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ATP- and NADH-dependent membrane potential generation in plasmalemma enriched vesicles from parenchyma of dormant and non-dormant Jerusalem artichoke tubers

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Abstract

Using a membrane potential probe, Oxonol VI, it was possible to demonstrate generation of ATP- and NADH-dependent membrane potential across the plasmalemma, with membrane vesicles derived from parenchyma cells of Jerusalem artichoke tubers (*Helianthus tuberosus* L.). It was shown that ATP- and NADH-dependent membrane potential generation was higher in dormant material than in non-dormant tissue and that the effects of ATP and NADH on membrane potential generation were additive. ATP-dependent potential generation was sensitive to vanadate, an inhibitor of plasmalemma ATPase activity. The results are discussed in relation to the properties of the different enzymes bound to the plasma membrane, the morphogenetic potentialities of tuber buds and the hypothesis that tuber dormancy could be an extreme case of nutrient deficiency induced by short-distance intercellular relationships.

Introduction

Previous studies from our laboratory led to the hypothesis that the morphogenetic potential of buds from Jerusalem artichoke tubers could be influenced by the properties of the parenchyma underlying the bud. In dormant tubers, it was found that plasmalemma ATPase activity (Pétel and Gendraud 1986) and cyanide (CN^-) sensitive transmembrane potential (Fol *et al.* 1989) of parenchyma cells were higher than in non-dormant tubers. Nutrient uptake is dependent on proton extrusion (Delrot and Bonnemain 1981) and transmembrane potential (Felle and Bentrup 1980). According to these authors, our previous results indicated that nutrient absorption

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Abbreviations: CCCP - carbonyl cyanide *m*-chloro-phenylhydrazone; ISO - inside-out; Oxonol VI - bis-(3-propyl)-5-oxoisoxazol-4-yl)pentamethine oxonol; RSO - right side-out;

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potential is higher in dormant material. This idea is also in accordance with the greater sucrose uptake observed in dormant tubers (Gendraud and Lafleuriel 1983).

Plasmalemma ATPase was found to be linked to proton extrusion and to be twice as active in dormant material (Pétel and Gendraud 1986). The same results were found in investigations on plasmalemma NADH dehydrogenase (Pétel and Gendraud 1987)

The plasmalemma H^+-ATPase hyperpolarizes (interior negative) the membrane potential. This was shown most recently using Oxonol probes:plasmalemma ATPase (Singh *et al.* 1987) and NADH dehydrogenase (Hassidim *et al.* 1987) were found to participate in membrane potential generation in higher plants.

The aim of the present work was to demonstrate the capability of the plasmalemma ATPase and a putative NADH dehydrogenase from Jerusalem artichoke to generate a transmembrane potential, using Oxonol VI (bis(3-propyl-5-oxoisoxanol-4-yl)pentamethine), and to determine the part of each enzymatic system in this potential generation, in both dormant and non-dormant tubers.

Material and methods

Plant material: Jerusalem artichoke tubers (*Helianthus tuberosus* L.) were harvested in October and kept dormant in moist sand, at 24 °C, in the dark. The dormancy can be broken by keeping the tubers at 4 °C, under the same conditions, for a period of 16 weeks (Courduroux 1967).

Membrane preparation: Homogenization of the tissue and centrifugation on linear sucrose gradient were carried out as described previously (Pétel and Gendraud 1986). Separation on sucrose gradient was chosen because this procedure yielded a large proportion of sealed inside-out (ISO) vesicles, while two-phase partitioning produced a sealed vesicle preparation that was 90 % right side-out (RSO) (Larsson *et al.* 1988), and thus not useful for exogenous ATP- and NADH-dependent potential generation measurements. Plasmalemma enriched fractions were free of NO_3^- sensitive ATPase and NAD(P)H cytochrome *c* reductase (Pétel and Gendraud 1989), indicating that contamination of these fractions is negligible.

Spectrophotometric assays: Membrane potential generation was monitored by the probe Oxonol VI as an increase of absorbance at 630 nm (Macri *et al.* 1987). The reaction medium was buffered with 10 mM HEPES Tris (pH 6.5) and contained 15 µM Oxonol VI. 2 mM ATP and 2 mM $MgSO_4$ or 0.5 mM NADH and 1.7 mM $K_3Fe(CN)_6$ were added in order to record ATP- and NADH-dependent potential generation, respectively. The reactions were started by addition of 100 µl of plasmalemma enriched fractions (12 µg protein) 3 or 4 min after subtrate addition. Controls were made as described in the Results section.

Results

Oxonol VI is a biochemical probe of transmembrane potential generation. The substrate is first added alone to the reaction medium. No response was recorded before membrane vesicle addition, indicating that the optical changes monitored after plasma membrane addition did not represent an interaction between the probe and the reaction medium (Fig 1, curve a). Measurements made with addition of *Triton X-100* treated vesicles showed that an intact, sealed membrane vesicle is required to observe the response of the probe (Fig 1, curve c). When ATP was added in an assay containing native plasmalemma vesicles, a potential generation was recorded. Moreover, the potential was collapsed by addition of CCCP (carbonylcyanide-*m*-chlorophenylhydrazone) (1 μM) at the end of the run. Finally, addition of vanadate to the system (120 μM) caused a great decrease in ATP-dependent potential generation (Fig 1, curve b). These results are in accordance with a transmembrane potential generation involving the plasmalemma H^+-ATPase.

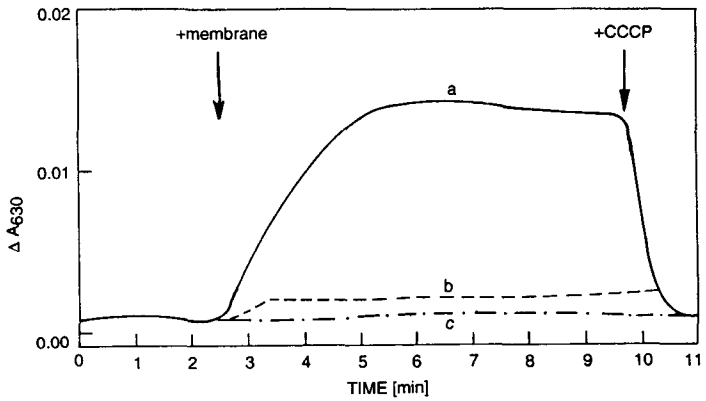

Fig. 1. ATP-dependent membrane potential generation monitored with Oxonol VI with native plasmalemma vesicles without (a) and with (b) vanadate (120 μM) added to the reaction medium. Control was made using Triton X-100 treated vesicles (c).

ATP- and NADH-dependent potential generation was measured in plasmalemma vesicles obtained from dormant and non-dormant tubers (Fig 2). CCCP was added at the end of the run to ensure that membrane vesicles were responsible for the response recorded. The results given (Fig 2) show that ATPase and NADH dehydrogenase were implicated in membrane potential generation and that these generations were higher in dormant material. ATP- and NADH-dependent potential generation were, respectively, 58 and 30 % higher in dormant material.

Comparing ATP- and NADH-dependent membrane potential generation, our results show that ATP-dependent generation is greater than the NADH-dependent one, in both materials: 63 % higher in dormant material and 33 % higher in non-

dormant one. This can be linked to our previous measurements of H^+ extrusion, monitored with the acridine orange probe, which was also twice as high with ATP than with NADH (Pétel and Gendraud 1986, 1987).

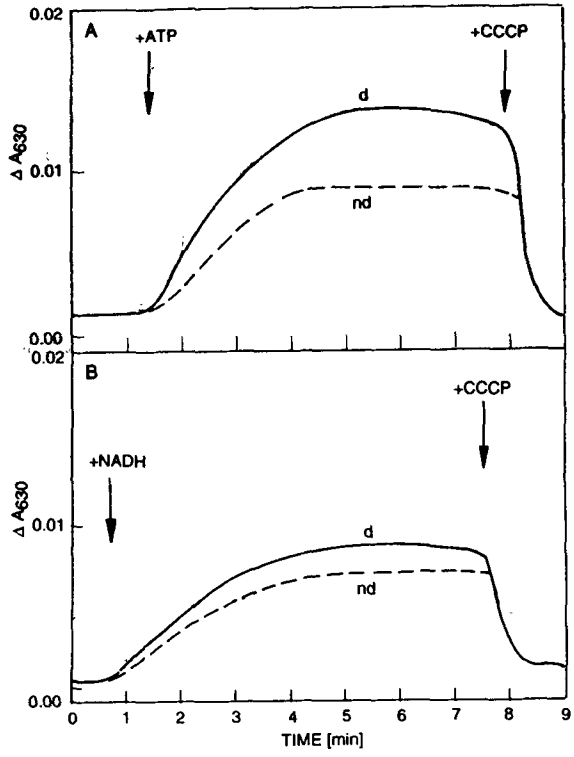

Fig. 2. ATP- (A) and NADH-dependent (B) membrane potential generation monitored on dormant and non-dormant materials.

Finally, additivity of ATP- and NADH-dependent generation was monitored in both dormant and non-dormant materials (Fig 3). In these experiments, it was found that adding both ATP and NADH generated an absorbance increase equal to the sum of the absorbance increase obtained when ATP and NADH were added separately.

Discussion

The results presented here indicate that the plasmalemma of parenchyma cells from Jerusalem artichoke tubers is electrically energized by two enzymatic systems : an ATPase and a NADH dehydrogenase, regarding the additivity of ATP- and NADH-dependent membrane potential generation.

Three different redox activities (Rubinstein *et al.* 1984) were previously identified at the plasmalemma: (a) reduction of exogenous ferricyanide by an endogenous electron donor; (b) oxidation of exogenous NADH by exogenous ferricyanide ; and (c) oxidation of endogenous NADH by endogenous electron acceptor. In the experimental conditions used here, systems (b) and (c) could be evidenced by adding NADH and ferricyanide to the reaction medium, involving, respectively, RSO and ISO plasmalemma vesicles.

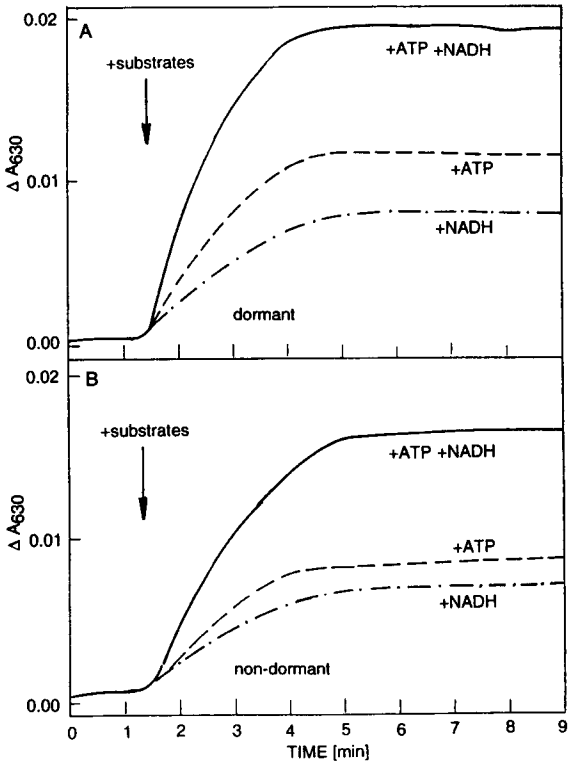

Fig. 3. Membrane potential generation recorded in dormant (A) and non-dormant (B) materials. Experiments were made adding ATP (+ATP) and NADH (+NADH) alone and adding both substrates together (+ATP+NADH).

It was demonstrated, with intact cells, that proton extrusion induced by exogenous ferricyanide reduction (system a) is dependent on plasmalemma ATPase (Belkoura and Marigo 1986, Rubinstein and Stern 1986). Other authors postulated that proton efflux linked to redox activity was independent of plasmalemma ATPase activity (Bown and Crawford 1988). These results could indicate that one plasmalemma redox system (a) could be linked to plasmalemma ATPase activity but not the others

(systems b and c). Obviously, the NADH-dependent potential generation described in the present paper is independent of plasmalemma ATPase as it was obtained without ATP addition to the medium.

It appears that transmembrane potential at the plasmalemma could be generated by redox systems and plasmalemma ATPase. Membrane potential generation was found to be higher in dormant than in non-dormant material. This result corroborates with the higher activities measured previously (Pétel and Gendraud 1986, 1987) and with the membrane potential measured in the intact tissue (Fol *et al.* 1988), where the membrane depolarization induced by CN^- addition was twice as large in dormant material.

More precisely, our results indicated that plasmalemma ATPase was more implicated in transmembrane potential generation than NADH dehydrogenase, in both types of material. Comparing dormant and non-dormant tubers, it appeared that the total decrease in transplasmalemma potential, observed after dormancy breaking, is mainly induced by the decrease of ATP-dependent potential generation. This result is in accordance with the decrease of plasmalemma ATPase (Pétel *et al.* 1992a) and NADH dehydrogenase (Pétel *et al.* 1992b) activities during the release of dormancy.

It appears that dormancy breaking in Jerusalem artichoke tubers is characterized by a decrease of proton extrusion (Pétel and Gendraud 1986, 1987) and transplasmalemma potential generation, that is to say by a decrease of the total proton motive force of parenchyma cells, involved in metabolite absorption.

References

Belkoura, M., Marigo, G.: The proton-pump at the plasmalemma of *Catharanthus roseus* cells. - Biochimie **68**: 1299-1302, 1986.

Bown, A.W., Crawford, L.A.: Evidence that H^+ efflux stimulated by redox activity is independent of plasma membrane ATPase activity. - Physiol. Plant. **73**: 170-174, 1988.

Courduroux, J.C.: Etude du mécanisme physiologique de la tubérisation chez le Topinambour (*Helianthus tuberosus* L.). - Ann. Sci. Nat. Bot. **8**: 215-356, 1967.

Delrot, S., Bonnemain, J.L.: Involvement of protons as substrate for the sucrose carrier during phloem loading in *Vicia faba* leaves. - Plant Physiol. **67**: 560-564, 1981.

Felle, H., Bentrup, F.W.: Hexose transport and membrane depolarization in *Riccia fluitans*. - Planta **147**: 471-476, 1980.

Fol, M., Frachisse, J.M., Pétel, G., Gendraud, M.: Effect of cyanide on the membrane potential of Jerusalem artichoke (*Helianthus tuberosus* L.) tuber parenchyma: different responses in relation to dormancy. - Compt. rend. Acad. Sci. Paris, Sér.III **309**: 551-556, 1989.

Gendraud, M., Lafleuriel, J.: Characteristics of sucrose and tetraphenylphosphonium uptake by parenchyma of *in vitro* grown dormant and non dormant Jerusalem artichoke tubers. - Physiol. vég. **21**: 1125-1133, 1983.

Hassidim, M., Rubinstein, B., Lerner, H.R., Reinhold, L.: Generation of a membrane potential by electron transport in plasmalemma-enriched vesicles of cotton and radish. - Plant Physiol. **85**: 872-875, 1987.

Larsson, C., Widell, S., Sommarin, M.: Inside-out plasma membrane vesicles of high purity obtained by aqueous two-phase partitioning. - FEBS Lett. **229**: 289-292, 1988.

Macri, F., Vianello, A., Cocucci, M.C.: Mycochrome decreases ATP-driven proton translocation in plant plasma membrane- and tonoplast-enriched vesicles. - Plant Cell Environ. **10**: 369-373, 1987.

Pétel, G., Gendraud, M.: Contribution to the study of ATPase activity in plasmalemma-enriched fractions from Jerusalem artichoke tubers (*Helianthus tuberosus* L.) in relation to their morphogenetic properties. - J. Plant Physiol. **123**: 373-380, 1986.

Pétel, G., Gendraud, M.: Activité d'un système oxydoréducteur au niveau du plasmalemme des cellules de parenchyme de Topinambour (*Helianthus tuberosus* L.) en relation avec les propriétés morphogénétiques des tubercules. - Compt. rend. Acad. Sci. Paris, Sér.III **305**: 51-54, 1987.

Pétel, G., Gendraud, M.: Localization in sucrose gradients of pyrophosphatase activities of Jerusalem artichoke (*Helianthus tuberosus* L.) tubers microsomal fractions. - J. Plant Physiol. **134**: 466-470, 1989.

Pétel, G., Lafleuriel, J., Dauphin, G., Gendraud, M.: Cytoplasmic pH and plasmalemma ATPase activity of parenchyma cells during the release of dormancy of Jerusalem artichoke tubers. - Plant Physiol. Biochem. **30**: 379-382, 1992a.

Pétel, G., Sueldo, R., Coudret, A., Gendraud, M.: Plasmalemma fluidity in parenchyma cells from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers during the break of dormancy. - Biol. Plant. **34**: 373-380, 1992b.

Rubinstein, B., Stern, A.I.: Relationship of transplasmalemma redox activity to proton and solute transport by roots of *Zea mays*. - Plant Physiol. **80**: 805-811, 1986.

Rubinstein, B., Stern, A.I., Stout, R.G.: Redox activity at the surface of oat root cells. - Plant Physiol. **76**: 386-391, 1984.

Singh, S.P., Sudershan Kesav, B.V., Briskin, D.P.: Reconstitution and rapid partial purification of the red beet plasma membrane ATPase. - Physiol. Plant. **69**: 617-626, 1987.

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