Energy-dependent auxin transport through the plasmalemma: the role of H^+-ATPase

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The aim of the present work has been to determine whether there is a relationship between the functioning of plasmalemma H^+-ATPase and auxin transport through this membrane in the process of its polar transport in plants. Inverted wheat coleoptile cell plasmalemma vesicles were employed for this purpose. Removal of Mg^{2+} from the incubation medium or treatment with the H^+-ATPase inhibitor dicyclohexylcarbodiimide as well as the auxin polar transport inhibitors 2,3,5-triiodobenzoic and N-1-naphthylphthalamic acids *in vitro* reduced the ATP-dependent auxin transport into the vesicles. The conclusion has been made that auxin export from the cell is related to the functioning of plasmalemma H^+-ATPase and that this way of efflux could be an important part of basipetal auxin transport, possibly defining its polarity.

Key words: wheat, plasmalemma, auxin transport, H^+-ATPase

INTRODUCTION

Auxin efflux from the cell, its molecular mechanism being not yet clear, is thought to define the polarity of basipetal phytohormone transport in plants. It proceeds in an active, ATP-dependent manner [1]. H^+-ATPase is one of the best-known enzyme hydrolysing ATP in the plasmalemma [2]. It is possible that it is this enzyme that provides energy for auxin efflux. This kind of mechanism of auxin export could be an important part of its polar transport. However, we failed to find in the literature reports on the relationship between auxin transport through the plasmalemma and the functioning of H^+-ATPase. The aim of the present work was to establish the relationship between the functioning of the plasmalemma H^+-ATPase and auxin transport through this membrane in the process of polar phytohormone transport.

MATERIALS AND METHODS

The object of the study was 4-d-old etiolated wheat (*Triticum aestivum* L., 'Selpek') coleoptiles. The fraction of plasmalemma vesicles was obtained by the method of differential centrifugation and purification on step sucrose gradient [1, 3]. Vesicles were inverted with *Brij 58* [4]. The intensity of IAA transport through the plasmalemma was evaluated depending on ^{14}C-IAA (sp. act. 29 GBq·g^{-1}) accumulation in vesicles [1, 5]. Active, ATP-dependent IAA transport into plasmalemma vesicles was evaluated as a difference between ^{14}C-IAA accumulation in the vesicles with and without 3 mM ATP, 50 mM K^+ and 3 mM Mg^{2+} in the incubation medium. The plasmalemma vesicles were screened with $5 \cdot 10^{-7}$ M unlabelled IAA to avoid ^{14}C-IAA sorption on membrane surface. Protein quantity was determined according to Bradford [6]. Data in the paper are presented as arithmetical means of no less than 3 tests and their standard deviations.

RESULTS AND DISCUSSION

Addition of ATP and the dephosphorylation reaction cofactors K^+ and Mg^{2+} enhanced the intensity of IAA transport through the plasmalemma *in vitro* [1]. Can this enhancement of IAA transport intensity be related to the functioning of plasmalemma H^+-ATPase? To answer this question, we employed the factors that influence the functioning of H^+-ATPase. Since the Mg–ATP complex is known to be a true H^+-ATPase substrate, exclusion of Mg^{2+} from the incubation medium abolishes H^+-ATPase activity. Removal of Mg^{2+} from the incubation medium reduced the intensity of ^{14}C-IAA accumulation in plasmalemma vesicles by 42.9% (Table). However, Mg^{2+} might influence also the functioning of other ATPases and even of hydrolases. To confirm

Table. **The effect of Mg^{2+}, DCCD and DES on the active, ATP-dependent ^{14}C-IAA transport into wheat coleoptile cell plasmalemma vesicles. Incubation temperature with Mg^{2+} +37 °C, with DCCD and DES +10 °C**

Variant	cpm/100 μg protein	%
3 mM ATP, 3 mM Mg^{2+}, 50 mM K^+	47.3 ± 8.9	100
3 mM ATP, 50 mM K^+	27.0 ± 5.7	57.1
3 mM ATP, 3 mM Mg^{2+}, 50 mM K^+	34.2 ± 7.8	100
3 mM ATP, 3 mM Mg^{2+}, 50 mM K^+, 0.1 mM DCCD	23.4 ± 2.8	68.4
3 mM ATP, 3 mM Mg^{2+}, 50 mM K^+, 0.1 mM DES	36.3 ± 4.9	106.1

a relationship between IAA transport through the plasmalemma and the functioning of H^+-ATPase, the specific H^+-ATPase inhibitors, dicyclohexylcarbodiimide (DCCD) and diethylstilbestrol (DES), were employed. Neither of them at 0.1 mM concentration, which inhibits H^+-ATPase activity about 50%, was found to exert any effect on ATP-dependent IAA transport at a temperature of the incubation medium +37 °C (data not shown). A possible explanation can be the sufficiency of the residual H^+-ATPase activity for providing energy for auxin transport. An attempt was made to reduce H^+-ATPase activity by lowering the temperature of the incubation medium to +10 °C. In these conditions the effect of the inhibition by DCCD became manifested, and it suppressed the active, ATP-dependent transport of auxin by 31.6%. The other inhibitor, DES, in these conditions did not exert any effect on the active transport of auxin, either (Table). Since this work has been carried out on inverted vesicles, the ATP-dependent import of IAA into the vesicles related to the functioning of H^+-ATPase mimicks the process of auxin export from the cell. Thus, we can conclude that auxin can be exported from the cell in an active way with the aid of the energy of ATP hydrolysis by plasmalemma H^+-ATPase.

What then is the role of this mechanism of IAA export from the cell in the process of auxin polar transport? To find the answer to this question, we employed the widely known auxin polar transport inhibitors, 2,3,5-triiodobenzoic (TIBA) and 1-N-naphthylphthalamic (NPA) acids, which suppress the polar transport of auxin *in vivo* [7] and influence the transport of auxin through the membrane *in vitro* [5]. Both inhibitors exhibited a reducing effect on ^{14}C-IAA accumulation in the vesicles when ATP was present in the incubation medium (Figure). As they are known to exert no effect on IAA diffusion through the plasmalemma [8], the influence on the transport related to the effect of ATP, probably related with the function of H^+-ATPase, is the best explanation. Thus, we can conclude that active, ATP-dependent IAA transport through the plasmalemma is part of auxin polar transport in plants.

Summarizing, we can state that H^+-ATPase is one of the energy sources for auxin transport through the plasmalemma in the process of its efflux from the cell. This mechanism of auxin export is an important part of auxin basipetal transport, which possibly defines its polarity.

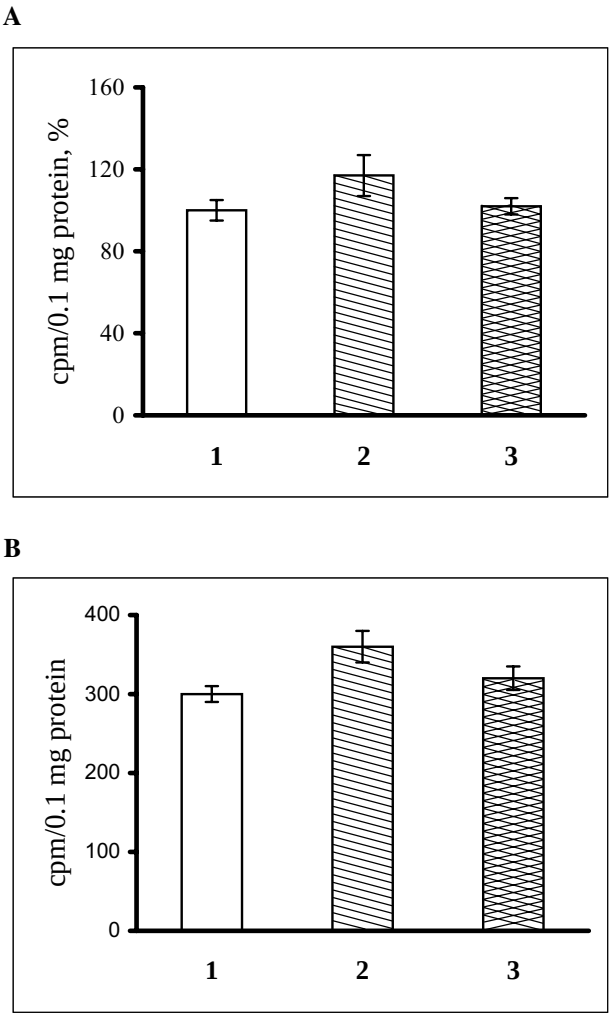

Figure. The *in vitro* effect of NPA and TIBA on ^{14}C-IAA transport into wheat coleoptile cell plasmalemma vesicles. Composition of incubation medium: 1 – no additives; 2 – 3 mM ATP, 50 mM K^+, 3 mM Mg^{2+}; 3 – 3 mM ATP, 50 mM K^+, 3 mM Mg^{2+}, $5 \cdot 10^{-6}$ M NPA (A) or $5 \cdot 10^{-6}$ M TIBA(B)

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ENERGETIŠKAI PRIKLAUSOMAS AUKSINO TRANSPORTAS PER PLAZMOLEMĄ: H^+-ATPazės VAIDMUO

S a n t r a u k a

Šio darbo tikslas – nustatyti, ar yra ryšys tarp plazmolemos H^+-ATPazės veiklos ir auksino pernešimo per šią membraną poliarinio fitohormono transporto metu.

Buvo panaudotos invertuotos plazmolemos vezikulės, išskirtos iš kviečių koleoptilių ląstelių. Pašalinus Mg^{2+} iš inkubavimo terpės arba paveikus plazmolemos vezikules H^+-ATPazės veiklos inhibitoriumi – dicikloheksilkarbodiimidu, poliarinio auksino transporto inhibitoriais – 2,3,5-trijodbenzoine rūgštimi arba N-1-naftilftalamine rūgštimi, nuo ATP priklausomas auksino pernešimas į vezikules sumažėjo.

Taigi buvo padaryta išvada, kad auksino pašalinimas iš ląstelės per plazmolemą yra susijęs su H^+-ATPazės veikla, o toks auksino pernešimo būdas yra svarbi poliarinio auksino transporto sudedamoji dalis.

Raktažodžiai: kvietys, plazmolema, auksino transportas, H^+-ATPazė