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# Plasmalemma fluidity in parenchyma cells from Jerusalem artichoke (*Helianthus tuberosus* L.) tubers during the break of dormancy

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## Abstract

Plasmalemma-enriched fractions were isolated from Jerusalem artichoke tubers along the time course of dormancy break produced by cold treatment. A decrease of membrane fluidity was noted from the 3rd to the 8th week of this treatment, as well as a decrease of plasmalemma NADH dehydrogenase activity from the 5th to the 8th week. The plasmalemma lipid extracts studied revealed two major phospholipidic components: phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Their respective quantities decreased until the 12th week, where the phosphatidylcholine level is lower than the phosphatidylethanolamine one. The observed changes are discused in relation to dormant and non-dormant states of tubers and the breaking of dormancy.

## Introduction

Dormancy is expressed by tubers of a number of higher plants. It is now accepted that the plasmalemma is the first cellular component affected by low temperatures, and that this membrane could have an essential role during the break of dormancy (Steponkus 1984). Previous work has investigated kparticular properties of the plasmalemma from parenchyma cells of dormant tubers where ATPase (Pétel and Gendraud 1986) and NADH dehydrogenase (Pétel and Gendraud 1987) activities, the transplasmalemma pH gradient and tetraphenylphosphonium ($TTP^+$) absorption in parenchyma cells (Gendraud and Lafleuriel 1983) were higher than in non-dormant material. These results are in accordance with a possible restructuring of the plasmalemma during the break of dormancy by low temperatures, implicating a modification of short distance intracellular relations and leading towards the morphogenesis. Plasmalemma restructuring could be due to a modification of phospholipid environment or of phospholipids/proteins ratio, that could lead to a variation in membrane fluidity.

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In this note we report the modifications of NADH dehydrogenase activity, phospholipidic composition, phospholipids/proteins ratio and membrane fluidity during the break of dormancy of tubers by cold treatment at 4 °C.

## Material and methods

**Plant material:** Dormant tubers of Jerusalem artichoke (*Helianthus tuberosus* L.) were harvested in October and kept at 4 °C, in the dark in moist sand for a period of 16 weeks, in order to break their dormancy (Courduroux 1967). Measurements were made each week as described hereafter.

**Membrane preparation:** 50 g of parenchyma were ground in 0.1 M Tris HCl buffer, pH 8.0, added with 0.5 M mannitol, 0.5 % (v/m) Bovine Serum Albumine (BSA), 2 mM EDTA and 10 mM β-mercaptoethanol and then filtered through four layers of cheesecloth. The homogenate was centrifuged for 20 min at 7500 *g* in order to remove cell wall, nuclei and mitochondria.

The supernatant was recentrifuged for 1 h at 100 000 *g* on a 66 % (v/m) saccharose cushion. The microsomal fraction was centrifuged for 4 h at 100 000 *g* on a continuous saccharose gradient (30 to 45 % - v/m). The plasmelemma enriched fraction was then sediment by a 45 min centrifugation at 100 000 *g*, resuspended in 12.5 mM MES Tris buffer, pH 8.0, supplemented with 1 mM β-mercaptoethanol and 20 % glycerol (v/v), and kept frozen at -76 °C before used for different measurements (for details see Pétel and Gendraud 1986).

**NADH dehydrogenase assays (Pétel and Gendraud 1987):** Measurements were made at 30 °C in 1 $cm^3$ of 20 mM MES Tris buffer, pH 6.5, supplemented with 1.7 mM $K_3Fe(CN)_6$.10 $mm^3$ of enriched fraction were added to the reaction medium and the reaction started by addition of 0.5 mM NADH. NADH oxidation was monitored by the decrease in absorbance at 340 nm, and quantified using a millimolar extinction ratio of 6.23.

**Protein determination:** Protein content of the fractions was determined by the Bradford method, using BSA as a standard (Bradford 1976).

**Lipids separation:** Lipid extract from the enriched fraction was obtained by the method of Folch *et al.* (1957). Separation of phospholipids (PL) was performed by monodimensional fractionation of polar lipids (Nouvelot *et al.* 1977), and the phosphorus of each band obtained was measured according to Bartlett (1959).

**Membrane fluidity:** The relative fluidity of plasmalemma enriched fractions was estimated by measuring the fluorescence depolarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) (Shinitzky and Inbar 1976). 50 µg of membrane proteins were mixed with DPH in a phosphate buffer sline (0.1 M PBS), pH 7.4. The final concentration of DPH was 2 mM. A 30 min incubation at 25 °C induced DPH

incorporation into the membrane. The steady state polarization was expressed as the fluorescence anisotropy, *r*, measured with a fluofluidimeter *SEFAM AMINCO*. Measurements were replicated at least 3 times, on 3 different plasmalemma-enriched fractions.

## Results

**Membrane purification.** The purity of the plasmalemma enriched fractions obtained by centrifugation on linear saccharose gradient was demonstrated previously using various membrane markers. Vanadate-sensitive ATPase and UDPG-sterol transferase, specifically located in plant plasma membranes (Hartmann-Bouillon and Benveniste 1978) were found in the same high density fractions of the gradient (Petel and Gendraud 1986). Moreover, we previously demonstrated, based on the distribution of other markers on this sucrose gradient (such as $NO_3^-$ sensitive ATPase, NAD(P)H cytochrome *c* reductase and different PPiases activities), that contamination of the plasmalemma enriched membrane fraction obtained from Jerusalem artichoke by this technique is negligible (Petel and Gendraud 1989). Finally, the purity of the fractions employed for each measurement was routinely estimated by the inhibition of ATPase activity by vanadate (120 μM). This inhibition was always about 90-95 % of the control activity (control : 18.6 mmol $mg^{-1}$ (protein) $min^{-1}$; vanadate added : 1.43 mmol $mg^{-1}$ (protein) $min^{-1}$: this result shows the high purity of the plasmalemma fractions obtained.

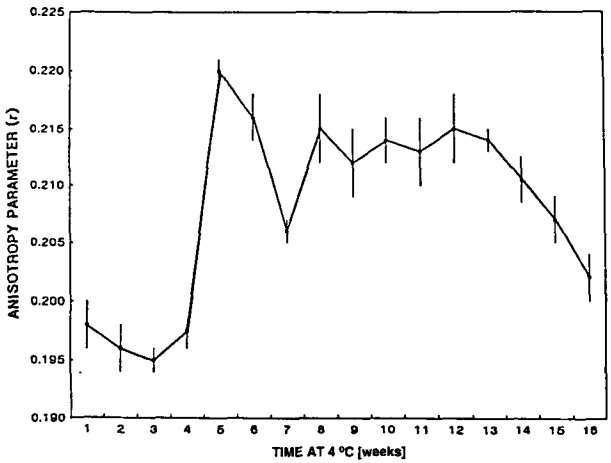


Fig. 1. Changes in the anisotropy parameter (*r*) of plasma membrane obtained from Jerusalem artichoke tubers, measured at 4 °C by fluorescence polarization, as a function of time (weeks of cold treatment). Each point is the mean of 9 measurements (3 measurements on 3 different plasmalemma-enriched fractions). Bars represent the standard deviation.

**Membrane fluidity.** Plasmalemma fluidity did not vary during the first 3 weeks (Fig.1). An important decrease (increase in the anisotropy parameter *r*) was noted from 3rd to 13th weeks, with a significant increase at the 7th week. From 8th to 11th weeks, no significative variation of membrane fluidity was recorded, but an increase was observed from the 13th week until the end of cold treatment.

**Phospholipids/proteins ratio.** This ratio (Fig. 2) decreased gradually during the first time of treatment and had a low value for 1 month from the 4th week of storage, with the exception of the increase recorded at the 7th week. At the end of storage, this ratio was higher than values measured the third month of cold, with a decrease at the very end of tubers storage.

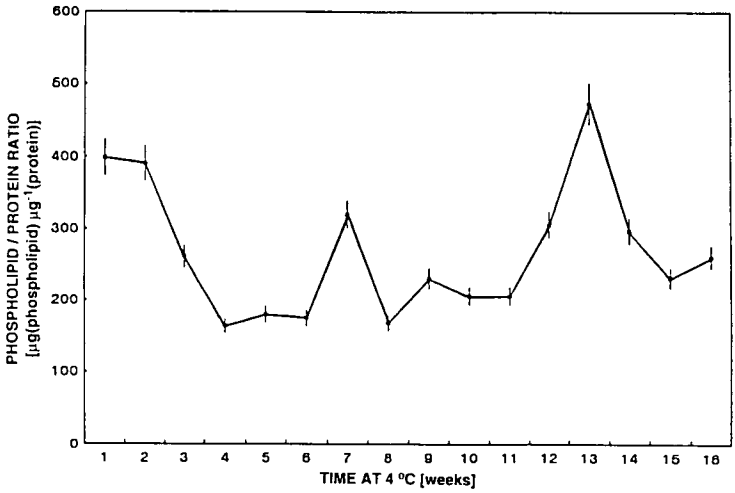


Fig. 2. Time-course of the phospholipid/protein ratio (µg (phospholipid) $\mu g^{-1}$ (protein)) of plasma membrane from Jerusalem artichoke tubers during cold treatment at 4 °C. Each phospholipid and protein detrmination was made in triplicate, on 3 different plasmalemma-enriched fractions. Bars represent the standard deviation of ratio.

**Phospholipid composition of membrane.** Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) are the major phospholipids found in plasmalemma-enriched fractions (Table 1), and their respective quantities decreased throughout the treatment, until the 12th week, where PC level was lower than PE.

Variations of the PC/PE ratio are indicated in the table, and may be linked to the membrane fluidity. Other phospholipids of fractions increased, as did phosphatidic acid, during the treatment, but a decrease of all lipids was noted at the end of the break of dormancy.

**NADH dehydrogenase activity.** The activity of NADH dehydrogenase in the plasmalemma, which could be linked to metabolite co-transport (Pétel and Gendraud

1987), decreased during the cold treatment, from 5th to 8th week. After the 8th week, NADH dehydrogenase activity was found to be constant.

Table 1. Comparison of phospholipid classes (in percent of total phospholipid), phosphatidylcholine/phosphatidylethanolamine (PC/PE) ratio of plasma membrane isolated from Jerusalem artichoke tubers during the break of dormancy. Each phospholipid determination was made in triplicate, on 3 different plasmalemma-enriched fractions. Variation of the experimental points did not exceed 10 %. (PA: phosphatidic acid; PE: phosphatidylethanolamine; PC: phosphatidylcholine; PI: phosphatidylinositol; PG: phosphatidylglycerol; PS: phosphatidylserine)

| Time at 4 °C [weeks] | PC | PE | PS+PI | PG | PA | PC/PE |
|---|---|---|---|---|---|---|
| 1 | 42.6 | 37.6 | 14.8 | 2.6 | 2.4 | 1.13 |
| 2 | 45.5 | 39.0 | 11.6 | 2.2 | 1.7 | 1.07 |
| 4 | 38.6 | 37.7 | 16.3 | 3.5 | 3.9 | 1.02 |
| 9 | 39.2 | 31.2 | 13.8 | 6.9 | 8.9 | 1.26 |
| 10 | 35.0 | 30.3 | 16.3 | 9.4 | 9.0 | 1.15 |
| 11 | 34.8 | 30.2 | 16.4 | 9.7 | 8.9 | 1.15 |
| 12 | 30.4 | 33.4 | 15.0 | 9.4 | 11.8 | 0.91 |
| 13 | 35.7 | 33.8 | 17.7 | 6.8 | 6.0 | 1.06 |
| 15 | 35.7 | 32.5 | 17.6 | 6.4 | 7.8 | 1.10 |
| 16 | 43.9 | 31.1 | 14.5 | 4.7 | 5.8 | 1.41 |

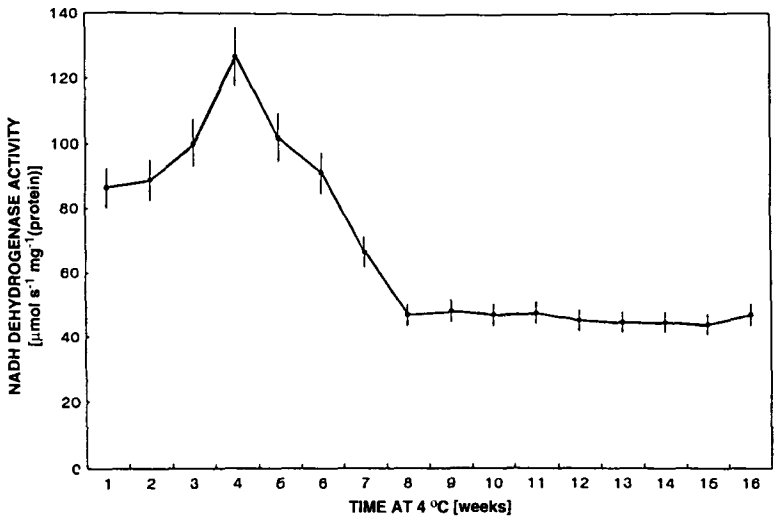


Fig. 3. Changes in NADH dehydrogenase activity in the plasmalemma isolated from Jerusalem artichoke during the break of dormancy by cold treatment, at 4 °C. Each point is the mean of 8 experiments made with 2 different plasmalemma-enriched fractions. Bars represent the standard deviation.

## Discussion

Three phases can be distinguished for the pattern of change in membrane fluidity:
(1) a decrease of fluidity (Fig. 1) that could be explained by an increase of the sterol/phospholipid ratio in the plasmalemma of Jerusalem artichoke (Ishikawa and Yoshida 1985). An another explanation could be the decrease in the phospholipid level or in the PC/PE ratio (Table 1). The decrease of the PC/PE ratio could be involved in the greater rigidity of membranes, because of the high viscosity of PE. The only exception to this pattern is the increase in the $7^{th}$ week, correlated with an increase of phospholipid/protein ratio (Fig. 2) and membrane fluidity.
(2) a phase of fluidity equilibrium, with a slight decrease, beginning at the 8th week of treatment and lasting for 20-30 days. This could be caused by a decrease in PC/PE ratio or phospholipid/protein ratio (Fig. 2). An increase in this last ratio during cold acclimation of tubers was reported previously (Ishikawa and Yoshida 1985).
(3) an increase of fluidity, corresponding to the capability of tubers to grow.

Ethanol treatment of Jerusalem artichoke tubers for 24 h is able to break their dormancy and an increase of membrane fluidity was observed at the same time (Candelier *et al.* 1989). This increase could be linked to a decrease in sterol level and is in accordance with the measurements made on the whole tubers (data not shown).

All these results point towards a major and critical reorganization of the plasmalemma at the time of break of dormancy.

An important decrease of PC and PE was noted (Table 1). Choline could be liberated by plasmalemma phospholipid degradation leading to a higher rigidity of plasma membrane. This could also be linked to the increase of mitochondrial membrane fluidity during the dormancy of tubers (Chapman *et al.* 1979), and with an increase of succinate oxidase activation energy (Chapman *et al.* 1979, Hannon and Raison 1979).

It was previously shown that NADH dehydrogenase activity is linked to proton extrusion out of the parenchyma cells (Pétel and Gendraud 1987). Moreover, this NADH-dependent proton translocation across the plasma membrane of plant cells occurs via the plasmalemma ATPase (Belkoura and Marigo 1986, Rubinstein and Stern 1986). The decrease of plasmalemma NADH dehydrogenase activity is so in accordance with the lower ATPase activity found in non-dormant tubers (Pétel and Gendraud 1986), and with our recent results showing that the decrease of ATPase activity is noted after 7 weeks of cold treatment of tubers (Pétel *et al.* 1992). In corn roots, cold stress was characterized by an increase of membrane proteins phosphorylation, provoking a lost of more than 50% of ATPase activity (Hanson *et al.* 1986).

Lower capability of ATPase to extrude protons in dormant tubers could be due to the great increase of phosphoproteins noted in the whole tuber (data not shown). A modification of the enzymatic lipidic environment (as a decrease in phospholipid level, a modification of PC/PE ratio or and increase of sterol level) could also be involved in this phenomena (Xie *et al.* 1986, Yoda and Yoda 1987).

Studying these parameters all along the cold treatment of Jerusalem artichoke, in order to characterize the break of dormancy, has led us to the conclusion that the plasmalemma is heavily reorganized during this period. These changes in membrane

fluidity and morphogenetic potentials of tubers are similar when tubers were treated by ethylene (Candelier 1989). All these results fit well with the hypothesis that new morphogenetic potentialities of tubers are the result of important modifications of short distance intracellular relations. After the break of dormancy, plasmalemma properties of parenchyma cells allow sufficient metabolite flux to the bud, thus enabling its growth.

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