# The effect of kinetin on cytochemical localization of Mg++ dependent ATP-ase in isolated lupine cotyledons

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## Abstract

ATP-ase activity stimulated with $Mg^{++}$ ions was localized cytochemically in lupine cotyledons. Studies were also made of the effect of kinetin on this activity. Activity of $Mg^{++}$ dependent ATP-ase was observed in plasmalemma, nucleus, nucleolus, endoplasmic reticulum, thylakoid membranes, prolamellar bodies, cell wall, and inter-cellular spaces. Kinetin (6-furfurylaminpurine) used in the experiment stimulated ATP-ase activity, but did not affect its localization.

## INTRODUCTION

ATP-ase is an membrane bound enzyme, occurring commonly both in plant and animal tissues. Localization of this enzyme is usually studied in young roots (H a l l 1971, W i n t e r - S l u i t e r et al. 1977, N a s s e r y and V a l a m a n e s h 1978, B e f f a g n a et al. 1979). Numerous papers deal with the activity of ATP-ase in conductive elements of plants. $Mg^{++}$ and $K^{+}$ dependent ATP-ase was localized in the phloem of: *Nicotiana tabacum* (G i l d e r and C r o n s h a w 1974, B e n t w o o d and C r o n s h a w 1976), *Cucurbita maxima* (G i l d e r and C r o n s h a w 1973), *Pinus nigra* (S a u t e r 1977), and *Pisum sativum* (B e n t w o o d and C r o n s h a w 1978), as also in xylem elements of *Hordeum vulgare* (W i n t e r - S l u i t e r et al. 1977). On the cell level ATP-ase activity stimulated with $Mg^{++}$ ions was observed in grana thylakoid and chloroplast envelopes (S a b n i s and G o r d o n 1970) in plasmodesmata, plasmolemma and tonoplast (H a l l 1969, S a b n i s and G o r d o n 1970, B e n t w o o d and C r o n s h a w 1978). ATP-ase activity was also discovered in mitochondria (S a b n i s and G o r d o n 1970,

Gilder and Cronshaw 1973), endoplasmic reticulum (Robards and Kidwai 1969, Gilder and Cronshaw 1973, Bentwood and Cronshaw 1978), nucleus envelope (Bentwood and Cronshaw 1978), nucleus and nucleolus (Robards and Kidwai 1969) and in cell wall and inter-cellular spaces (Sabnis and Gordon 1970, Dexheimer 1978). $Mg^{++}$, $Ca^{++}$, $K^+$, $Rb^+$, $Na^+$ and $Li^+$ ions activate ATP-ase present in the fraction of plasmic membranes (Leonard and Hotchkiss 1976). It is also known that there is a positive correlation between ATP-ase activity and ion uptake (Leonard and Hotchkiss 1976, Beffagna et al. 1979). Consequently, in the recent years there was a great interest of the role of this enzyme in ion transport through cell membranes (Hodges 1975). Cytokinins also stimulate permeability of many substances through plasmalemma (Livne and Graziani 1972, Feng 1973). In the present work a study was made of the possible dependence between exogenous cytokinin and localization and activity of $Mg^{++}$ stimulated ATP-ase in lupine cotyledon cells.

## MATERIAL AND METHODS

Experiments were carried out on isolated lupine (*Lupinus luteus* L. cv. "Topaz") cotyledons. After surface sterilization in 0.2% $HgCl_2$ and swelling in bi-distilled water for 14 hours the seeds were placed in darkness in Petri dishes on filter paper moistened with bi-distilled water (in 22° C). On the sixth day axis was cut, and cotyledons were transferred to Petri dishes with filter paper moistened either with bi-distilled water or 500 μM kinetin solution in water. The dishes were kept in darkness for 2 days in 22° C. Material for analyses was taken from the middle part of axial cotyledon side, which was in direct contact with the solution. Cotyledon fragments were fixed in 3% glutaraldehyde buffered with 0.05 M sodium-cacodylate buffer of pH 7.2 (1.5 h in 0°-4° C). After washing with 0.05 M sodium-cacodylate buffer of pH 7.2 (three times each 15 min) cotyledon fragments were incubated applying Wachstein Meisel method, as modified by Coulomb and Coulomb (1972). Incubation medium contained 3.6 mM lead nitrate, 10 mM magnesium sulphate, 80 mM tris-maleic buffer of pH 7.2 and 1 mM ATP (sodium salt) as a substrate. Control fragments were incubated in a medium without substrate. A two-step incubation was made: preincubation — for 0.5 h in 0°-4° C, and incubation for 2.5 h in 37° C. After washing with 0.05 M tris-maleic buffer of pH 7.2 material used for light-microscopic studies were treated with 2% ammonium sulphate for 2 min, and washed with bi-distilled water. Material used for electron microscopy was postfixed in 2% $OsO_4$ buffered with 0.05 M sodium-cacodylate buffer of pH 7.2 (for 14 hours) in 0°-4° C. Re-washing was

made with the same buffers. The sections were then dehydrated with a series of acetones and propylene oxide, embedded in Epon 812, and cut with a glass knife in a LKB "Ultrotom III" ultramicrotome. Ultrathin sections were viewed and photographed unstained using a JEM 7A electron microscope at 80 KV.

## RESULTS AND DISCUSSION

Especially high activity of $Mg^{++}$ dependent ATP-ase was noted in light microscopy in cotyledon cells situated near the axis. As results from previous observations (Młodzianowski and Gezela 1974, Jakubek and Młodzianowski 1978) storage materials are most early mobilized in this part of cotyledons. Also chloroplast development (after exposing isolated cotyledons to light) is most advanced here. Cotyledon sections treated with kinetin were characterized by more intensive ATP-ase activity than section treated with water (Fig. 2). Zone of cells with high ATP-ase activity was also broader and more deeply extending into the cotyledons. Especially strong enzymatic reaction was noted in endodermis cells and cells of the palisade mesophyll (Figs. 1, 2), mostly in the near-wall areas and in cell nucleus. More deeply situated cells had visibly lower activity of this enzyme. Similarly, significantly higher activity of ATP-ase stimulated with one- and bivalent ions was noted in the cells of primary cortex of lupine root (Potopov et al. 1978). Control sections possessed only rare precipitates resulting from a reaction of lead salts (contained in the incubation medium) with free phosphate groups (Fig. 3).

Subepidermal cells of palisade mesophyll were analysed with electrone microscopy. ATP-ase activity was localized in: plasmalemma (Figs. 5, 6, 7), nucleus (Figs. 4, 6), nucleolus (Fig. 6), prolamellar bodies and etioplast thylakoids (Figs. 4,7) and, sporadically, in endoplasmic reticulum (Fig. 4). ATP-ase activity was also observed in cell walls and inter--cellular spaces (Figs. 6, 7). Such localization is in accordance with numerous studies, carried out on various materials, for instance on *Arachis hypogaea* (Stanley et al. 1973), *Suaeda maritima* (Hall and Davie 1975), *Pinus nigra* (Sauter 1977), *Drosera capensis* (Dexheimer 1978), *Hordeum vulgare* (Nassery and Valamanesh 1978); mostly as regards conductive tissue — *Nicotiana tabacum* (Gilder and Cronshaw 1974, Bentwood and Cronshaw 1976) and *Pisum sativum* (Bentwood and Cronshaw 1978), and young roots — *Hordeum vulgare* (Hall 1969, Winter-Sluiter et al. 1977) and *Glycine max* (Travis et al. 1979).

As it has been already mentioned, ATP-ase is activated by several ions (Leonard and Hotchkiss 1976). Also localization of this en-

Figs. 1-3. Cytochemical localization of ATP-ase in light microscopy. Hand made transverse sections through lupine cotyledon fixed in 3% glutaraldehyde

Fig. 1. Cotyledon kept in water — incubation with ATP. In all cells of the cotyledon precipitating lead sulphide is visible. 320 ×

Fig. 2. Cotyledon kept in 500 $\mu$M water solution of kinetin — incubation with ATP. Precipitates of lead sulphide in cotyledon cells more intensive compared with material kept in water. 240 ×

Fig. 3. Cotyledon kept in 500 $\mu$M water solution of kinetin — incubation without ATP. No precipitates of lead sulphide. 320 ×

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zyme differs depending on the activating ion. So far, most attention has been paid to $Mg^{++}$ and $K^+$. ATP-ase activity stimulated with $K^+$ ions was observed mostly in cell membranes. It was localized in plasmalemma, tonoplast, endoplasmatic reticulum, nucleus envelope, and external membrane of mitochondrial envelope (Winter-Sluiter et al. 1977). $Mg^{++}$ dependent ATP-ase was noted — apart from cell membranes (Bentwood and Cronshaw 1978) — also in cell wall and inter--cellular spaces (Dexheimer 1978), and within nucleus and nucleolus (Robards and Kidwai 1969). Additionally, $Mg^{++}$ stimulated ATP--ase was localized in plastids, i.e. in granal and intergranal thylakoids and in envelope (Sabnis and Gordon 1970). In the present study $Mg^{++}$ stimulated ATP-ase was localized in the prolamellar bodies and in the forming prothylakoids. Hence, it may be stated that both structures are not differentiated as regards ATP-ase activity. This fact should be pointed out as according to some papers, these two development--dependent components of the etioplast may exhibit qualitatively different enzymatic activity (Wrischer 1978). Significant ATP-ase activity was found in cell nuclei of lupine cotyledons. This fact is supported by studies of Vasileva et al. (1978), who also noted high activity of this enzyme in isolated nuclei of barley embryos.

Localization of ATP-ase activity within nucleolus may create some controversy. According to some papers (Libanati and Tandler 1969, Tandler and Solaria 1969) nucleolus accumulates significant amounts of free phosphate groups, which can give unspecific reactions. In fact, numerous unspecific precipitates were observed in nucleoli in lupine cotyledon sections incubated without the substrate. However, their higher numbers after incubation with the substrate, as also total lack of precipitates in many control preparates (Figs. 8, 9) suggest that $Mg^{++}$ dependent ATP-ase is present also in nucleolus.

Van Steveninck's (1979) comments were taken into account in analysing the sections namely that apart from granular precipitates formed as a result of cytochemical reaction (specific for the reaction with lead), also globular precipitates containing osmium may form. Furthermore, no calcium was used as, according to this author, calcium favour
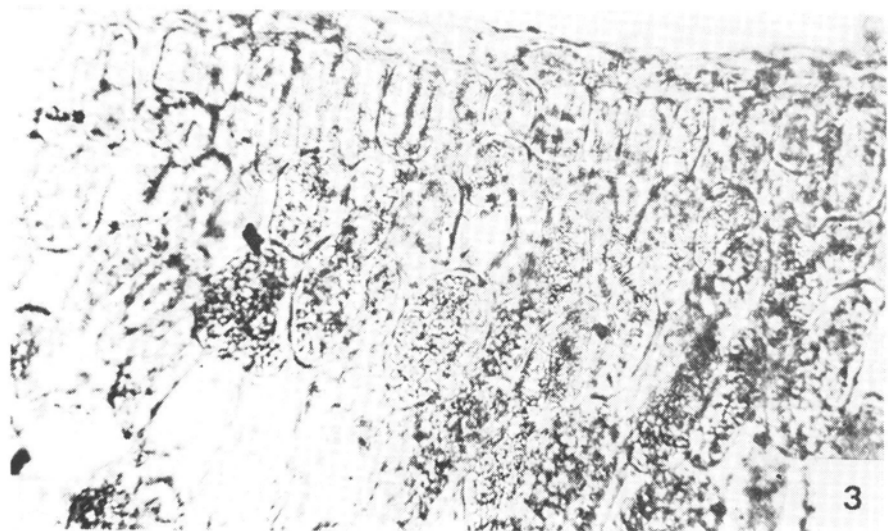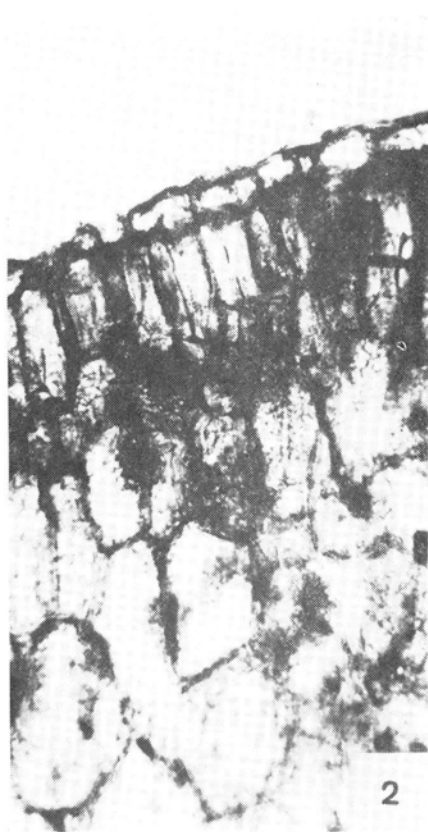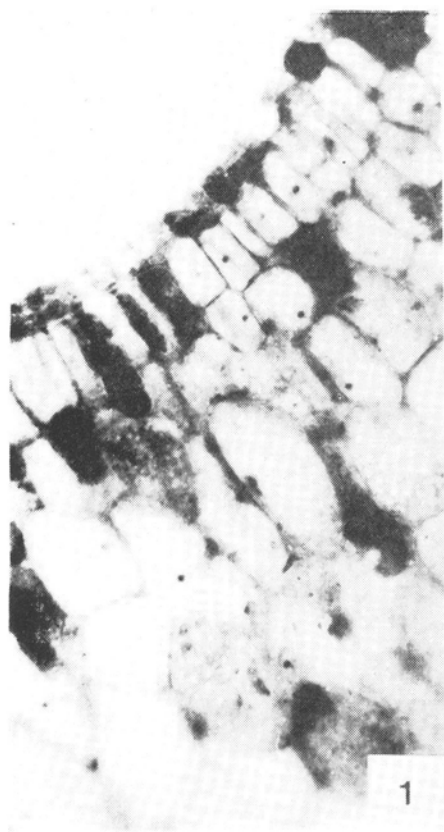
Figs. 4-9. Cytochemical localization of Mg⁺⁺ ATP-ase in electrone microscopy. Epon sections. Glutaraldehyde, OsO₄. C — prolamellar body, CW — cell wall, E — etioplast, ER — endoplasmatic reticulum, IS — inter-cellular space, M — mitochondrium, Nu — nucleolus, N — nucleus, NE — nuclear envelope, Pb — PbHPO₄ precipitate, PM — plasmalemma, S — starch
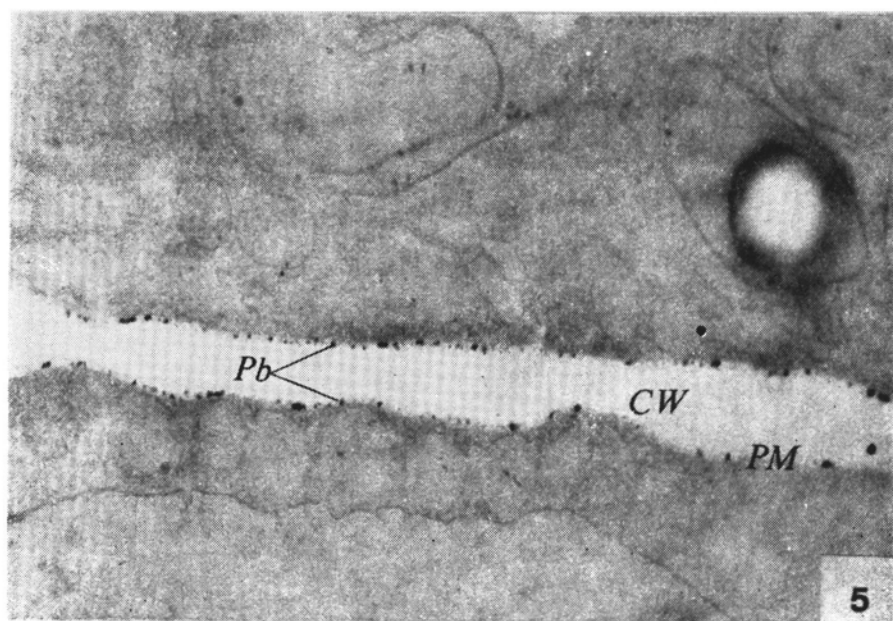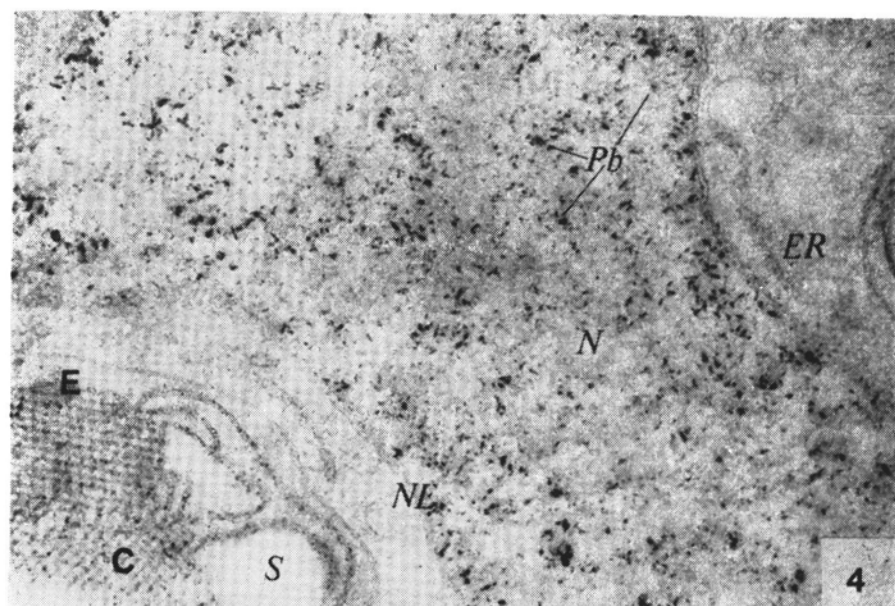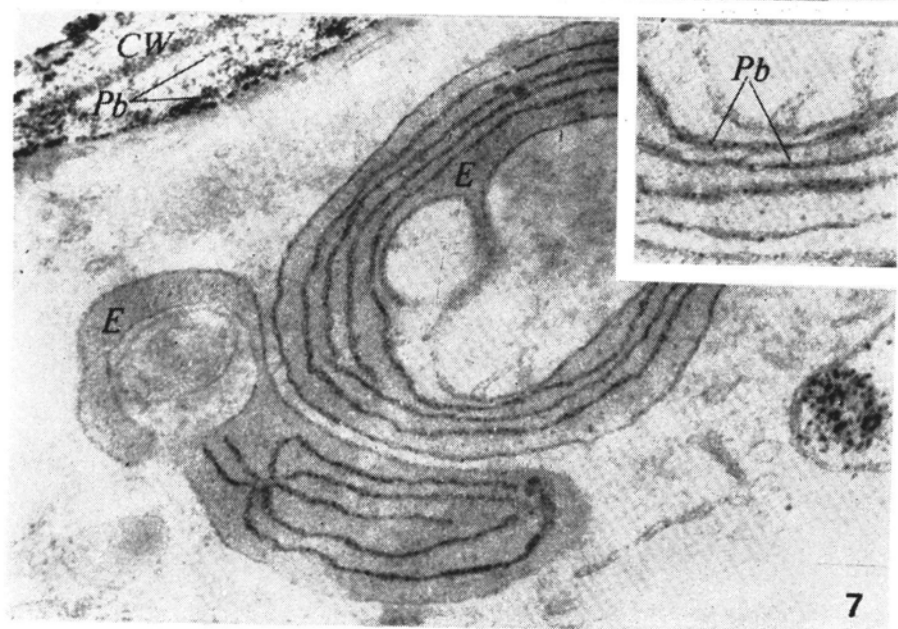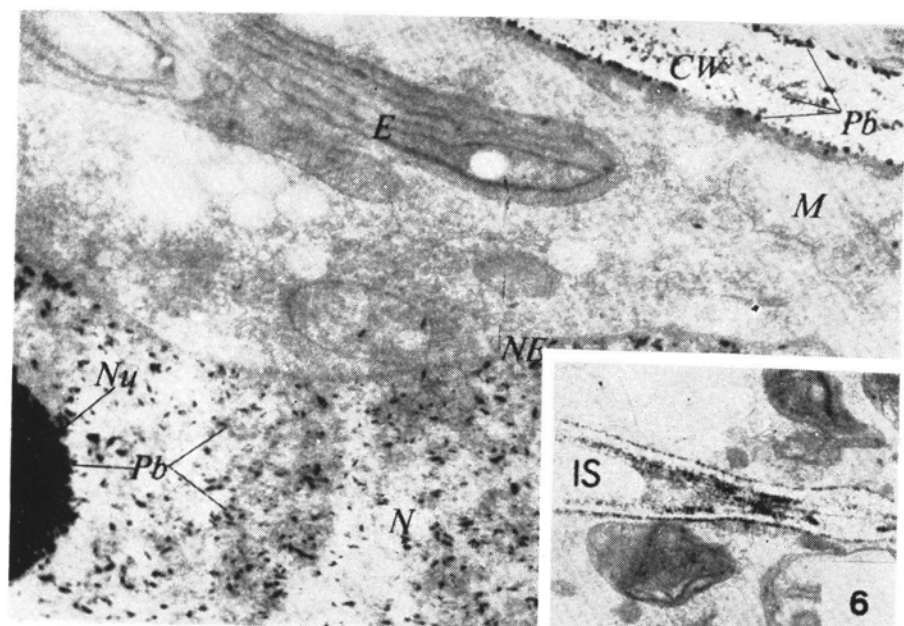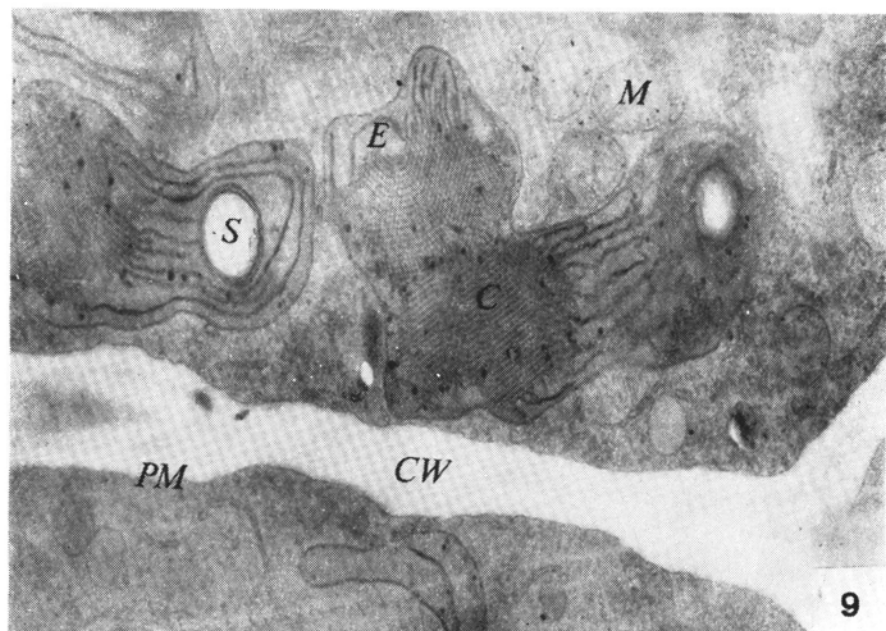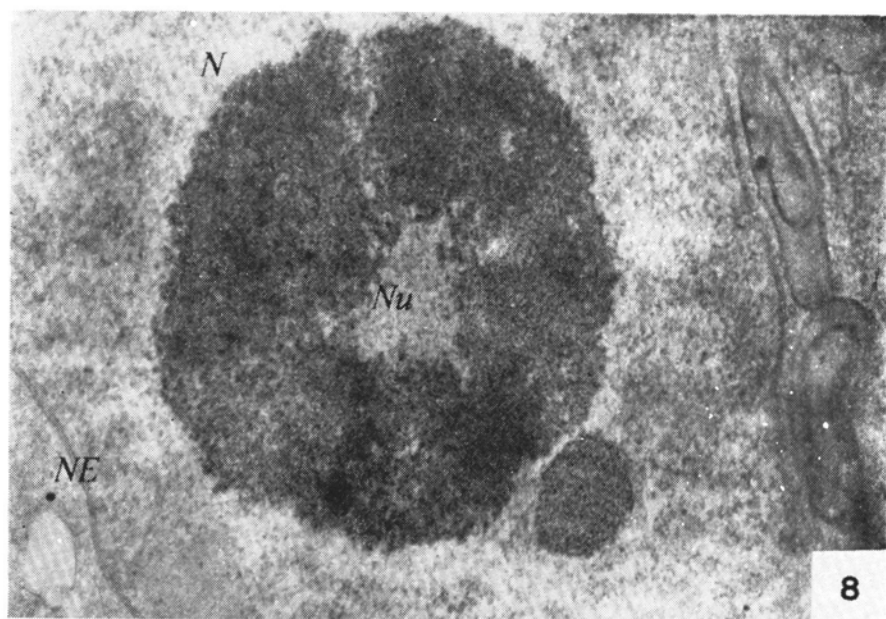
Fig. 4. Cotyledon kept in water — incubation with ATP. Lead precipitates visible within the nucleus, and in membranes of prolamellar body of etioplast. 24 000 ×
Fig. 5. Cotyledon kept in water — incubation with ATP. Lead precipitates visible in plasmalemma. 22 400 ×

Title and designations as in Plate II

Fig. 6. Cotyledon kept in 500 μM water solution of kinetin — incubation with ATP. Positive reaction is visible within plasmalemma, cell wall, nucleus and nucleolus, 14 000 ×.
Insert — reaction in the inter-cellular space. 5 800 ×

Fig. 7. Cotyledon kept in 500 μM water solution of kinetin — incubation with ATP. Lead precipitates visible in cell wall, plasmalemma and etioplast thylakoids. 22 000 ×. Insert — enlarged fragment of a thylakoidal system. 44 000 ×

Title and designations as in Plate II

Fig. 8. Cotyledon kept in 500 μM water solution of kinetin — incubation without ATP. Lack of lead precipitates within nucleus and nucleolus. 28 000 ×

Fig. 9. Cotyledon kept in 500 μM water solution of kinetin — incubation without ATP. Lack of lead precipitates. 16 000 ×

formation of globular precipitates during material fixation. Moreover, washing time was doubled compared with Van Steveninck's (1979) procedure.

Higher activity of $Mg^{++}$ dependent ATP-ase was found both in light microscopy and in electrone microscopy in the material treated with kinetin. On the other hand, there were no differences in the localization of this enzyme activity under the effect of kinetin. Increased ATP--ase activity under the effect of kinetin may be connected with mechanisms of transport through the membranes. It is possible that this dependence explains stimulating effect of kinetin on mobilization of storage substances and chloroplast development in lupine cotyledons (M ł o d z i a n o w s k i and G e z e l a 1974, J a k u b e k and M ł o d z i a n o w s k i 1978).

Results obtained in this study with cytochemical methods are supported by biochemical works. S c h n e i d e r et al. (1980) using moss protonema (which is a convenient test material for cytokinin) proved that exogenous cytokinins also stimulate activity of $Mg^{++}$ dependent ATP-ase.

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# Wpływ kinetyny na cytochemiczną lokalizację ATP-azy zależnej od $Mg^{++}$ w izolowanych liścieniach łubinu

Streszczenie

Zlokalizowano cytochemicznie aktywność ATP-azy stymulowanej jonami $Mg^{++}$ w izolowanych liścieniach łubinu oraz zbadano wpływ kinetyny na poziom jej aktywności. Aktywność ATP-azy zależnej od $Mg^{++}$ wykazano w plazmalemmie, jądrze, jąderku, siateczce śródplazmatycznej, w błonach tylakoidów i ciałach prolamellarnych etioplastów oraz w ścianie komórkowej i przestworach międzykomórkowych. Użyta w doświadczeniach kinetyna (6-furfuryloaminopuryna) stymulowała aktywność ATP-azy, nie wpływała jednak na zmianę jej lokalizacji.