ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 49, nr 3: 269-280
1980

Reduction of nitrates in *Cucumis sativus* L. seedlings

II. Influence of tungsten and vanadium on nitrate reductase and adenosine triphosphatase activities

JÓZEF BUCZEK

Department of Plant Physiology, Institute of Botany, Wrocław University, ul. Kanonia 6/8, 50-328 Wrocław, Poland

(Received: December 1, 1979)

Abstract

ATPases isolated from the roots of cucumber seedlings activated by Mg^{2+} ions in experiments *in vitro*, were fairly distinctly inhibited by Ca^{2+} ions, very slightly inhibited by fluorides and molybdenum ions while NO_3^- anions had no effect on the level of ATPase activity studied. Introduction into the nutrient of 10^{-4} M Na_2WO_4 or 10^{-3} M $Na\,VO_3$ (inhibitors of nitrate reductase NR) distinctly inhibited activity of the ATPase under study especially of fractions IIa and III, and inhibited NR activity and lowered uptake of NO_3^-. WO_4^{-2} and VO_3^- inhibited to the same extent absorption and reduction of NO_3^- in the initial phase of NR induction, whereas at a later stage both inhibitors checked reduction to a greater degree than uptake of NO_3^-. The results indicate the possibility of certain ATPase participation in assimilating nitrates, and suggest that in the initial stage of biosynthesis of the NR enzyme system, activity of the enzyme is distinctly dependent upon NO_3^- transport and the level of NR activity limited by the amount of nitrate taken up. At a later an additional mechanism of NO_3^- transport probably functions, not connected with simultaneous reduction of nitrates. On the basis of results the Butz and Jackson (1977) hypothesis concerning a model for the absorption and reduction of NO_3^- by plant tissues is discussed.

INTRODUCTION

In a previous study (Buczek et al., 1980) it was found that tungsten and molybdenum given to plants during the period of nitrate induction inhibited NR activity after 24 hours, and significantly lowered NO_3^- uptake. However inhibition of NR activity was not possible to explain as being due to lowered nitrate absorption. On the other hand a certain correlation was found between the amount of NO_3^- taken up and its reduction, if WO_4^{-2} and VO_3^- were given 24 hours before induction of the NR system was begun. Butz and Jackson (1977) proposed a model of the mechanism of nitrate uptake and reduction by plant tissues. This model includes a sub-unit structure of the enzyme and participation of a membrane bound ATPase.

There is a lack of data in literature concerning the influence of NO_3^- ions on activity of plant ATPase with the exception of reports by Falkowski (1975a, b). The mentioned author found two species of sea algae ATPase which required nitrates for maximum activity within the pH range he investigated. The dependence of NO_3^- reduction upon simultaneous transport of nitrates to plant cells is a consequence of the Butz and Jackson proposal. Hence inactivation of any one of the links in the system of nitrate reduction should inhibit both transport as well as reduction of nitrates. The purpose of this study was to prove whether the inhibiting action of WO_4^{-2} and VO_3^- on NR activity is connected with ATPase activity of root extracts and with nitrate absorption by the roots of cucumber seedlings.

MATERIAL AND METHODS

Plant material

Five day cucumber seedlings of the "Monastyrski" variety (*Cucumis sativus* L.) were prepared and cultivated as described in a previous study (Buczek et al., 1980). 0.1 mM $Na_2WO_4 \cdot 2H_2O$ and 1 mM $NaVO_3 \cdot H_2O$ were added to the nutrient medium.

Analytical methods

Nitrate reductase extracts were prepared from fresh leaves and roots by means of a procedure described previously (Buczek, 1976; Buczek et al., 1980). NR activity was expressed in nmoles of NO_2^- produced in one hour per 100 mg of fresh tissue matter.

Extracts of adenosine triphosphatase (ATPase) were prepared from fresh roots washed many times in cold deionized water. Roots from around 20 plants (1 g of fresh weight) were homogenized in a cold mortar in 10 ml of a medium containing 50 mM Tris-maleate buffer (pH 7.5), 0.25 M saccharose and 3 mM EDTA. The homogenate was pressed through four layers of cheese-cloth and the filtrate centrifuged for 10 minutes at 1500 × g. The sediment was discarded and the supernatant centrifuged for 15 minutes at 13000 × g. The sediment from the 13000 × g centrifuging was washed in the extract medium and after centrifuging suspended in the same solution, marking it as fraction IIa (enriched in mitochondria). The supernatant from this centrifuging was marked as fraction III. In some cases the sediment from fraction IIa was suspended in the extract medium with an addition of 0.1 % (final concentration) Triton X-100 and shaken in a temperature of 3°C for 30 minutes, then centrifuged at 20000 × g for 25 minutes, marking the sediment after suspension in the extract medium as fraction IIb and the supernatant as fraction IIc. All of the above described preparations were carried out at 0°-4°C.

ATPase activity was assayed in the total volume of 4 ml over 30 minutes of incubation at a temperature of 37°C. Four ml of the incubation mixture contained 0.1 M Tris-maleate (pH 6.5), 25 mM KCl, 3 mM ATP, 2 mM $MgCl_2$ and 0.2 to 0.5 ml enzyme extract (40 - 70 µg protein). The incubation medium for fraction IIa additionally contained 25 mM saccharose. The reaction was stopped by adding 1 ml of cold trichloroacetic acid (5% final concentration) and the test tubes were placed in an ice bath for ten minutes. Protein was removed by centrifuging, and inorganic phosphate (P) released from ATP was determined by the Fiske and Subbarow (1925) method. ATPase activity was expressed in nmols P_i liberated per mg of protein per hour, or per g fresh tissue matter per hour. Soluble protein was determined by the procedure of Lowry et al. (1951).

Absorption and accumulation of nitrates were determined by means of the method described in the previous study (Buczek et al., 1980). The reagents used were of analytical grade and supplied by POCH (Gliwice). ATP as a disodium salt and NADH were obtained from the Sigma Chemical Co.

Abreviations used. ATPase — adenosine triphosphatase, NR — nitrate reductase.

RESULTS

The preliminary experiment referred to measurement of ATPases — isolated from the roots of five day old cucumber seedlings grown on a nutrient medium devoid of nitrates — at various pH ranges, and of the influence of some ions on enzyme activity. The optimum pH for ATPases of fraction IIa and fraction III (Fig. 1) was 6.5, although enzyme activity for fraction III was relatively high in the pH range between 6.0 and 7.0.

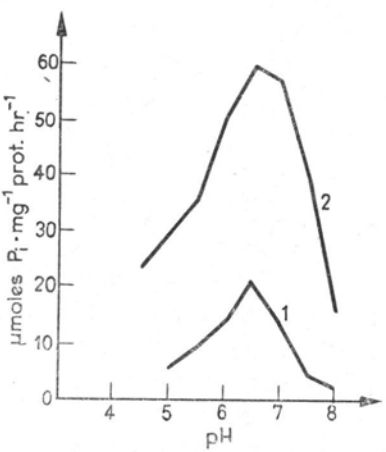

Fig. 1. Effect of pH on ATPase activity.
The incubation medium contained 100 mM Tris-maleate, 3 mM ATP and 25 mM KCl. 1 — fraction IIa, 2 — fraction III.

Both sodium fluoride as well as molybdate, typical inhibitors of acid phosphatase only decreased ATPases from root extracts (Table I). This can suggest that most of the phosphatase activity refers to ATPases and that in the pH range acid phosphatase to a lesser degree hydrolyse the substrate. Tungsten and vanadium added to the incubation medium distinctly inhibited ATPase activity. This inhibition was especially high for ATPase fraction III. Introduction of NO_3^- ions into the incubation medium had no influence on activity of the ATPases under investigation.

Table I

Effect of various ions on the activity of ATPases extracted from cucumber roots

Substance added		ATPase activity μmoles $P_i \cdot mg^{-1}$ protein $\cdot hr^{-1}$		
		fraction IIa	fraction IIc	fraction III
None		18.36	51.83	25.17
NaF	10^{-2} M	16.25 (88)	36.17 (70)	21.16 (84)
Na_2MoO_4	10^{-3} M	15.02 (82)	43.19 (83)	17.51 (69)
Na_2WO_4	10^{-4} M	14.94 (81)	35.09 (68)	13.86 (55)
$NaVO_3$	10^{-3} M	12.76 (69)	34.01 (66)	11.67 (46)
KNO_3	10^{-3} M	17.92 (98)	—	25.31 (100)
KNO_3	10^{-4} M	18.43 (100)	—	25.83 (103)

The seedlings were grown 5 days on pretreatment solution without nitrogen, then the roots were harvested, homogenized and enzymatic activities were assayed in separate fractions. The investigated salts were added to the reaction mixture (see Fig. 3). Values in brackets reflect the effect of the treatment relative to control.

As can be seen from Fig. 2 the investigated ATPases of fraction II were activated by Mg^{2+} ions, whereas Ca^{2+} ions distinctly lowered activity of the enzyme. A similar dependence was noted with respect to fraction IIb and fraction III.

The subsequent experiment consisted of measurements of the activity of ATPases isolated from the roots of cucumber seedlings which after growth of five days in an initial nutrient medium not containing nitrogen, were transferred to a nitrogen nutrient. Measurements at intervals of several hours were made of ATPase activity in root extracts, nitrate reductase activity in extracts from roots and cotyledons, and at the same time of the absorption and accumulation of nitrates.

The results presented in Fig. 3 show that placing the plants in a nutrient medium containing nitrates effected a significant increase in ATPase activity in both of the enzyme fractions under investigation as compared to activity of ATPases extracted from the roots of plants growing in a nutrient medium without nitrates. In analysing changes taking place in ATPase activity during 24 hour growth of plants, a similar character in changes was observed, however the presence of NO_3^- in the nutrient always significantly stimulated activity of the ATPases under study.

Introducing tungsten and vanadium salts into the nitrate nutrient (nitrate reductase inhibitors) has its influence also on ATPase activity. WO_4^{-2} and VO_3^- distinctly inhibited ATPase activity of fraction IIa (Fig. 4b), but only WO_4^{-3} significantly

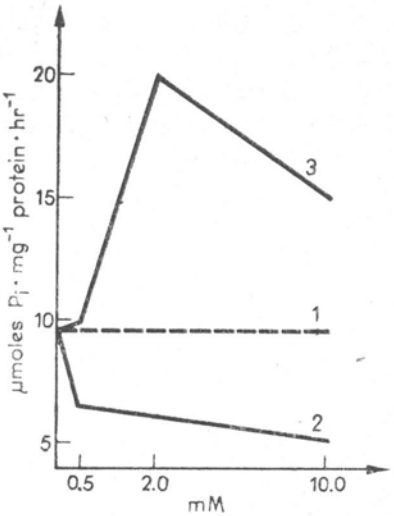

Fig. 2. Effect of divalent cations on ATPase activity of fraction IIa.
The incubation medium contained 100 mM Tris-maleate (pH 6.5), 3 mM ATP, 0.1 M sucrose, 25 mM KCl and various concentrations of $CaCl_2$ and $MgCl_2$. 1 — control, 2 — $CaCl_2$, 3 — $MgCl_2$

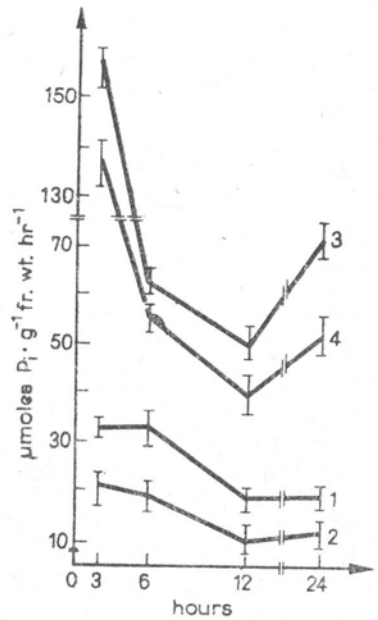

Fig. 3. *In vivo* effect of NO_3^- ions on the kinetics of ATPase activity extracted from roots of cucumber seedlings.

The seedlings were grown on medium with or without nitrates. At designated times, the roots were harvested, homogenized and enzymic activities assayed immediately. The incubation medium contained 100 mM Tris-maleate (pH 6.5), 3 mM ATP, 25 mM KCl, 2 mM $MgCl_2$ and 0.2-0.5 ml of the preparation from given fractions. 1 — $+NO_3^-$, fraction IIa; 2 — $-NO_3^-$, fraction IIa; 3 — $+NO_3^-$, fraction III; 4 — $-NO_3^-$, fraction III.

inhibited enzyme activity of fraction IIb (Fig. 4a). Furthermore tungsten distinctly inhibited ATPase activity of fraction III (Fig. 5). Inhibition of the activity of fraction III by vanadium ions was less effective and significant differences as compared to controls were evident only after 12 hours of growth of plants in a nitrate nutrient medium with an addition of VO_3^- salts.

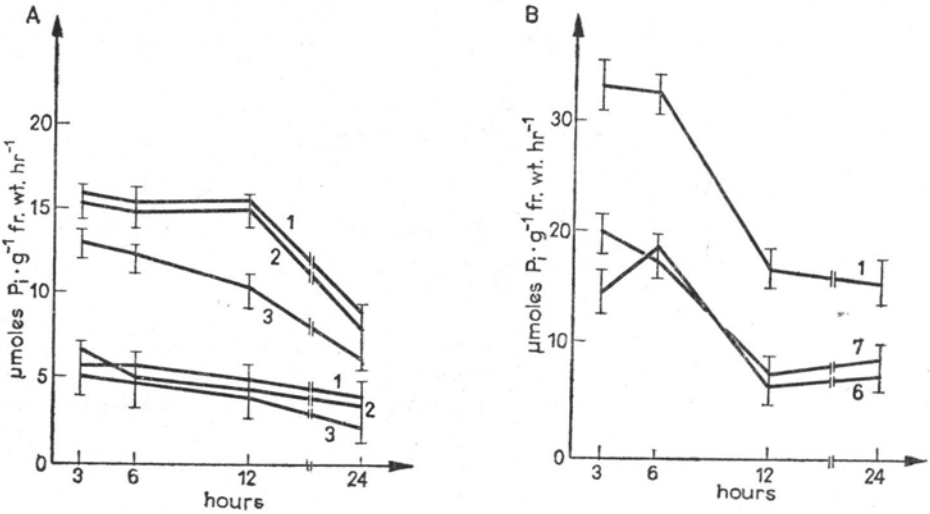

Fig. 4. Effect of tungstate and metavanadate on ATPase activity extracted from cucumber roots. The seedlings were grown on NO_3^- medium with or without 10^{-4} M WO_4^{-2} or 10^{-3} M VO_3^-. For measuring details of enzymic activities see Fig. 3. A — fractions IIb and IIc,, B — fraction IIa; 1 — control; 2 — VO_3^-, fraction IIc; 3 — WO_4^{-2}, fraction IIc; 4 — WO_4^{-2}, fraction IIb; 5 — VO_3^-, fraction IIb; 6 — WO_4^{-2}, fraction IIa; 7 — VO_3^-, fraction IIa.

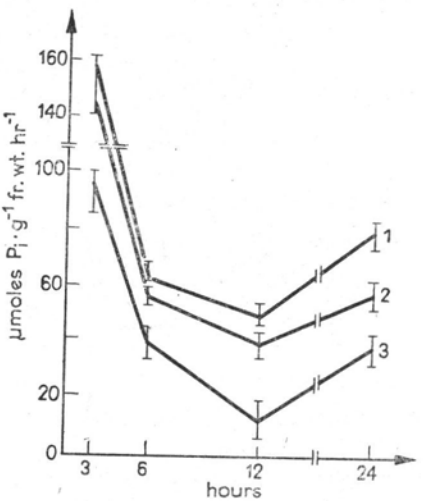

Fig. 5. Effect of Na_2WO_4 and $NaVO_3$ on the kinetics of fraction III ATPase activity extracted from cucumber roots.
Experimental details see Fig. 4. 1 — control, 2 — VO_3^-, 3 — WO_4^{-2}.

The kinetics of changes in nitrate reductase activity in the cotyledons and roots of cucumber plants are presented in Fig. 6. As was to be expected both inhibitors (WO_4^{-2} and VO_3^-) distinctly inhibited NR activity in both types of tissue. This inhibition was not full in cotyledons even after 24 hours of plant growth in the presence of the inhibitors. On the other hand as concerns roots, NR activity in the presence of WO_4^{-2} and VO_3^- disappeared completely after 24 hours of growth in comparison to the controls.

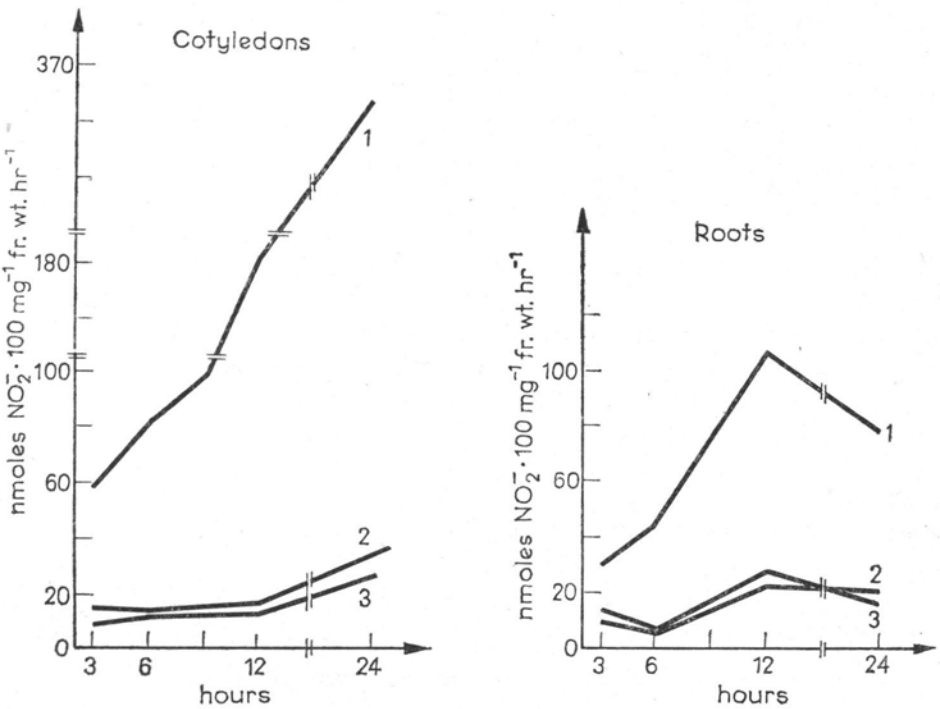

Fig. 6. Nitrate reductase induction kinetics in the presence or absence of 10^{-4} M WO_4^{-2} or 10^{-3} M VO_3^-.
The seedlings were grown on NO_3^- induction medium with or without inhibitors. At designated time intervals the roots were harvested and *in vitro* NR activity measured immediately (see Material and methods). 1 — NO_3^-, 2 — NO_3^-+WO_4^{-2}, 3 — NO_3^-+VO_3^-.

Both inhibitors likewise decreased NO_3^- uptake (Fig. 7), in which case inhibition of NO_3^- absorption, as compared to the controls, increased with time, and was more effective in the presence of VO_3^- than in the presence of WO_4^{-2}.

The analyses of absorption, accumulation and reduction of NO_3^- during the 24 hour growth of seedlings are shown in Table 2. As can be noted from the results there is in fact a distinct decline in NO_3^- uptake and reduction during the first six hours of reaction of the inhibitors in the nutrient medium, but the ratio of NO_3^- absorbed to reduced in the presence of WO_4^{-2} and VO_3^- differs but slightly in compa-

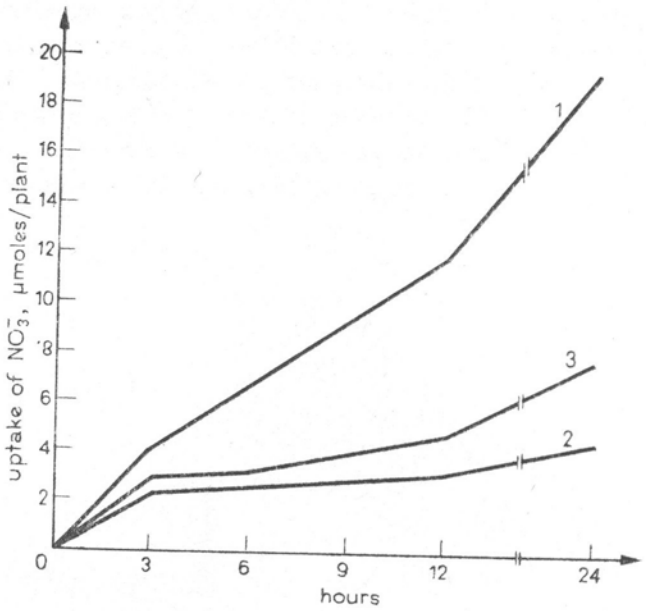

Fig. 7. Effect of WO_4^{-2} and VO_3^- on absorption of NO_3^-.
1 — control, 2 — 10^{-3} M VO_3^-, 3 — 10^{-4} M WO_4^{-2}

Table 2

Kinetics of absorption, accumulation and reduction of NO_3^- ions by cucumber seedlings as affected by tungsten and metavanadium

Absorption medium	Time of measurements (hr)	Absorption	Accumulation	Reduction	Absorbed / Reduced
		µmoles NO_3^- per seedling			
NO_3^-	3	4.1	0.8	3.3	1.2
$NO_3^-+WO_4^{-2}$		3.0	1.1	1.9	1.6
$NO_3^-+VO_3^-$		2.4	0.9	1.5	1.6
NO_3^-	6	6.8	1.1	5.7	1.2
$NO_3^-+WO_4^{-2}$		3.1	1.2	1.9	1.6
$NO_3^-+VO_3^-$		2.7	1.1	1.6	1.7
NO_3^-	12	12.0	3.7	8.3	1.4
$NO_3^-+WO_4^{-2}$		4.8	3.6	1.2	4.0
$NO_3^-+VO_3^-$		3.2	2.1	1.1	2.9
NO_3^-	24	19.4	8.1	11.3	1.7
$NO_3^-+WO_4^{-2}$		8.2	6.8	1.4	5.8
$NO_3^-+VO_3^-$		4.5	3.5	1.0	4.5

Five-days-old seedlings were grown on nitrate medium with or without 10^{-4} M Na_2WO_4 or 10^{-3} M $NaVO_3$. At designated time intervals the absorption of NO_3^- was measured and plant parts hervested to assay NO_3^- accumulation. Reduction was determined by subtracting the total amount of nitrate in whole seedling from the total uptake of nitrate at each assay period.

rison to the controls. Only after around 12 hours of plant growth is nitrate reduction inhibited to a greater degree than nitrate uptake, as indicated by the almost three times higher ratio of NO_3^- absorbed to that reduced, as compared to the controls.

DISCUSSION

The above described experiments show that vanadium and tungsten — inhibitors of nitrate reductase (Heimer et al., 1969; Wray, Filner, 1970; Vega et al., 1971; Notton et al., 1974; Buczek, 1973; Rao, Rains, 1976; Buczek et al., 1980) likewise inhibit activity of certain ATPases isolated from the roots of cucumber seedlings both *in vitro*, as well as *in vivo*. Inhibition of ATPase activity *in vivo* also took place distinctly for fraction IIa, rich in mitochondria, and also for fraction III. On the other hand no significant influence of the inhibitors under study was observed on ATPase activity within fractions IIb and IIc, that is after treating the sediment of fraction IIa with Triton X-100. It therefore appears that WO_4^{-2} and VO_3^- chiefly inhibit membrane bound ATPases. Occurrence of distinct inhibition of ATPase activity in fraction III can be explained by the presence here of ATPase linked with cytoplasmatic membranes. The fact that tungsten and vanadium salts may inhibit the some ATPases activity and at the same time absorption of NO_3^- ions, can suggest — in accordance with the hypothesis of Butz and Jackson (1977) — that there is a correlation between ATPase activity and nitrate absorption and reduction. The mentioned authors proposed a model of the mechanism for the absorption and reduction of NO_3^- by plant tissues. In accordance with their assumptions membrane bound ATPases participate in nitrate reductase structural subunit which functions as carrier for nitrate transport and as reductase. Hence if the Butz and Jackson hypothesis is correct, then factors which change the structure of the enzyme reducing nitrates, or ATPases associated with this enzyme, should inhibit both intake and reduction of NO_3^-.

In a previous study (Buczek et al., 1980) it was found that after 24 h growth of seedlings in a nitrate nutrient medium and presence of WO_4^{-2} or VO_3^-, nitrate reductase was totally inhibited in the roots and almost completely in the cotyledons, whereas NO_3^- uptake dropped by 50 percent in the presence of WO_4^{-2} and by around 70 percent in the presence of VO_3^-. These results suggested that absorption and reduction of nitrates are partially independent processes. A kinetic analysis of uptake and reduction of NO_3^- in the presence of WO_4^{-2} and VO_3^- in the present study showed, on the one hand, a drop in absorption of nitrates by the roots of plants growing in a nutrient medium with an addition of NR inhibitors, but the ratio of NO_3^- absorbed to that reduced changed during the course of the experiment. Relatively distinct similarity between the value of the NO_3^- absorption/reduction coefficient was found up to the first 6 hours in both control plants and plants subjected to inhibitors, while during a further lapse of the time of the experiment almost a three times greater gain in the value of the NO_3^- absorption/reduction ratio in the presence

of WO_4^{-2} and VO_3^- was noted than in the controls. It therefore appears that during the initial period of NR induction its activity depends distinctly upon the NO_3^- taken up from outside. Hence WO_4^{-2} and VO_3^- modify either the NR system or the ATPases linked with it, limiting absorption and consequently leading to a lowering of NR activity. After formation of the NR system both inhibitors inhibit to a greater extent nitrate reductase than NO_3^- uptake. These results are thus in accordance with previous investigations (Buczek et al., 1980) and suggest participation of certain ATPases in the process of absorption and reduction of nitrates.

The above assumptions are confirmed by the results of our experiments, indicating that the presence of nitrates in the nutrient medium is linked with a significant rise in the ATPase activity of fractions IIa and III from root extractions in comparison to the activity of ATPase in root plants growing in a nutrient medium devoid of nitrogen (Fig. 3).

Experiments showed that ATPase activity in the fractions under investigation declines more or less to the 12th hour of plant growth irrespective of the presence of nitrogen in the nutrient, and then increases, this being especially true for fraction III. Convergence in the rise of ATPase activity after 12 hours of plant growth with increased NO_3^- uptake (Fig. 7) can suggest participation of certain ATPase in the absorption of nitrates.

Hence in line with the results investigations of a number of authors, functioning of NR depends upon the continuous transport of NO_3^- from outside to the plant cell (Filner et al., 1969; Oaks et al., 1972), or depends upon the so called nitrate metabolic pool (Ferrari et al., 1973; Jackson et al., 1973; Aslam, Oaks, 1975). In our experiments the ratio of NO_3^- absorbed/reduced in the presence of WO_4^{-2} or VO_3^- did not differ from the controls during the initial 3 to 6 hours of plant growth. At the same time, however, inhibition of ATPase and NR activity took place. The above data allow for the assumption that in the initial phase of induction NR activity depends principally upon the transport of NO_3^- from outside, and the level of nitrate reductase is limited by the amount of nitrates taken up. This assumption is in agreement with the results of investigations by Aslam and Oaks (1975) who did not note an increase of the nitrate metabolic pool in the roots of maize during the initial phase of enzyme induction.

The above data to some extent support the Butz and Jackson (1973) hypothesis suggesting a correlation between nitrate reduction and activity of certain ATPases in linkage with the transport of NO_3^- in the initial phase of induction of the NR system. At a later phase, probably after formation of the NR enzyme system, presence of WO_4^{-2} and VO_3^- in the external solution significantly inhibit ATPase and NR activity, but do not inhibit nitrate intake to such an extent as to assume a distinct correlation between uptake of NO_3^- and functioning of the NR system and ATPase associated with it. The above data rather suggest existence of a different mechanism of nitrate transport than that linked with simultaneous reduction of NO_3^-. It is not improbable that WO_4^{-2} and VO_3^- modify the structure of certain proteins or

enzymes active in the intercellular transport of nitrates, which does not remain without influence upon NR activity.

Our experiments thus suggest participation of certain ATPases of cells in the assimilation of nitrates, however the problem of NO_3^- uptake and its reduction by the same enzyme complex requires further investigation.

This study was financed from problem MR-II/7.

REFERENCES

Aslam M., Oaks A., 1975. Effect of glucose on the induction of nitrate reductase in corn roots. Plant Physiol. 56: 634-639.

Buczek J., 1973. Effect of vanadium on nitrate reductase activity in tomato leaves. Acta Soc. Bot. Pol. 42: 223-232.

Buczek J., 1976. The role of light in the induction of nitrate reductase and nitrite reductase in cucumber seedlings. Acta Soc. Bot. Pol. 45: 77-92.

Buczek J., Kowalińska E., Kuczera K., 1980. Nitrate reduction by *Cucumis sativus* L. seedlings. I. Influence of tungsten and vanadium on absorption and reduction of nitrates. Acta Soc. Bot. Pol. 49: 259-267.

Butz R. G., Jackson W.A., 1977. A mechanism for nitrate transport and reduction. Phytochem. 16: 409-417.

Falkowski P. G., 1975a. Nitrate uptake in marine phytoplankton: Comparison of half saturation conctants from seven species. Limnol. and Oceanogr. 20: 412-417.

Falkowski P. G., 1975b. Nitrate uptake in marine phytoplankton: (nitrate, chloride)-activated adenosine triphosphatase from *Skeletonema costatum* (*Bacillariophyceae*). J. Phycol. 11: 323-330.

Ferrari T. F., Yoder O. C., Filner P., 1973. Anaerobic nitrate production by plant cells and tissues: evidence for two nitrate pools. Plant Physiol. 51: 423-431.

Filner P., Wray J. L., Varner J. E., 1969. Enzyme induction in higher plants. Science. 165: 358-367.

Fiske C. H., Subbarow Y., 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66: 375-400.

Heimer Y. M., Wray J. L., Filner P., 1969. The effect of tungstate on nitrate assimilation in higher plant tissues. Plant Physiol. 44: 1197-1199.

Jackson W. A., Flesher D., Hageman R. H., 1973. Nitrate uptake of dark-grown corn seedlings: some characteristics of apparent induction. Plant Physiol. 51: 120-127.

Lowry O., Rosenbrough N. J., Farr A. L., Randall R. J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193: 265 - 275.

Notton B. A., Graf L., Hewitt E. J., Porey R. C., 1974. The role of molybdenum in the synthesis of nitrate reductase in cauliflower (*Brassica oleraeae* L. var. *botrytis* L.) and spinach (*Spinacea oleracea* L.). Biochim. Biophys. Acta. 52: 45-58.

Oaks A., Wallace W., Stevens D., 1972. Synthesis and turnover of nitrate reductase in corn roots. Plant Physiol. 50: 649-654.

Rao K. P., Rains D. W., 1976. Nitrate absorption by barley. II. Influence of nitrate reductase activity. Plant Physiol. 57: 59-62.

Vega J. M., Herrera J., Aparicio P. J., Paneque A., Losada M., 1971. Role of molybdenum in nitrate reduction by *Chlorella*. Plant Physiol. 48: 294-299.

Wray J. L., Filner P., 1970. Structural and functional relationships of enzyme activities by nitrate in barley. Biochem. J. 119: 751-725.

Redukcja azotanów w siewkach Cucumis sativus L.

II. Wpływ wolframu i wanadu na aktywność reduktazy azotanowej i adenozynotrójfosfatazy

Streszczenie

Wyodrębnione z korzeni siewek ogórka ATPazy, w doświadczeniach *in vitro*, aktywowane były jonami Mg^{2+}, dość wyraźnie hamowane przez jony Ca^{2+}, słabo hamowane przez fluorki i jony molibdenu, podczas gdy aniony NO_3^- nie miały wpływu na poziom aktywności badanych ATPaz. Wprowadzenie do pożywki 10^{-4} M Na_2WO_4 czy 10^{-3} M $NaVO_3$ (inhibitory reduktazy azotanowej NR), wyraźnie zahamowało aktywność badanych ATPaz, zwłaszcza frakcji IIa i frakcji III, oraz zahamowało aktywność NR i obniżyło pobieranie NO_3^-. WO_4^{-2} i VO_3^- zahamowały w jednakowym stopniu pobieranie i redukcję NO_3^- w początkowej fazie indukcji NR, natomiast w późniejszym okresie, obydwa inhibitory bardziej hamowały redukcję niż pobieranie NO_3^-. Wyniki wskazują na możliwość udziału pewnych ATPaz w asymilacji azotanów oraz sugerują, że w początkowej fazie biosyntezy systemu enzymu NR, aktywność enzymu jest wyraźnie zależna od transportu NO_3^- a poziom aktywności NR limitowany jest ilością pobranych azotanów. W późniejszym etapie, funkcjonuje prawdopodobnie dodatkowy mechanizm transportu NO_3^-, nie związany z równoczesną redukcją azotanów. W oparciu o wyniki dyskutowano hipotezę Butz i Jackson (1977) dotyczącą modelu dla pobierania i redukcji NO_3^- przez tkanki roślin.