP1), but not to UCP2 or UCP3 (Nicholls and Rial, 1999; Klingenberg and Echtay, 2001).

Besides the possible thermogenic role, PUMP (as well as AOX) activity may represent a protective response to oxidative stress (Vercesi et al., 1997; Kowaltovski and Vercesi, 1999; Ecthay et al., 2002; Hourton-Cabassa et al., 2002; Brandalise et al., 2003).

In soybean and pea mitochondria, H_2O_2 generation was initiated by succinate addition and was strongly enhanced by addition of AA and subsequent addition of SHAM, suggesting the involvement of AOX in mitochondria protection against oxidative stress (Popov et al., 1999). In addition, increased oxygen peroxide level is generally followed by increased APX and CAT activities (Okuda et al., 1991; O'Kane et al., 1996).

In this work, no significant differences were observed for mitochondrial APX activity when tubers were stored at

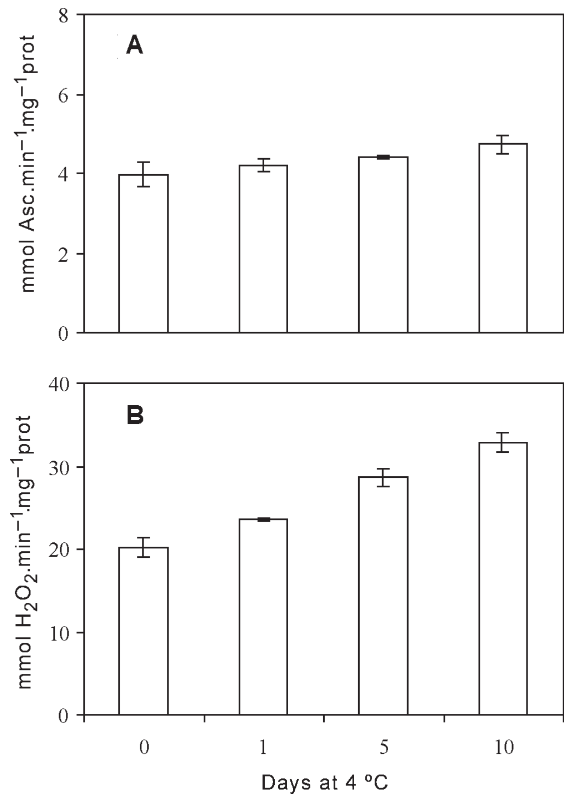

Figure 6. Ascorbate peroxidase (APX) and catalase (CAT) activities in mitochondria obtained from potato tubers submitted to low temperature. Mitochondria were partially purified from tubers stored at 4°C for 1, 5 or 10 days. Values are the means ± SE of three replicates, from three different mitochondrial preparations.

28°C or at 4°C for one day (figure 6A). In contrast, mitochondrial CAT activity increased to a high level after exposing tubers for ten days at 4°C (figure 6B). Despite the fact that H_2O_2 levels were not determined, the increase of CAT activity paralleled by the increase of AOX and PUMP suggests that oxidative stress occurred after storing tubers for five and ten days at 4°C. These results support the hypothesis that AOX and PUMP activities are stimulated in response to oxidative stress caused by low temperature.

In conclusion, both AOX and PUMP activities in potato tuber mitochondria were shown to be stimulated by low temperatures in a time dependent manner. AOX and PUMP activities did not change after storing the tubers at 4°C for one day. However, high AOX and PUMP activities were observed in potato tuber mitochondria after storage at 4°C for five and ten days. In addition, increased CAT activity was also observed and it paralleled the increase of AOX and PUMP activities. As AOX and PUMP can inhibit reactive oxygen species production in mitochondria and CAT is an antioxidant enzyme, our results indicate that these proteins may play an important role in protection against oxidative stress at low temperatures.

Acknowledgments: The authors wish to thank Dr. Thomas Elthon (Michigan University, USA) for providing the antibody against AOX and Dr. Elizabeth Fontes for the use of the facilities of the Plant Molecular Biology Lab. (BIOAGRO/UFV). The authors also thank Dr. Everaldo Gonçalves de Barrros for reviewing the manuscript. H.A.P. and D.C.C. were recipients of scholarships granted by CNPq, and R.B. was the recipient of a scholarship from CAPES (Brazilian Government).

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