Changes in ATPase activity in meristematic cells of *Allium cepa* L. roots caused by oncostatics

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Abstract

Specific changes in the activity of ATPases: mitochondrial and transport associated with the plasmalemma and membranes of endoplasmic reticulum have been observed during experiments on the meristematic tissue of *Allium cepa* root apices subject to cytostatics (methotrexate, cyclophosphamide, dacarbazine). It seems that an observed increase in the ATPase activity is correlated with a decrease in the level of cell respiration caused by oncostatics.

Key words: root meristems, oncostatics action, ATPase activity

INTRODUCTION

From the previous studies on oncostatics it has been inferred that oncostatics of the group of metabolites and alkylators cause, apart from changes in the ultrastructure and disturbance of mitotic cycle, inhibition of cell respiration (Podbielkowska and Nowaczek 1979, Podbielkowska and Wałęza 1980, Podbielkowska et al. 1980, 1981, 1982).

The lowered level of high-energy compounds in a cell may result in a number of consequences, an increase in the ATPase activity is the one (Bednarski et al. 1977). The ATPase activity may be also regulated by pharmaceuticals (Rachmilevitz et al. 1978, Segawa et al. 1978, Bryła 1981). On the grounds of the data mentioned above an assumption has been made that oncostatics should cause similar effects.

The ATPase activity, linked with the regulation of an active transport, has been studied in cell systems by the lead precipitation method (Wachstein and Meisel 1957). Electron-opaque, lead phosphate precipitates are easily identified under an electron microscope as dense aggregates in these cell structures where enzymatic hydrolysis of ATP takes place with ATPases.

Our previous studies on the localization of active ATPases in the meristematic cells of root apices have showed that some ATPase types may be identified by the modified method of Wachstein and Meisel, i.e. transport ATPase within the plasmalemma and active ATPase associated with the membranes of mitochondria and ER (Podbielkowska and Wałęza 1987). In the present studies previously tested methods of ATPase localization have been applied; formaldehyde fixatives have been used, and K^+ and Mg^{2+} ions have been introduced into incubation medium to stimulate ATPase activity.

MATERIAL AND METHODS

ATPase activity was studied in the apical meristems of *Allium cepa* adventitious roots grown at room temperature in aerated tap water. 3-4 cm long, growing roots were treated for 12 hrs with water solutions of following oncotics: 1.5×10^{-2} M cyclophosphamide, 1×10^{-5} M methotrexate, 2.7×10^{-2} M dacarbazine. Roots growing in tap water were the control.

0.5 mm long root apical meristem fragments, devoid of root cap were fixed according to two variants:

1. In 1% glutaraldehyde, prepared in 50 μ M cacodylic buffer at pH 7.0, at 0°C for 2 hrs. The tissues were then rinsed in 50 μ M Tris buffer at pH 7.0, at 23°C for 2 hrs. The enzymatic reaction was conducted in the incubation medium containing 50 μ M Tris buffer at pH 7.0, 2 μ M $Mg(NO_3)_2$, 2 μ M $Pb(NO_3)_2$ and 12 μ M KNO_3 , at 23°C for 2 hrs. The control treatment was carried out in the incubation medium lacking ATP.
2. In 1% glutaraldehyde + 4% formaldehyde, prepared in 100 μ M cacodylic buffer at pH 7.2, at 0°C for 2 hrs. The tissues were then rinsed in 100 μ M Tris buffer at pH 7.2, at 23°C for 2 hrs. The enzymatic reaction was conducted in the incubation medium containing 100 μ M Tris buffer at pH 7.2, 2 μ M ATP (sodium salt), 2 μ M $Mg(NO_3)_2$ and 2 μ M $Pb(NO_3)_2$, at 23°C for 2 hrs. The control treatment was carried out in the incubation medium lacking ATP.

Next, in both variants, the tissues were rinsed in distilled water for 2 hrs, postfixed at 2-3°C for 12 hrs in 1% OsO_4 , prepared in 20 μ M cacodylic buffer at pH 7.2, and dehydrated in alcohol series and propylene

oxide. The fixed tissues were embedded in epon, sectioned with LKB ultramicrotome and contrasted according to Reynold's method. The observations were conducted under a Tesla BS 500 electron microscope.

RESULTS

In the experiments two fixative media (weak, pure glutaraldehyde and glutaraldehyde+formaldehyde) were applied to eliminate possible artefacts, which could have resulted from the fixation method. Therefore, also the reaction used to identify active ATPases was conducted doubly. In this case ATPases of different type, which were activated by various ions (Ca^{2+} , Mg^{2+} , K^{+}) were also to be localized. The control reaction, used to show the specificity of the methods that identified active ATPases, was conducted in the incubation medium lacking a substrate (ATP sodium salt). The result of the conducted reaction was negative.

In the meristematic cells of root apices treated with cytostatics transport ATPase in the plasmalemma and active ATPases within ER and in the outer mitochondrial membrane were found. The course of the reaction which indicated the presence of active ATPases was independent of the method of tissue fixation and of the composition of incubation medium, however, to a large extent it depended upon the cytostatic used. The reactions which identified the active enzyme differed not only in the degree of their intensity, but also in their location.

In the meristematic cells subjected to dacarbazine (DTIC) the reaction was observed that indicated the presence of the active enzyme in contact with the plasmalemma (Figs. 1-3) and close to the outer membrane in numerous mitochondria (Figs. 1, 3, arrows). It is worth mentioning that under the conditions of the conducted experiment dacarbazine caused only slight changes in the protoplast structure in comparison with the control treatment. Among them a reduction in the number of ER cisternas and ribosomes, as well as the presence of numerous mitochondria were considered the most significant (Figs. 1-3).

In the presence of methotrexate (MTX) the reaction indicating active ATPases was very clear in the plasmalemma (Figs. 4, 5) and only slightly weaker within ER cisternas (Fig. 4). No reaction was observed within mitochondria. Moreover, in the presence of methotrexate transport ATPase activity was markedly higher than in the presence of dacarbazine. Cyto-morphological changes caused by methotrexate lay in the presence of numerous cisternas of rough ER and of numerous mitochondria at clearly low optical density of cytoplasm and reduced number of cytoplasmic ribosomes (Figs. 4, 5).

In the root meristematic cells subjected to cyclophosphamide a very strong reaction indicating the presence of active ATPases in the outer mitochondrial membrane (Figs. 6-10) and trace reaction in the plasmalemma (Fig. 8) were noticed. The observed reaction in the outer mitochondrial membrane was much more intensive in comparison with the analogous reaction in the cells subjected to dacarbazine.

In the presence of cyclophosphamide the cytoplasm of meristematic cells exhibited considerable optical density. Numerous mitochondria and ER cisternas were observed within it (Figs. 6-10)

DISCUSSION

The cytostatics used in the experiments belong to the group of antimetabolites (methotrexate) and alkylators (dacarbazine and cyclophosphamide). They cause chemical changes in DNA particles which restrain or prevent the replication process. Apart from the specific effect on the synthesis of deoxyribonucleoproteids (Deysson et al. 1981a, 1981b) these compounds cause a considerable decrease in the cell respiration level (Kaminskas and Nussey 1978, Podbielkowska et al. 1980, 1981, 1982) correlated with characteristic changes in the protoplast structure. The present experiment has confirmed these conclusions on the specific reorganization of a protoplast in meristematic cells, brought about by the analysed cytostatics.

Cell energy metabolism reflected, among others, in the synthesis of adenosine triphosphate can considerably influence the course of many processes such as mitotic activity dependent upon basic biosyntheses (DNA, RNA, proteins), or active transport — also energy-consuming process. The mentioned above data, when taken into account, have seemed to justify the studies on the activity of enzymes that regulate a cell transport through releasing energy in the ATP hydrolysis. Active H⁺ ATPases act in cells within their plasmalemmas as regulators of a sodium-potassium pump and within ER cisternas as regulators of the flow of some ions e.g. Ca²⁺. Mitochondrial F₁ ATPase that catalyses the reaction of ATP synthesis is characterized by different specificity.

As it has been proved in the previous experiment most of the active ATPases present in the root meristematic cells can be identified by the lead precipitation method (Podbielkowska and Wałęza 1987).

In the present experiment, applying previously tested techniques of ATPase localization, there have been demonstrated changes in the ATPase activity that depend upon the cytostatics action. The results so obtained clearly indicate that each of the analysed cytostatics effects the activity of the studied enzymes differently. It concerns, though to a various degree, both

Figs. 1-3. *Allium cepa* — 1% glutaraldehyde + 4% formaldehyde, dacarbazine 2.7×10^{-2} M; the reaction indicates the presence of active ATPases in the plasmalemma and outer mitochondrial membrane (arrows). 18000 $\times$

Figs. 4, 5. *Allium cepa* — 1% glutaraldehyde + 4% formaldehyde, methotrexate 10^{-5} M; the reaction indicates the presence of active ATPases in the plasmalemma (arrows). Fig. 4 — the reaction product in ER, 18000 $\times$

PLATE III

Figs. 6-10. *Allium cepa* — 1% glutaraldehyde + 4% formaldehyde, cyclophosphamide $1.5 \times 10^{-2} \text{M}$; the reaction indicates the presence of active ATPases in the outer mitochondrial membrane, 18000 $\times$

transport ATPase stimulated by K^+ and Mg^{2+} ions and ATPase localized within the outer mitochondrial membrane, found in plant cells by Winter-Sluiter et al. (1977); no uniform view on its role has been expressed so far.

The modified method of Wachstein and Meisel, used in this experiment, enabled to localize transport ATPase in the plasmalemma with relatively close accuracy. Its highest activity was observed in the presence of methotrexate, lower in that of dacarbazine and trace under the influence of cyclophosphamide. Similarly the strongest reaction indicating the presence of active ATPases was observed in the methotrexate treatment within ER. In the presence of dacarbazine, or cyclophosphamide the analogous reaction was not observed or was a trace one.

On the other hand, cyclophosphamide exhibited clear specificity in the activation of ATPases localized in the outer mitochondrial membrane. In the presence of other cytostatics the reaction in the mitochondrial membrane was weaker (DTIC) or not observed at all (MTX).

The interpretation of the obtained results is faced with some difficulties. If the part of cytostatics in the activation of transport ATPase, localized in the plasmalemma, can be explained as to reflect the regulation of flow through membranes under the conditions of energy deficiency, an increase in the activity of ATPase associated with ER membranes and especially of that present in the outer mitochondrial membrane is equivocal.

Endoplasmic reticulum, apart from playing a significant role in the synthesis of proteins and fatty acids, takes part in the processes of detoxication, which involves oxido-reductases (such as cytochrome B_5 and P-450) constituting within ER as incomplete electron transfer chain. Detoxication is a complex process (Szukalski et al. 1973). Its first stage, biotransformation is composed of the reactions of oxidation, reduction and hydrolysis that take place both within ER and in the lysosome interval. The second phase comprises coupling reactions which involve specific enzymes and ATP as an energy source.

Under the conditions of the conducted experiments an increase in the activity of ATPases associated with ER membranes might have been governed by an increased need for the energy utilized in the detoxication processes.

The outer mitochondrial membrane constitutes the specific kind of a molecular sieve. Into mitochondria it lets pass only micromolecular compounds whether to macromolecular compounds is impermeable. Instead, the inner mitochondrial membrane, as a typical semipermeable membrane, is characterized by a considerably higher selectivity towards micromolecular compounds. The transport of adenine nucleotides (ADP into mitochondria and ATP into cytoplasm) is of a considerable importance to a normal cell metabolism.

This transport, such as the flow of phosphates (intermediate in the Krebs cycle) and pyruvate, as well as amino acid transfer, involve specific carriers and are regulated by the energy status of mitochondria. All systems responsible for the transport through mitochondrial membranes cooperate due to the proton gradient produced by the electron transfer in the respiratory chain.

In the states of energy deficiency the membrane potential decreases. Therefore, it seems that in this case an active transport could have been regulated by the increased activity of ATPases hydrolysing ATP to release some energy. A change in the membrane potential, as it appears, may also concern the outer mitochondrial membrane and may be followed by a reduction of its permeability. The fact, that in the low-energy states the flow of nucleotide adenilates in mitochondria has to be intensified in aim to maintain a right energy balance, ought also to be taken into account. It seems that just in this process the role of active ATPases associated with the outer mitochondrial membrane might be possibly looked for.

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Zmiany aktywności ATPaz w komórkach merystematycznych korzeni Allium cepa L. wywołane działaniem onkostatyków

Streszczenie

Stwierdzono zmiany aktywności ATPaz w tkankach merystematycznych z wierzchołków wzrostu korzeni *Allium cepa* poddanych działaniu cytostatyków z grupy antymetabolitów (metotreksat) i związków alkilujących (cyklofosamid, dakarbazyna). Dakarbazyna i metotreksat w różnym stopniu stymulowały aktywność ATPazy transportowej związanej z plazmalemą, cyklofosamid, natomiast, wywołał spadek jej aktywności. W obecności cyklofosamidu i dakarbazyny wystąpiła charakterystyczna reakcja świadcząca o aktywności enzymu na terenie zewnętrznej błony mitochondrialnej. Działanie metotreksatu, natomiast stymulowało aktywność ATPaz związanych z błonami ER. Ponieważ użyte w eksperymencie cytostatyki powodują inhibicję oddychania komórkowego, wydaje się, że zmiany aktywności ATPaz związane były z regulacją aktywnego transportu w stanie niedoborów energetycznych.