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ATPase, ADPase and p-nitrophenylphosphatase activities in microsomal preparations from various plant materials. I. Effects of the presence of magnesium in the homogenization medium

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Biochimica vegetale. — *ATPase, ADPase and* p-*nitrophenylphosphatase activities in microsomal preparations from various plant materials.* I. *Effects of the presence of magnesium in the homogenization medium* (*). Nota di PIERANTONIO PESCI e NICOLETTA BEFFAGNA, presentata (**) dal Corrisp. E. MARRÈ.

RIASSUNTO. — I - Con un mezzo di omogenizzazione contenente Mg^{2+} 10 mM si ottengono frazioni microsomiali, sia da fusto che da radice, che in genere sono meno ricche di proteine rispetto a quelle preparate con un mezzo privo del catione. In fusti di orzo, cece, lenticchia e ravanello le proteine microsomiali subiscono una diminuzione sino del 60%; per contro l'attività ATPasica, riferita a grammo di peso fresco iniziale, è fortemente aumentata dalla presenza del magnesio nel mezzo di omogenizzazione nelle preparazioni provenienti da fava e da pisello. L'attività specifica risulta pure essere incrementata nelle leguminose in genere, mentre nelle altre specie esaminate (dicotiledoni non leguminose e graminacee) subisce diminuzioni dal 20% ad oltre il 50%.

II - La sensibilità alla presenza del magnesio nel saggio utilizzato per la misura dell'attività ATPasica microsomiale è molto elevata in fava, pisello e nelle radici di cece, mentre risulta molto ridotta per gli altri materiali considerati.

INTRODUCTION

In recent years considerable attention has centered on the presence and on the nature of electrogenic, energy-dependent proton pumps in higher plants. Consistent evidence indicates that in fungi (*Neurospora crassa*, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) ATPases localized at the plasmalemma provide the energy for the uphill transport of protons [1, 2].

An involvement of plasmalemma ATPases in active H^+ transport in higher plants is strongly suggested by the finding that: *i*) - H^+ extrusion " in vivo " is influenced by treatments lowering the intracellular ATP level as well as by membrane (non mitochondrial) ATPase inhibitors; *ii*) - plasmalemma-enriched membrane preparations from a number of higher plants contain a relevant ATPase activity; *iii*) - these preparations, rich in " sealed " membrane vesicles, are able to carry out ATP-dependent electrogenic proton transport [3, 4, 5, 6].

Further development in this field requires the identification and the characterization of plasmalemma ATPases of higher plants: a difficult task in view

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of the difficulty of separating plasmalemma from other organelles. In fact, the plant cell membrane preparations as yet available are heavily contaminated by fragments or vesicles from tonoplast, endoplasmic reticulum, Golgi apparatus and, probably, mitochondrial or plastidial outer membranes. The study of plasmalemma ATPase might be facilitated by the identification of the most suitable plant materials. Moving from this consideration we thought it useful to carry out a preliminary survey of the ATPase, ADPase and *p*-nitrophenylphosphatase activities detectable in the so called " microsomes " (fraction sedimenting between 13000 and 80000 × g), prepared in the presence or not of magnesium in the grinding medium, from several plant materials. The rational of preparing the microsomal fractions in these two different conditions and of considering the activity against these substrates is that: *i*) – ATP seems the specific substrate for fungi plasmalemma ATPase [2]; *ii*) – enzymes hydrolyzing both ATP and ADP have been extensively purified from membrane preparations from at least 3 plant stems [7, 8, 9]; *iii*) – the level of ATP-ADPase present in pea stem microsomal preparations and its specific activity is remarkably influenced by the Mg^{2+} concentration in the grinding medium [10]; moreover, Mg^{2+} seems important in maintaining the original characteristics of the membrane ATPase [11, 12]; *iiii*) – a largely aspecific acid phosphomonoesterase attacking *p*-nitrophenylphosphate and also nucleoside phosphates is usually present in plant microsomal preparations, and its localization at the tonoplast has been suggested [13]. It seemed therefore that some information on the relative activities of crude microsomes from several plant materials towards these three substrates, and on the possible effects of Mg^{2+} on the amount of these activities in microsomal preparations might be a good premise for the further development of studies on plasmalemma ATPases.

This first note deals with the results about protein and ATPase activity yields of microsomal preparations obtained, from 11 species, in the presence or not of 10 mM Mg^{2+} in the grinding medium. ATPase activity was also studied in relation to its dependence upon the presence of Mg^{2+} in the enzyme assay medium.

A further characterization of the ATPase activity (sensitivity to dicyclohexycarbodiimide and to diethylstilbestrol, effect of some solubilizing treatments) is reported in a second note, where the ADPase and the acid phospatase activity of these microsomal preparations are discussed.

MATERIALS AND METHODS

The screening was performed on 11 different plant materials, namely, 6 leguminous species: horse bean (*Vicia faba*), pea (*Pisum sativum*, cv. Alaska), chickpea (*Cicer arietinum*), lentil (*Lens esculenta*), kidney bean (*Phaseolus vulgaris*, var. Borlotto), Azuki bean (*Vigna angularis*), and other 5 species: zucchini squash (*Cucurbita pepo*), radish (*Raphanus sativus*), barley (*Hordeum vulgare*), oat (*Avena sativa*) and corn (*Zea mays*, cv. Dekalb XL 640).

The seeds of these species, after a 20 min washing in running water, were germinated on three layers of filter paper, previously washed and kept imbued with a 0.5 mM $CaSO_4$ solution, and the seedlings were grown at 26 °C in the dark for 5 to 8 days. Stem subapical internode segments, approx. 1 cm long, or shoots were harvested at 4 °C for 10 to 30 min; in barley and in oat the coleoptile was included. Root segments, approx. 2 cm long, were harvested after removing the apical portion (ca. 2 mm long). The plant material was immediately homogenized after repeated washings in cold distilled water.

Preparation of the microsomal fractions.

The excised stem, shoot or root segments were ground (mortar and pestle) at 4 °C in a medium (5 ml/g of tissue) consisting of 250 mM sucrose and 20 mM Hepes (pH 7) with or without the addition of 10 mM $MgCl_2$. The homogenate was filtered through three layers of cheesecloth and the suspension was centrifuged at 80000×g for 18 min, after a previous run at 13000×g for 15 min, to give the " microsomal " fraction. This fraction was re-suspended by using a teflon pestle in a medium containing 250 mM sucrose and 5 mM Hepes (pH 7) in the proportions of 30 ml buffer/g initial fresh weight. The whole extraction was performed at 4 °C.

ATPase activity assay.

The incubation mixture for the assay of the ATPase activity was routinely composed of 100 μl of extract, 10 μmol KCl, 1 μmol $MgCl_2$ and 1 μmol ATP (sodium salt) in a final 1 ml volume of 250 mM sucrose, 10 mM Hepes buffer (pH 6). The microsomal preparations employed for the enzyme test were always diluted with appropriate amounts of the same re-suspension buffer so to maintain the values of substrate hydrolysis lower than 15–20 %. The reaction was started by the addition of the substrate and was carried out for 30 min at 28 °C. The released phosphate was evaluated by the colorimetric procedure of Tausski and Shorr, partially modified as previously described [10].

Protein assay.

Protein was determined by the colorimetric Bio-Rad assay technique [14] with crystalline bovine serum albumine as a standard.

Results

I – *Protein content of microsomal fractions.*

The data of Table I show a large variability in the protein content of microsomal fractions from different species, while only negligible differences in the protein content are detectable if we compare the microsomal fractions from roots and from shoots within the same species.

TABLE I

Protein contents of the microsomal fractions obtained in the absence or in the presence of Mg^{2+} in the homogenization medium (data are the average of three separate experiments run in triplicate).

	Root $\mu g \cdot g^{-1}$ fresh wt.			Shoot $\mu g \cdot g^{-1}$ fresh wt.		
	$-Mg^{2+}$	$+Mg^{2+}$	Δ%	$-Mg^{2+}$	$+Mg^{2+}$	Δ %
Leguminous dicotyledons:						
Horse bean	476	326	— 31	310	300	— 3
Pea	760	760	0	750	540	— 28
Chickpea	1080	800	— 26	1320	460	— 65
Lentil	750	700	— 7	680	410	— 40
Kidney bean	1020	618	— 39	—	—	—
Azuki bean	—	—	—	960	640	— 33
Other dicotyledons						
Zucchini squash	460	490	+ 6	—	—	—
Radish	100	96	— 3	90	45	— 50
Monocotyledons						
Barley	380	270	— 29	420	140	— 66
Oat	540	460	— 15	1380	820	— 40
Corn	350	210	— 40	—	—	—

" $+Mg^{2+}$ " and " $-Mg^{2+}$ " indicate the presence of 10 mM Mg^{2+} or, respectively the absence of it in the grinding medium; Δ % is the difference between the two protein contents referred to the " $-Mg^{2+}$ " values taken equal to 100.

The presence of 10 mM Mg^{2+} in the grinding medium usually leads to a decrease in the microsomal protein content of both shoots and roots, the greatest differences being observed in shoots of barley, chickpea, radish, lentil and oat.

II – *Effect of Mg^{2+} in the homogenization medium on the ATPase activity (at pH 6) of the microsomal fraction.*

Table II reports the effects of the presence of 10 mM Mg^{2+} in the homogenization medium on the ATPase activity (assayed at pH 6 in the presence of 1 mM ATP.Mg^{2+}) in the microsomal fractions from the various materials

TABLE II

ATPase activity in microsomal fractions obtained with or withouth Mg^{2+} in the homogenization medium. Experimental conditions as described in Methods; data are the average of three separate experiments run in triplicate.

	Root				Shoot			
	$-Mg^{2+}$		$+Mg^{2+}$		$-Mg^{2+}$		$+Mg^{2+}$	
	total*	spec.**	total	spec.	total	spec.	total	spec.
Horse bean	21600	45.4	78000	239.2	37058	119.4	235484	784.0
Pea	9848	13.0	52352	68.0	53470	71.2	144132	266.8
Chickpea	28994	26.8	24386	30.4	18314	13.8	8332	18.0
Lentil	2580	3.4	2950	4.2	2366	3.4	3446	8.4
Kidney bean	14072	13.8	12660	20.4	—	—	—	—
Azuki bean	—	—	—	—	2948	3.1	2380	3.7
Zucchini squash	9076	19.6	7500	15.2	—	—	—	—
Radish	1714	17.0	726	7.6	792	8.6	344	7.6
Barley	110402	290.0	49676	184.0	53656	127.6	6960	49.6
Oat	770	1.4	380	0.8	3502	2.4	1078	1.2
Corn	4090	11.6	2040	9.6	—	—	—	—

* Total ATPase activity expressed as mμmol $Pi \cdot h^{-1} \cdot g^{-1}$ fresh wt.

** Specific ATPase activity expressed as mμmol $Pi \cdot h^{-1} \cdot \mu g^{-1}$ protein.

"$+Mg^{2+}$" and "$-Mg^{2+}$" indicate the presence or not of 10 mM Mg^{2+} in the grinding medium.

investigated. The data show that both the total ATPase activity per g initial fresh weight recovered in this fraction and the specific ATPase activity per μg microsomal protein are heavily influenced by Mg^{2+} in the homogenization medium, in a way which is not correlated with the effects of Mg^{2+} on the protein contents in the same fractions. It is seen in fact, that the presence of Mg^{2+} in the homogenization medium: a) – strongly increases the total (per g initial fresh weight) ATPase activity in horse bean and pea root and stem tissues, only minor changes being induced in all of the other materials; b) – markedly increases the specific ATPase activity (per μg microsomal protein) in all of the 10 materials from the 6 leguminous plants, while it decreases it (from 20 to over 50%) in the non leguminous dicotyledons and in the monocotyledons (5 materials).

III – *Mg^{2+}-dependence of the ATPase activity.*

The data of Figure 1 show that the presence of Mg^{2+} in the assay medium induces a significant increase of ATPase activity only in the leguminous horse bean, pea and chickpea. On the other hand, in all of the materials here considered, the presence or the absence of Mg^{2+} during homogenization has only

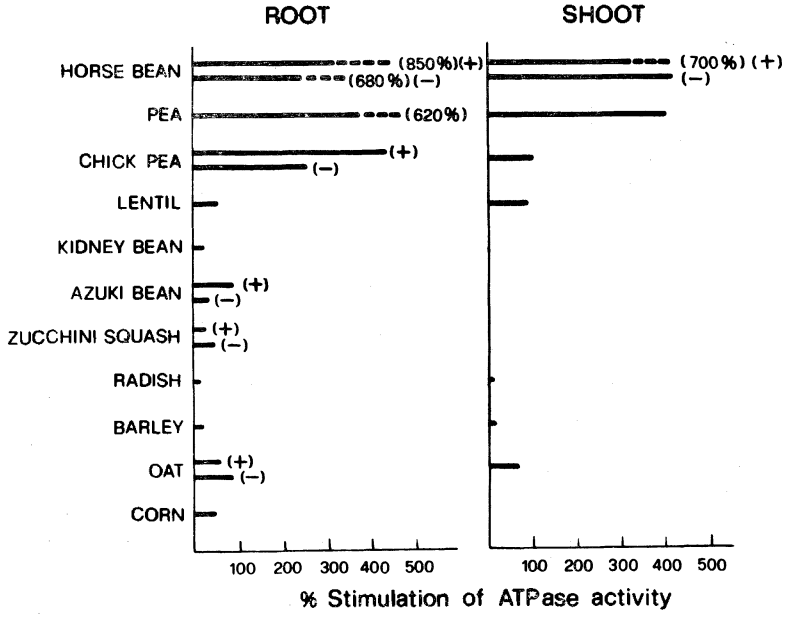

Fig. 1. – Effects of the presence of Mg^{2+} in the assay mixture on the ATPase activity. (+) and (—) indicate the presence of 10 mM Mg^{2+} or, respectively the absence of it in the grinding medium; the lack of any sign indicates that the values are almost equal in the two conditions.

little influence on the effect of this ion in the ATPase assay. These data on the Mg^{2+}-dependence of the ATPase activity must be taken with caution, as some Mg^{2+} is present in the crude microsomal preparations rich in partially sealed membrane vesicles (free Mg^{2+} concentration in plant tissue is of the order of

ca. 5×10^{-3} M). In fact, washing the microsomes strongly increases the Mg^{2+} requirement in some (but not all) of the materials here investigated (data not shown).

Conclusions

The results reported in this paper can be summarized as follows:

I – The presence of 10 mM Mg^{2+} in the grinding medium induces, in both shoots and roots preparations, a change in the protein content of the microsomal fractions which results lowered (up to 60% in shoots of barley, chickpea, lentil and radish) as compared with that of the microsomal preparations obtained in the absence of the cation.

II – The amount of ATPase activity recovered (on an initial tissue fresh weight basis) in horse bean and pea microsomal preparations is, on the contrary, strongly increased by the presence of the cation in the homogenization medium; moreover, the specific ATPase activity of the 6 leguminous plants investigated is enhanced in this condition, whereas in the case of the other 5 species a decrease in the specific ATPase activity is observed.

III – The stimulating effect due to the presence of magnesium in the ATPase assay medium is very high for the microsomal preparations from horse bean and pea, and from roots of chickpea, while no significant increase is induced by the presence of the cation in the other investigated materials.

Other information on the characteristics of the ATPase, and of the ADPase and *p*-nitrophenylphosphatase activities of the microsomal preparations from the materials here investigated will be presented in a second note.

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