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The effect of calcium to magnesium ratio on adenosine triphosphatases from wheat roots

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Abstract:

The activities of ATPase isolated from 4 days old wheat roots grown on distilled water were found to be associated with the ratio of Ca : Mg ions added in different proportions to the incubation medium. This relationship was stated in the crude cell wall free homogenate (fraction I) as well as subcellular fraction II and fraction III under investigations. It is stated moreover that activity of ATPases extracted from wheat roots grown on deficient nutrient solution with different Ca : Mg ratio is dependend on exogenic ratio of these cations. These results support the concept that activity of plant ATPases depends not only on mineral nutrition and concentrations of Ca^{2+} and Mg^{2+} ions but also on the Ca : Mg ratio in soil or in nutrient solution and in separate subcellular fractions of plant cells.

INTRODUCTION

It is well known that many plant ATPases are ion sensitive (Brown et al. 1965, Greuner and Neumann 1966, Fisher and Hodges 1969). Hanson and Kylin (1969) and Kylin and Gee (1970) have stated that ATPases from sugar beet roots and *Avicennia nitida* leaves showed a specific ($Na^+ + K^+$) requirement for optimal activity. However Fisher and Hodges (1969) have found that monovalent cations showed only a unspecific stimulatory effect on ATPases prepared from oat roots and were ineffective in the absence of the divalent cations such as Mg^{2+} or Mn^{2+}. A number of investigations have stated that the activity of plant ATPases may be stimulated either by Mg^{2+} (Greuner and Neumann 1966, Fisher and Hodges 1969, Hall 1971) or Ca^{2+} ions (Hall and Butt 1969, Sexton and Sutcliffe 1969) or by both divalent cations (Kylin and Kahr 1973).

Although the effect of Ca^{2+} and Mg^{2+} ions on the activity of many plant phosphatases has been stated in quite a number of plant species, there are however, few data (Kylin and Kahr 1973) on the activity of those enzymes within a wide range of divalent cations ratios. This problem seems to be of importance since both cations may occur in plant tissues in different proportions depending on the ratio of Ca^{2+} and Mg^{2+} ions in soil or nutrient solution (Buczek and Leonowicz-Babiak 1971).

The present study was undertaken in order to examine the effect of Ca^{2+} and Mg^{2+} ions and of Ca : Mg ratio on the ATPase activity in particular subcellular fractions isolated from wheat roots, and the effect of exogenic Ca : Mg ratio in nutrient solution on phosphatase activity in wheat roots.

MATERIAL AND METHODS

Plant cultures. Wheat (*Triticum vulgare*, var. *Ostka popularna*) seedlings after germination on filter paper were placed on cheesecloth stretched over glass crystallisers filled either with distilled water (dark cultures) or with deficient nutrient solutions (light cultures) at constant light of 10.000 lx. The seedlings were grown at 25° with gentle aeration of solutions. The deficient nutrient solution contained either 4 mM KCl + 1 mM $CaCl_2$ or 4 mM KCl + 1 mM $MgCl_2$, or 4 mM KCl wit Ca^{2+} and Mg^{2+} ions given at different proportions. The roots of seedlings 4 days old were used in all experiments.

Preparation of enzyme extracts. Enzyme extracts were prepared by grinding the roots, washed previously in cold deionized water, in cold 0.05 M Tris-maleate buffer at pH 6.0 and 0.25 M sucrose. For one gram of tissue 10 ml of medium was used. The debris was sqeezed through four layers of cheesecloth and, subsequently subjected to successive centrifugation at 1.500 g for 10 min. (fraction I — cell wall free homogenate) and 18.000 g for 15 min. (fraction II). Supernatant, from the last centrifugation was referred to as the fraction III. The pellet from fraction II centrifugation was washed in the extraction medium, resedimented, and, finally suspended in fresh extraction medium. All described above preparations were carried at 0°—4°.

Analytical methods. The mesurements of ATPase activity were carried out in a total 4 ml volume. The incubation medium (3 ml) consisted of 0.1 M tris-maleate buffer at pH 6.0, mM KCl, 3 mM of ATP, 0.2—0.4 ml of preparation from given fraction and various concentrations of tested ions. Incubation was carried out at 37° for 30 min. The reaction was stopped by addition of 1 ml of cold trichloroacetic acid (10% of final concentration) and cooling the tubes in ice bath. The proteins were re-

moved by centrifugation and inorganic phosphate (P_i) released from ATP was determined colorimetrically (Fiske and Subbarow 1925). The ATPase activity was expressed in μmoles of inorganic phosphate liberated per mg of protein per hour. Soluble protein was determined according to Lowry at al. (1951). All figures given are the means of three samples
Source of reagents. ATP, ADP, AMP (5′), GTP (as disodium salt) and p-NPP (disodium salt) were obtained from Sigma. Tris and bovine albumin from Serva.
Abbreviations: ATPase — adenosine triphosphatase; p-NPP — p-nitrophenyl phosphate.

RESULTS

Preliminary investigations concerned the activity of ATPase at different pH intervals, measured in extracts obtained from the roots of 4 day old dark grown seedlings. The pH optimum for the cell wall free homogenate (fraction I) ATPase was close 6.0 (Fig. 1), although the activity of this enzyme was relatively high at the pH ranging from 5.0 to 7.0 The addition of Ca^{2+} or Mg^{2+} ions given to reaction medium in optimal concentrations did not change the optimum pH; stimulating effect of both ions being only observed. Similar results were also found with the fraction II (Fig. 2) and fraction III preparations.

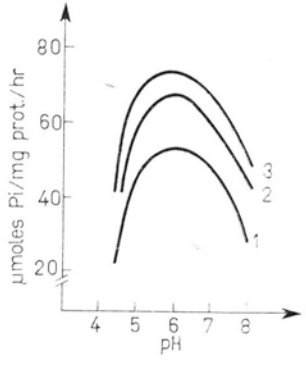

Fig. 1. Effect of pH on the ATPase activity of fraction I

The incubation medium contained 3 mM ATP, 100 mM Tris-maleate, 25 mM KCl and when added 2 mM $CaCl_2$ or 2 mM $MgCl_2$
1 — KCl; 2 — KCl+$MgCl_2$; 3 — KCl+$CaCl_2$

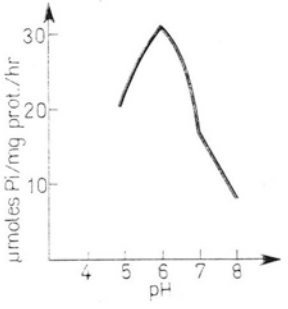

Fig. 2. Effect of pH on the ATPase activity of fraction II (sediment after 18000 g centrifugation)

The incubation medium contained 100 mM Tris-maleate, 25 mM KCl and 3 mM ATP

The optimum Ca^{2+} and Mg^{2+} concentration for the fraction I ATPase in the presence of 25 mM KCl was about 2 mM (Fig. 3). The obtained results are showing, however, that fraction I ATPase preparation was distinctly stimulated by Ca^{2+} ions. This stimulation was about 29% higher

than in control, reaching its optimum for $CaCl_2$ concentration ranging within 2 — 10 mM. The Mg^{2+} ions however, given in low concentrations have a slight stimulating effect for the ATPase activity and in high concentrations inhibited the activity of ATPase from this fraction. Tabl. 1 shows that for fraction II ATPase, Ca^{2+} and Mg^{2+} ions have similar effect as for fraction I. The action of divalent cations for fraction III ATPase

Table 1

Effect of various ions on the ATPase activity of the fraction II and fraction III preparations

Substance	ATPase activity µmoles P (mg prot./hr	
	fraction II	fraction III
No addition	16.62	67.58
KCl	20.38	55.79
NaCl	19.82	—
$CaCl_2$	30.85	86.02
$MgCl_2$	24.64	112.13
NaF	14.95	38.40
KCl+NaCl	21.02	—
NCl+$CaCl_2$	57.68	89.09
KCl+$MgCl_2$	34.49	119.29

The concentrations of various additives were: 3 mM ATP, 25 mM KCl, 25 mM NaCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM NaF, and 100 mM Tris-maleate, pH 6.0.

are opposite: The action of Mg^{2+} ions are mor effective than Ca^{2+} ions. The influence of K^+ ions given alone, have slight effect, however, potassium ions given with Ca^{2+} or Mg^{2+} thogether into incubation medium

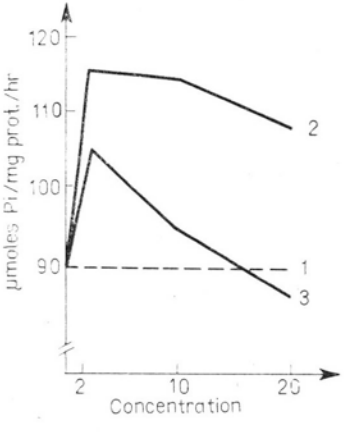

Fig. 3. Effect of divalent cations on fraction I ATPase activity

The incubation medium contained 3 mM ATP, 100 mM Tris-maleate at pH 6.0, 25 mM KCl and various concentrations of $CaCl_2$ and $MgCl_2$
1 — KCl; 2 — KCl+$CaCl_2$; 3 — KCl+$MgCl_2$

increased action of divalent cations on ATPase activity of fraction II, and had slight effect on fraction III ATPase system. Fluoride ions inhibited very weakly fraction II ATPase and relatively greatly fraction III ATPase.

At pH 6.0 in the presence of 3 mM various substrates, fraction II preparations hydrolysed a number of phosphate esters (Tab. 2). The enzyme activity towards ATP was taken as 100 per cent. The highets activity being observed with ATP, GTP ADP and AMP. Para-nitrophenyl phosphate and pyrophosphate were hydrolysed much less intensively, whereas glucose-6-phosphate and fructose-1,6-diphosphate proved just quite resistant to the action of enzyme.

Table 2

Substrate speceficity of fraction II ATPase in the presence of 2 mM Ca^{2+} or Mg^{2+} ions

Substrate	Ca^{2+}	Mg^{2+}
	Relative activity %	
ATP	100	100
GTP	85	124
ADP	85	99
AMP (5′)	47	57
p-NPP	21	30
glucose-6-phosphate	3	4
fructose-1,6-phosphate	0	11
sodium pyrophosphate	19	33

The incubation medium contained 3 mM substrates (sodium salt) and 100 mM Tris-maleate (pH 6.0). Enzyme activity in the presence of various substrates expressed as per cent of activity towards ATP.

The effect of Ca : Mg ratio in the reaction system on ATPase activity in the extracts of 4 days old wheat roots grown in distilled water was investigated using optimal concentrations of Ca^{2+} and Mg^{2+} ions (2 mM) given at different proportions. The ATPase activity was strongly stimulated by Ca^{2+} ions in fraction I and fraction II preparations (Fig. 4).

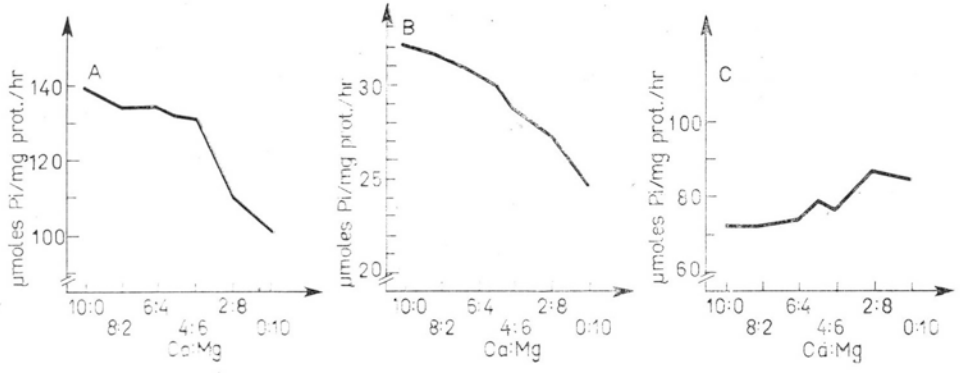

Fig. 4. ATPase activity of fraction I (A), fraction II (B) and fraction III (C) at different Ca : Mg ratios

Incubation medium contained 3 mM ATP, 100 mM tris-maleate (pH 6.0), 25 mM KCl and 2 mM of both divalent cations given at different proportions.

The addition of Mg^{2+} ions to the incubation medium with a simultaneous decrease in the Ca^{2+} concentrations brought about a gradual and continuous decrease in fraction I and fraction II ATPase activity. It seems that the Ca : Mg ratio equal to 4 : 6 is for these subcellular fractions a critical one, since further increase in concentration of Mg^{2+} ions results in a sudden decrease in the enzyme activity in fraction I, or decrease decesively ATPase activity in fraction II preparations. On the ather hand, a peak of ATPase activity may be distinguished for fraction III at Ca : Mg ratio equal to 2 : 8 (Fig. 4), what may indicate the dependence of fraction III ATPase system on the ratio of both divalent cations in the medium.

Table 3

Effect of divalent cations on ATPase activity of wheat roots grown at deficient nutrient solutions containing either $K^+ + Ca^{2+}$ or $K^+ + Mg^{2+}$ ions or both divalent cations at different proportions

Growth medium	Ca:Mg	ATPase activity µmoles P /mg prot./hr	
		fraction II	fraction III
$KCl + CaCl_2$	—	39.0*	65.8
$KCl + MgCl_2$	—	31.1	69.6
$KCl + CaCl_2 + MgCl_2$	10:1	38.9*	87.8
$KCl + CaCl_2 + MgCl_2$	1:10	30.4	101.8

* Differences aignificant.

Incubation medium include only 100 mM Tris-maleate at pH 6.0 and 3 mM ATP. The deficient nutrien solution contained either 4 mM KCl+1 mM $CaCl_2$ or 4 mM KCl+1 mM $MgCl_2$ or 4 mM KCl+1 mM $CaCl_2$+0.1 mM $MgCl_2$, and or 4 mM KCl+0.1 mM $CaCl_2$+1 mM $MgCl_2$ respectively.

The next steep was to see how the ATPase activity of fraction II and fraction III, depends on the different proportions of investigated divalent cations in nutrient solution. The deficient nutrient solution contained 4 m M KCl supplemented either with 1 mM $CaCl_2$ or 1 mM $MgCl_2$, or both divalent cations at different ratios. The obtained fraction II and fraction III preparations were tested toward ATP and the reaction medium was not supplemented with calcium or magnesium ions. From the data presented in Tabl. 3, it may be read that calcium ions added alone to the growth medium induce the fraction II ATPase activity in comparison to the conditions with magnesium ions. Results obtained for supernatant (fraction III) are not so distinct, although a stimulating tendency of magnesium may be observed. The data show also that the fraction II ATPase activity is higher in combination of Ca : Mg = 10 : 1 than for the reverse ratio of Ca : Mg ions (1 : 10) in nutrient solution, while in fraction III a certain increase in ATPase activity have been observed, for the roots from growth solution at Ca : Mg ratio equal to 1 : 10.

DISCUSSION

The results presented above show some characteristic properties of wheat roots ATPases. They concern the influence exerted by the egzogenic ratio of Ca : Mg ions in growth solution or in incubation medium on ATPase activity of subcellular fractions prepared from wheat roots.

It is stated that addition of Ca^{2+} or Mg^{2+}ions into incubation medium containing extracts of enzymes, caused astimulating effect on ATPase activity from fraction I and fraction II, and in weaked degree in fraction III. These results show that ATPases prepared from wheat roots exhibit a greater specificity for Ca^{2+} than for Mg^{2+} ions. The above hypothesis have been confirmed by our experiments cunducted on roots of wheat seedlings grown in solutions containor or KCl with $CaCl_2$ or with $MgCl_2$ (Tabl. 3). The fraction II prepared from these roots, exhibit ATPase activity that was activated by calcium ions presented in nutrient solution in relation to combination with magnesium ions.

The above results are not in agreement with experiments of Fisher and Hodges (1969) and Leonard and Hodges (1973). The quoted authors have stated that membrane-associated ATPases isolated from oat roots was activated by Mg^{2+} ions, whereas Ca^{2+} was ineffective in replacing Mg^{2+} for activation of plasma membrane ATPases. However, the experiments of Kylin and Kahr (1973) conducted with wheat roots and the early findings of Hall and Butt (1969) and Hall (1971) concerned the extracts from barley roots, have stated that subcellular fractions from these species contain more Ca^{2+} than Mg^{2+} activated ATPases.

The differences in properties of the ATPases prepared from wheat and oat roots imply that each species must be considered separately and perhaps these differences are results of ecological adaptation of species. The wheat, like barley is more dependend on calcium in soil, whereas oat has a high need for magnesium (Goralski 1965). The above properties of wheat ATPases can be further confirmed by ours experiments which shows that activity of all investigated fractions depend on Ca : Mg ratio in incubation mixture.

The sudden break in the activity of ATPase in fraction I and fraction II occurring at Ca : Mg ratio equal 4 : 6 (Fig. 4) suggests that the of ATPase activity depends on the ratio of the both divalent ions. Similar relationship has been stated by Kylin and Kahr (1973) for the fraction of microsomes isolated from wheat roots. Bearing in mind that Ca^{2+} and Mg^{2+} ions are always present in plant tissues and that their endogenic concentrations are controlled by egsogenic ratio of these ions either in nutrient solutions or in soil (Buczek and Leonowicz-Babiak 1971), it should be expected that the activity of ATPases may be controlled by the endogenic Ca : Mg ratio.

The above hypothesis have been confirmed by our experiments on the activity of ATPases in subcellular fractions isolated from the roots of wheat seedlings grown in solution containing calcium and magnesium ions given in different proportions. The dominance of Ca^{2+} ions (Ca : Mg = 10 : 1) in nutrient solution increased the ATPase activity in fraction II and decreased it in fraction III, analogically to the experiments *in vitro*, whereas the reverse Ca : Mg ratio (1 : 10) decreased the enzyme activity in the fraction II and slightly increased the ATPase activity in supernatant fraction (fraction III).

The Ca^{2+} and Mg^{2+} ions concentrations in plant tissues may assume different proportions, depending upon the species of plant (Madhok and Walker 1969), on separate organs of plants (Buczek and Leonowicz-Babiak 1971) as well as on pH, exogenic ratio of those ions and the presence of other elements in soil Mass and al. 1969, Buczek and Leonowicz-Babiak 1971, Mas and Ogata 1971). At a certain critical Ca : Mg ratio in the medium one observes the changes in endogenic proportions of those ions in cells due to differences in the accumulation of both ions. Our previous investigations (Buczek and Leonowicz-Babiak) have shown that this fact affects many metabolic processes. Present investigations have shown that the activity of plant ATPases which are widely spread within plant tissues, depend on actual endogenic Ca : Mg ratio in cells and separate subcellular fractions.

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Wpływ stosunku wapnia do magnezu na aktywność adenozyno-trójfosfatazy z korzeni pszenicy

Streszczenie

Doświadczenia przeprowadzono na 4-ro dniowych korzeniach pszenicy odmiany 'Ostka popularna', wyhodowanych w ciemności w wodzie destylowanej względnie na świetle w niekompletnej pożywce mineralnej zróżnicowanej pod względem stosunku Ca : Mg. Korzenie homogenizowano rozdzielając homogenat na trzy frakcje z czego frakcja I odpowiadała surowemu homogenatowi pozbawionemu ścian komórkowych, frakcja II odpowiadała osadowi po odwirowaniu przy 18000 g a supernatant z tego wirowania określano jako frakcję III.

Stwierdzono, że aktywność ATPazy wszystkich trzech frakcji wyodrębnionych z 4-ro dniowych korzeni wyrosłych na wodzie destylowanej wykazywała zależność od obecności jonów Ca^{2+} i Mg^{2+} w roztworze inkubacyjnym, przy czym aktywność enzymu we frakcji I i II była bardziej specyficzna w stosunku do Ca^{2+} niż Mg^{2+}, natomiast jony Mg^{2+} okazały się bardziej aktywne w stosunku do ATPazy frakcji III. Wszystkie badane frakcje wykazywały również zależność od stosunku Ca : Mg w roztworze inkubacyjnym. Przewaga jonów wapnia nad jonami magnezu zwiększała aktywność ATPazy frakcji I i II, podczas gdy we frakcji III obserwowano zależność odwrotną. Stwierdzono również, że ATPazy wyodrębnione z korzeni pszenicy rosnących na niekompletnej pożywce o różnym stosunku Ca : Mg zależne były od egzogennego stosunku tych jonów. Stosunek Ca : Mg równy 10 : 1 spowodował istotne zwiększenie aktywności ATPazy we frakcji II, podczas gdy odwrotny stosunek rzędu 1 : 10 spowodował obniżenia aktywności enzymu w tej frakcji. Wyniki wskazują, że aktywność roślinnych ATPaz zależna jest zarówno od mineralnego żywienia roślin jak i od stosunku jonów Ca : Mg w glebie czy pożywce oraz od stosunku tych kationów w poszczególnych subkomórkowych frakcjach tkanek.