Isolation and characteristics of closed inverted fragments of plant cell plasmalemma

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The aim of the present work was to obtain a membrane fraction consisting practically entirely of inverted vesicles of the plasmalemma isolated from wheat coleoptile (*Triticum aestivum* L., 'Selpek') cells. To this end, treatment of a plasmalemma fraction obtained from a step sucrose gradient by using the detergent *Brij 58* at a concentration of 0.025% was applied. The orientation of the obtained fraction was checked according to the activity of the asymmetric integral enzyme H^+-ATPase. It has been shown that in the presence of *Brij 58* the activity of the enzyme doubles without changing the ATP-permeability of the plasmalemma fragments, since the ATP-dependent proton potential measured with a potential-sensitive fluorescent probe dis-C_3-(5) increased to the same degree. It is concluded that the proposed comparatively simple model system can be used in experiments *in vitro,* when there is a necessity to affect by some factors the cytosol plasmalemma surface.

Key words: plasmalemma, fragments, isolation, orientation

INTRODUCTION

While isolating plasmalemma fragments from plant objects we applied the method based on [1] with differential centrifugation and subsequent purification in sucrose density gradient. The ratio of the quantity of inverted and of normally oriented vesicles under these conditions varies in a rather wide scale (from 1:1 and more rarely up to 3:1) depending on the object and method of isolation [2–4]. In this situation, a strict interpretation of the results of experiments *in vitro* is rather an intricate task. The method for obtaining a fraction consisting of differently oriented plasmalemma vesicles has been well elaborated [4–6]. However, it is much more promising to use a fraction of the plasmalemma whose vesicles are inverted, which is of particular significance while studying the processes initiated *in situ* on the internal (cytosolic) surface of the plasmalemma (energy-dependent transduction of ions, physiologically active substances – phytohormones, ATP-dependent processes, etc.) [7–8]. We think that the importance of such a model system can hardly be overestimated. At the same time there are only single works devoted to this problem [9, 10].

In this relation, the present work is an attempt to elaborate the method of obtaining, from wheat coleoptile, fragments fit to mimic the native cell processes *in situ*, *i.e.* able to generate a transmembrane potential adequate to an intact cell and to carry out an energy-dependent transduction of protons, phytohormones, etc.

MATERIALS AND METHODS

The object of the study was 4-d etiolated wheat (*Triticum aestivum* L., 'Selpek') coleoptiles. The fraction of plasmalemma vesicles from wheat coleoptile cells was obtained by the method of [1, 11–15]. The K^+, Na^+-diffuse and ATP-dependent H^+ potentials were measured according to [4]. Activity of K^+, Mg^{2+}-ATPase was evaluated by the formation of inorganic phosphate whose quantity was assessed according to [16]. IAA transport through the plasmalemma was evaluated depending on ^{14}C-IAA accumulation (sp. act. 29 GBk·g^{-1}) in plasmalemma vesicles [17–19]. Active IAA transport into plasmalemma vesicles was evaluated as a difference between ^{14}C-IAA accumulation in the vesicles in the presence in the incubation medium of 3 mM ATP and dephosphorylation cofactors (50 mM K^+ and 3 mM Mg^{+2}) and accumulation of ^{14}C-IAA in the vesicles in the absence in the incubation medium of ATP and dephosphorylation cofactors. The plasmalemma vesicles in the IAA transport experiments were 'screened' with $5 \cdot 10^{-7}$ M unlabeled IAA to avoid ^{14}C-IAA sorption on membrane surfaces [8]. Protein quantity was measured according to [20]. Data in the paper are presented as arithmetical means of no less than 3 tests and their standard deviations [21].

RESULTS AND DISCUSSION

It has been mentioned above that the orientation of plasmalemma vesicles isolated and purified in sucrose density gradient may vary. In our tests, 50–60% of vesicles were in normal, noninverted position, and thus experiments *in vitro* and all the more interpretation of their results were impeded. To elaborate a model with all or nearly all vesicles inverted, we applied a method [9] for treating the membrane fraction with the detergent *Brij 58* at comparatively low concentrations. We modified the method of these authors and applied *Brij 58* (at concentrations shown in the related tables and figure legends) to plasmalemma fractions during hypoosmotic shock by adding *Brij 58* into a shock solution.

As is shown in Table, *Brij 58*, even at a conc. of 0.025%, increased by 73% the activity of K^+, Mg^{2+}-ATPase. Increasing the concentration up to 0.05% enhanced the effect of *Brij 58*, however, it is evident that this increment of ATPase activity is not reliable, although we cannot exclude a direct effect of the detergent as an activator of ATPase activity, as is noted in some works [4]. We have suggested that in this case, indeed, reorientation of plasmalemma vesicles occurs, based on the facts that, first, the plasmalemma is weakly ATP-permeable, and, second, the active centres of K^+, Mg^{2+}-ATPase are located on the cytosol surface of the plasmalemma and at the normal (as *in situ*) orientation of vesicles are inaccessible for the ATPase reaction substrate. All seems to be clear; however, there is a minute detail able to ruin the whole scheme. Detergents are known for their ability to affect protein-lipid interactions and thus to break the integrity of the membrane. In this case, as we have described earlier [15], ATP and co-factors of ATPase reaction can penetrate into the intravesicular space and to switch on the active ATPase centres located on the internal surface of the normally oriented vesicles.

To deny or to support this version, we carried out an experiment in which, on the background of treating with *Brij 58*, we introduced alameticine, a canalogenic polypeptide forming in the membrane channels permeable for ATP, K^+ and Mg^{2+} [4]. Our results show that K^+, Mg^{2+}-ATPase activity undergoes no reliable changes in the presence of alameticine. Hence follows the consequence that treating the plasmalemma fraction with *Brij 58* results in the formation of inverted plasmalemma vesicles, although accompanied by a certain loss (up to 27%) of protein, *i.e*. probably also the detergent action of *Brij 58* affording a partial solubilization of proteins becomes revealed. Analogous results were obtained also by other authors, *e.g.* [9] on spinach leaf plasmalemma fractions.

Of course, we can neglect the very fact of partial solubilization of protein under the effect of *Brij 58*, all the more as the activity of the membrane-bound labeling enzyme (K^+, Mg^{2+}-ATPase) is not reduced as has been mentioned above; however, some other, maybe more important questions arise: does the membrane integrity withstand such an effect, does it not become more permeable for one-valent cations and does the proton pump of the ATPase nature function on it in an *in vitro* system? It is evident that the proposed model is promising in studying the transplasmalemmal transport of ions in an *in vitro* system, provided the answer to these questions is positive. The integrity of membranes treated with *Brij 58* was checked by the possibility to generate on them the diffusion potential and by the activity of the proton pump [8]. Table presents the results of fluorescence analysis. One can see that *Brij 58* at a concentration of 0.025% and even 0.05% reliably did not reduce the Na^+ diffusion potential, thus testifying to the integrity of the membrane and to the absence of a considerable current of cations escape. As to the effect of *Brij 58* on the function of the proton pump of the ATPase nature, here, on adding ATP, a doubling of the ATP-dependent H^+ potential is noted, – the fact which is in good agreement with the results concerning changes in the ATPase activity of plasmalemma vesicles treated with *Brij 58*. Formation of inverted plasmalemma vesicles from normally oriented ones leads to increased numbers of active ATP centers on the external surface of vesicles and thus to an enhanced transport of protons into the intravesicular space.

The proposed method of working on plasmalemma vesicles inverted with the aid of *Brij 58* was approbated by us on transmembrane ^{14}C-IAA transport. As could be expected, passive (according to the concentration gradient) ^{14}C-IAA transport, under the effect of treating isolated plasmalemma fragments with a detergent, increased only insignificantly – by about 5–10%. An in principle different picture is observed for active ^{14}C-IAA transport, which in the presence of

Table. **Effect of *Brij 58* on the hydrolytic activity of K^+, Mg^{2+}-ATPase and Na^+ diffusion and ATP-dependent H^+ potentials of plasmalemma vesicles from wheat coleoptile cells (% from K^+ diffusion potential)**

Variant	K^+, Mg^{2+}-ATPase activity		Na^+ diffusion potential	ATP-dependent H^+ potential
	μMP_i/mg protein/h	%		
Control	24.9 ± 2.9	100	100 ± 15.5	100 ± 23.1
Brij 58, 0.025%	43.1 ± 2.2	173	120 ± 24.0	195 ± 55.0
Brij 58, 0.05%	52.3 ± 7.4	210	129 ± 15.9	235 ± 44.5

ATP increased from 338.9 cpm/100 μg protein in the initial vesicle suspension to 465.3 in the variant with the *Brij 58* applied, *i.e*. 1.4 times, which is in a rather good agreement with the above data on changes in ATPase activity and ATP-dependent transport of protons (Table).

Thus, the sum total of the obtained results allows to consider the proposed modification of obtaining a fraction of inverted plasmalemma vesicles to be applicable in model experiments, when there is a necessity to affect by some factors the cytosol surface of the plasmalemma under *in vitro* conditions.

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UŽDARŲ INVERTUOTŲ AUGALO LĄSTELĖS PLAZMOLEMOS FRAGMENTŲ IŠSKYRIMAS IR CHARAKTERISTIKA

S a n t r a u k a

Darbo tikslas – išskirti kviečių (*Triticum aestivum* L.) koleoptilių ląstelių plazmolemą, sudarytą praktiškai vien iš invertuotų plazmolemos vezikulių. Tam panaudotas detergentas *Brij 58* (0,025% koncentracija), kuriuo buvo paveikiama laiptiniame sacharozės gradiente gauta plazmolemos frakcija. Gautosios frakcijos vezikulių orientacija patikrinta pagal asimetriško integralinio fermento H^+-ATPazės aktyvumą. Parodyta, kad, panaudojus *Brij 58*, fermento aktyvumas padidėjo dvigubai, ir tai įvyko ne dėl plazmolemos pralaidumo ATP pokyčių, nes tiek pat išaugo ir nuo ATP-priklausomas protoninis potencialas, kuris buvo matuojamas potencialui jautriu fluorescentriniu zondu dis-C_3-(5).

Daroma išvada, kad pasiūlyta palyginti nesudėtinga modelinė sistema gali būti taikoma bandymams *in vitro*, kai vienais ar kitais veiksniais reikia paveikti į citozolį nukreiptą plazmolemos paviršių.