Wound-Induced Respiration and Pyrophosphate:fructose-6-phosphate Phosphotransferase in Potato Tubers

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A seven fold increase in the rate of respiratory O_2 uptake was observed 24 h after slicing of potato tuber disks. The maximum activity of pyrophosphate:fructose-6-phosphate phosphotransferase (PFP) was 5–7 times greater than that of ATP-dependent phosphofructokinase (PFK) in fresh or aged potato slices. Thus, PFP may participate in glycolysis which supplies respiratory substrate in potato tubers. The PFP activity of desalted extracts determined in the absence of fructose-2,6-bisphosphate (F2,6BP) increased by 4.5 fold 24h after slicing. However, maximal PFP activity determined with saturating (1 μM) F2,6BP was not changed. The K_a values of PFP for F2,6BP was lowered from 33 to 7 nM after 24 h of aging treatment. This increased susceptibility of the PFP activity to its allosteric activator, F2,6BP, may be involved in the increased respiration in wounded disks of potato tubers. Imunoblotting experiments indicated that both the α (66 kDa) and the β (60kDa) subunits of PFP were present in fresh or 24h aged tuber slices.

Introduction

The metabolism of dormant plant storage organs, such as potato tubers and carrot roots, can be activated by slicing the tissue into thin disks and incubating of these segments in a moist atmosphere (Kahl, 1974; Uritani and Asahi, 1980). There are many reports concerning the activity of the glycolytic pathway in plant storage tissues in response to mechanical wounding (see review by Kahl, 1974). Involvement of the allosteric properties of ATP-dependent phosphofructokinase (PFK, EC 2.7.1.11) on the respiration raise during wounding has been reported, although different mechanisms have been proposed (Black and Wedding, 1968; Ashihara *et al.*, 1972). After these papers were published, a plant specific PPi: fructose-6-phosphate 1-phosphotransferase (PFP, EC 2.7.1.90) which also catalyzes the key step of glycolysis, conversion of fructose-6-phosphate to fructose-1,6-bisphosphate, has been discovered (Carnal and Black, 1979). In contrast to mammalian cells, fructose-2,6-bisphosphate does not activate plant PFK, but stimulates PFP activity (Sabularse and Anderson, 1981). Involvement of PFP as a glycolytic enzyme of potato tubers has been argued by Hajirezaci and Stitt (1991) . In potato tubers, PFP is a heterooctomer composed of α (66kDa) and β (60kDa) subunits (Moorhead and Plaxton, 1991). It has been suggested that the β-subunit, but not α-subunit, has a binding site for PPi, while the F2,6BP binding site may be present in the α-subunit (Carlisle *et al.*, 1990; Cheng and Tao, 1990). Thus, the α- and β-subunits of PFP may have regulatory and catalytic functions, respectively.

In the present study, we examined whether the catalytic and molecular properties of PFP were changed following wounding of potato tubers.

Materials and Methods

Mature tubers of potato (*Solanum tuberosum* L. cv 'Danshaku') were obtained from Kyogoku-cho, Hokkaido, Japan. The wounding treatment of potato tubers was performed according to the method of Dyer *et al.* (1989). Briefly, cylinders (diameter 7 mm) were cut from tubers with a sterilized cork borer, the epidermis was removed, and the cores were sliced into 1.5-mm-thick sections with a razor blade. All tissues were maintained on moist sterile filter paper in Petri dishes at 26 °C for 24h. Respiratory oxygen uptake was monitored by

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a revised version of Warburg manometric techniques with an oxygen uptester (Taitec Ltd., Tokyo, Japan).

Fresh and wounded potato tuber tissue (1 g fresh weight) were homogenized in 5 ml of 50 mM imidazole-HCl buffer (pH 7.6) including 1 mM NaEDTA, 2 mM $MgCl_2$, 0.1% (v/v) 2-mercaptoethanol, 0.5% (w/v) phenylmethylsulfonyl fluoride (PMSF) and 0.25 g insoluble polyvinylpolypyrrolidone with a mortar and pestle. The homogenate was centrifuged at 20,000×*g* for 20 min at 4 °C, and the aliquot of the supernatant (2.5 ml) was desalted on a column of Sephadex G-25 (PD-10 column, bed vol. 9.0 ml, Pharmacia, Uppsala, Sweden) that had been equilibrated with the same buffer used for extraction. The eluted protein fraction (3.5 ml) was used immediately for assays of enzyme activity. Protein was quantified by the method of Bradford (1976) with bovine serum albumin as standard.

Activity of PFP was determined by the method of Ashihara and Horikosi (1987) with slight modification. The standard reaction mixture (total volume 1.0 ml) composed of 50 mM *N*-2-hydroxyethylpiperazine-2-ethanesulfonic acid (HEPES)-NaOH buffer (pH 7.2), 10 mM F6P, 1 μM F2,6BP, 1 mM PPi, 5 mM $MgCl_2$, 0.2 mM NADH, and sufficient amount (>1 unit) of desalted aldolase, triose phosphate isomerase and glycerol 3-phosphate dehydrogenase. In the case of the determination of the activity of PFK, 1 mM PPi was replaced by 1 mM ATP, and F2,6BP was removed from the reaction mixture.

K_a values (concentration for half maximal activation) for F2,6BP was calculated using 'Enzyme Kinetics' software (Trinity Software, Campton, NH, USA).

Electrophoresis, immunoblotting and immunoquantification were carried out according to Theodorou *et al.* (1992). The final acrylamide monomer concentration in the separating gel was 10% (w/v). The separated polypeptides were electrophoretically transferred to an Immobilon transfer membrane (Millipore, Bedford, MA, USA). The membrane was soaked in blocking solution (5% non-fat dry milk and 0.25% Tween 20 in tris [hydroxymethyl] aminomethane (Tris)-buffered saline) for 1 h. The membrane was then incubated for 1 h with a mixture of 1:25,000 dilution of anti-(potato tuber α-subunit PFP), and 1:1,000 dilution of anti-(potato tuber β-subunit PFP) immune serum. After washing with blocking solution, the blot was incubated for 1 h with alkaline phosphatase-conjugated goat anti-rabbit IgG (Organon Teknika, West Chester, PA), which was diluted 1:2,000. The colour reaction was performed by incubation of the membrane in 100 mM Tris-HCl, pH 9.6, 100 mM NaCl, 4 mM $MgCl_2$, 0.1 mg ml^{-1} nitro blue tetrazolium, 0.05 mg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate, and 1% dimethylformamide.

Results and Discussion

Maximum rise of respiratory O_2 uptake in potato disks was observed 24 h after slicing (Table I). This observation confirmed that the wound respiration occurred in our potato tuber disks as found in several storage organs, such as tubers of potato and Jerusalem artichoke, and roots of carrot and chicory (Kahl, 1974; Hajirezaci and Stitt, 1991).

The activities of PFP and PFK were determined using desalted extracts from fresh or 24h aged potato tuber disks (Table I). PFP activity in the presence of 1 μM F2,6BP was 5–7 times greater than PFK activity in fresh and wounded disks. Thus, PFP may be functional as a glycolytic enzyme. These results are similar to those of Hajirezaci and Stitt (1991) who also suggested that PFP catalyses the glycolytic reaction in potato tubers. After 24 h-

Table I. Changes in respiratory O_2 uptake, activity of PPi:fructose-6-P phosphotransferase (PFP), and of phosphofructokinase (PFK) during aging of potato tuber slices. The K_a values of PFP for fructose-2,6-bisphosphate (F2,6BP) are also shown.

Time after slicing (h)	0	24
O_2 uptake (μ mol g FW^{-1} h^{-1})	1.25±0.56 (100)	9.23±2.63 (738)
PFP (nkat mg $protein^{-1}$)		
No F2,6BP	0.031±0.0017 (100)	0.140±0.052 (452)
1 μM F2,6BP	1.59±0.34 (100)	1.69±0.25 (106)
PFK (nkat mg $protein^{-1}$)	0.219±0.028 (100)	0.359±0.047 (164)
K_a value of PFP for F2,6BP (nM)	33±1*	7±1*

All values represent mean ± SD (n = 4). The values in parentheses show the percentage of the initial value (0 h).

* K_a values obtained from Hanes-Woolf plots. Kinetic data were subject to linear regression analysis and the correlation of the points to the lines was more than 0.995.

wounding, maximum catalytic activity of PFP in the presence of 1 μM F2,6BP was similar to that of fresh disks. However, the PFP activity without F2,6BP increased 4.5-fold 24 h after slicing and K_a values of PFP for F2,6BP declined from 33 nM to 7 nM during wounding. Typical Hanes-Woolf plots (Cornish-Bowden 1976) of the enzyme for F2,6BP are shown in Fig. 1. The activity of ATP-dependent PFK increased approximately 1.6-fold during wounding (Table I).

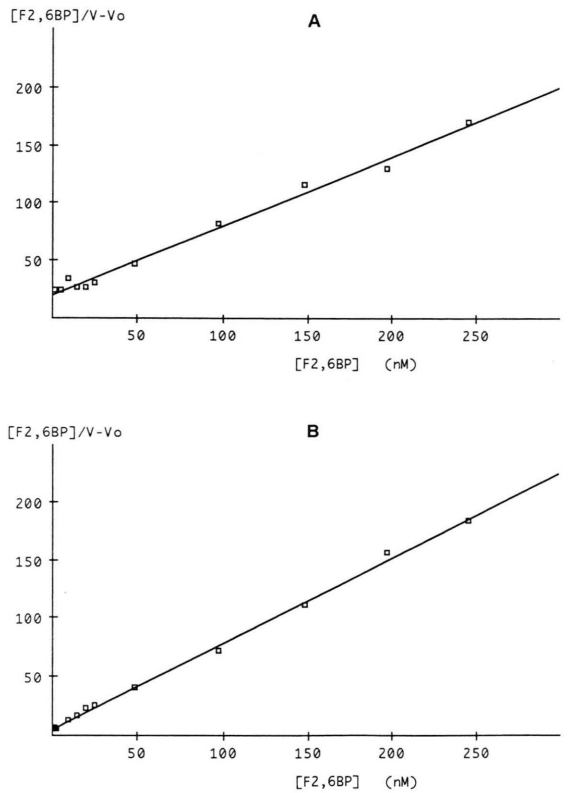

Fig. 1. Typical Hanes-Woolf plots ([F2,6BP] vs. [F2,6BP]/[v-v_0]) of PPi:fructose-6-P phosphotransferase from fresh and 24-h aged potato disks. Velocity is expressed as nkat mg^{-1} protein. v_0 means velocity without F2,6BP. (A) Fresh disks, (B) aged disks.

Compared with the marked increase in respiration, only no or little increase in the maximum activity of PFP and PFK was observed. Thus, it is unlikely that these respiratory rises are caused by the simple coarse control, i.e., increase in PFP and PFK proteins. The PFP from 24 h-wounded potato disks exhibited a more than 5-fold greater affinity for F2,6BP than the PFP from fresh disks did. Similar changes in K_a values of PFP have been reported in *Brassica nigra* suspension cells during Pi starvation (Theodorou *et al.*, 1992) and in castor bean cotyledons during germination (Podesta and Plaxton, 1994). The PFP from Pi-starved *B. nigra* cells exhibited a greater affinity for F2,6BP (K_a: 90 nM) than the enzyme from nutrient-sufficient cells (K_a: 320 nM). These changes in *B. nigra* cells were accompanied by apparent changes in subunit composition of PFP. The PFP from Pi-sufficient cells consisted of β-subunits, but PFP from Pi-deficient cells which exhibited high affinity for F2,6BP had both α- and β-subunits.

Immunoblotting studies using rabbit anti-(potato tuber PFP) immune serum were carried out to examine the molecular properties of the PFP from fresh and wounded potato tuber disks. Figure 2 showed the results of the immunoblots. Both α- (66 kDa) and β- (60 kDa) subunits were detected in the extracts from fresh and wounded potato disks. The ratio of α- and β-subunits was almost constant when different amounts of proteins (15–120 μg) were used. Thus, it is concluded that the different K_a values observed in PFP from fresh and wounded potato disks are not related their subunit composition. This is contrast to the phenomena observed in Pi-starvation of *B. nigra* cells. In *B. nigra* cells, in addition to greater affinity of PFP for F2,6BP, the 20-fold increase in V_{max} of PFP has been observed after Pi-starvation (Theodorou *et al.*, 1992).

The molecular mechanism responsible for the reduction in the K_a value of PFP for F2,6BP during wounding of potato disks has not yet been elucidated. Changes in this property of PFP may be involved in the control mechanisms of wound respiration of potato tubers. Enhanced activation of PFP by F2,6BP during wound respiration of potato tuber disks has been supported from the fluctuation of glycolytic metabolites during wounding, because the levels of the products of PFP, fructose-

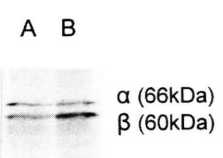

Fig. 2. Immunoblots of PPi:fructose-6-P phosphotransferase from fresh and 24-h aged potato disks. Equal amounts of protein (30 μg) were loaded on each lane. (A) Fresh disks, (B) aged disks.

1,6-bisphosphate and triose phosphate, increased, but that of a substrate, PPi, decreased after slicing. The potent activator, F2,6BP, itself also increased by ca. 5-fold during early phase of potato tuber wounding (Hajirezaci and Stitt, 1991). Thus, PFP activity may be greatly stimulated during wounding potato tubers.

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Ashihara H., Komamine A. and Shimokoriyama M. (1972), Phosphofructokinase of cultured and aged carrot-root slices. Phytochemistry **11**, 2717–2721.

Ashihara H. and Horikosi T. (1987), Role of pyrophosphate:fructose-6-phosphate 1-phosphotransferase in glycolysis in cultured *Catharanthus roseus* cells. Z. Naturforsch. **42c**, 1215–1222.

Black M. K. and Wedding R. T. (1968), Effect of storage and aging on properties of phosphofructokinase from Jerusalem artichoke tubers. Plant Physiol. **43**, 2066–2069.

Bradford M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **7**, 248–254.

Carlisle S. M., Blakeley S. D., Hemmingsen S. M., Trevanion S. J., Hiyoshi T., Kruger N. J. and Dennis D. T. (1990), Pyrophosphate-dependent phosphofructokinase. Conservation of protein sequence between the α- and β-subunits and with the ATP- dependent phosphofructokinase. J. Biol. Chem. **265,** 18366–18371.

Carnal N. W. and Black C. C. (1979), Pyrophosphate-dependent 6-phosphofructokinase, a new glycolytic enzyme in pineapple leaves. Biochem. Biophys. Res. Commun. **86,** 20–26.

Cheng H. F. and Tao M. (1990), Differential proteolysis of the subunits of pyrophosphate-dependent 6-phosphofructo-1-phosphotransferase. J. Biol. Chem. **265,** 2173–2177.

Cornish-Bowden A(1976), Principles of Enzyme Kinetics. Butterworths, London, pp. 1–206.

Dyer W. E., Henstrand J. M., Handa A. K. and Herrmann K. M. (1989), Wounding induces the first enzyme of the shikimate pathway in Solanaceae. Proc. Natl. Acad. Sci. USA **86,** 7370–7373.

Hajirezaci M. and Stitt M. (1991), Contrasting roles for pyrophosphate:fructose-6-phosphate phosphotransferase during aging of tissue slices from potato tubers and carrot storage tissues. Plant Sci. **77,** 177–183.

Kahl G. (1974), Metabolism of plant storage tissue slices. Bot. Rev. **40,** 263–314.

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

Moorhead G. B. G. and Plaxton WC (1991), High-yield purification of potato tuber pyrophosphate:fructose-6-phosphate 1-phosphotransferase. Protein Expr. Purification **2**, 29–33.

Podesta F. E. and Plaxton W. C. (1994) Regulation of cytosolic carbon metabolism in germinating *Ricinus communis* cotyledons. I. Developmental profiles for the activity, concentration, and molecular structure of the pyrophosphate- and ATP-dependent phosphofructokinases, phospho*enol*pyruvate carboxylase and pyruvate kinase. Planta **194,** 374–380.

Sabularse D. C. and Anderson R. L. (1981), D-fructose-2,6-bisphosphate:a naturally occurring activator for inorganic pyrophosphate:D-fructose-6-phosphate 1-phosphotransferase in plants. Biochem. Biophys. Res. Commun. **103,** 848–855.

Theodorou M. E., Cornel F. A., Duff S. M. G. and Plaxton W. C. (1992), Phosphate starvation-inducible synthesis of the α-subunit of the pyrophosphate-dependent phosphofructokinase in black mustard suspension cells. J.Biol.Chem. **267,** 21901–21905.

Uritani I. and Asahi T. (1980), Respiration and related metabolic activity in wounded and infected tissues. In: The Biochemistry of Plants (Stump, P. K. and Conn, E. E., eds). Academic Press, New York, Vol. 2, pp. 463–485.