Influence of hypoxia on protoplast structure in the plant cell

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Abstract

An influence of hypoxia on the protoplast's structure in the root tips meristematic cells of onion (*Allium cepa* L.) and of *Tradescantia bracteata* Small has been investigated. Hypoxia was caused either by respiratory inhibitors (sodium azide, 2,4-dinitrophenol), phosfon-D or by anaerobic conditions. In both cases characteristic membranization of cytoplasm was observed. It appeared as spherical and parallel structures of rough endoplasmic reticulum. The observed hypertrophy was not connected with the increase of nucleic acids and proteins synthesis. In the examined cells the membranization was accompanied by an increase of the lipids content.

INTRODUCTION

Numerous reports showed, that the changes in cell's ultrastructure caused by physical and chemical factors, very often, involve membranes of endoplasmic reticulum (ER), and this response is very fast. The quality of these changes depends of the kind of acting factor. It was described for example the degeneration of ER membranes, to myelin structures (David, 1970), or even, the change of membranes configuration. The typical example is the formation of extended spherical structures, which was observed in both the plant and animals tissues. Falck (1962), Perner (1966), Esau and Gill (1971), Dereuddre (1971, 1972), Duckett (1972) have observed such structures in the plant cells in the normal conditions.

Similar configurations of ER was occasionally described, in experimentally caused hypoxia (Wrisher, 1960, Gavaudan et al., 1960), however the role and origin of such structures remained unexplained. Podbielkowska and Kacperska-Palacz (1971), Podbielkowska et al. (1975) studying the effect of low temperature, respiratory inhibitors and phosfon-D on the onion root tips meristematic cells,

described the formation of membrane spherical structures consisting of RNA and proteins well visible by light and electron microscope. They suggested that these structures were due to the inhibition of respiration.

The experiments described here were designed in order to elucidate how far the process of cytoplasm membranization is the result of cell hypoxia and whether the enlargement in membrane structures involves nucleic acids and protein synthesis.

MATERIAL AND METHODS

Either the living or fixed material has been examined. Hypoxia was caused either by anaerobic conditions (nitrogen atmosphere), or by respiratory inhibitors (2,4-dinitrophenol, sodium azide) and phosphon-D.

In vivo observations were carried out on the onion bulb scale or hairs from stem and leaves of pumpkin. Material treated with inhibitors (in concentration 10 to 20 ppm during 15 to 120 min) was examined by means of phase contrast microscope ("Amplival" Carl Zeiss — Jena).

Before fixation the meristematic tissues of onion and *Tradescantia bracteata* root tips were treated with 2,4-DNP in concentration 50 or 100 ppm during 12 or 24 hours, and NaN_3 50 or 100 ppm during 12 or 24 hours, phosphon-D 100, 200 or 400 ppm during 12 or 24 hours, and in N_2 atmosphere during 3 or 6 hours.

In order to check the reversibility of the induced changes, some of the previously treated roots were transferred to the tap water for 24 hours.

The fixation of the experimental material was carried out as follows: chrom-aceto-formalin (CrAF) 0,5-1-20, chrom-formalin (CrF) 0,5-20, aceto-formalin (AF) 0,5-20, 2,5% glutaraldehyde in 0,5 M phosphate buffer pH 5,8 (for peroxidase fixation) or in 0,05 M cocadylate buffer pH 7,0. For autoradiographic examination the material was fixed in the mixture of ethanol (99,8%), glac. acetic acid, 40% formalin and chloroform 5-1-1 (v/v).

Longitudinal, microtome 3—5 μm thick sections were stained with:

1. Heidenhain Haematoxylin
2. Toluidine Blue (pH 3,4) for nucleic acids
3. Toluidine Blue (pH 7,4) for nucleic acids and basic proteins
4. Fast Green (pH 3,4) for proteins
5. Fast Green (pH 8,0) for histons
6. Acid Haematein for lipids
7. Leucofuchsin (Feulgen method) for DNA.

The level of nucleic acids and proteins was estimated by means of autoradiography using H^3 -thymidine, H^3 -uridine and H^3 -leucine in the following scheme: after the maximal time of action of respiratory inhibitors (24h) or the atmosphere of the nitrogen (6h) labelled thymidine,

leucine or uridine were added for 1h in the last hour of incubation, and extra labelled uridine in the first hour of incubation. In order to check the reversibility of the changes induced, labelled thymidine, uridine and leucine were added after 24 hours postincubation in water. The isotope was added for 1h in the last hour of postincubation.

For preliminary enzymatic analysis the tissues were stained with: benzidine according to Hall and Sexton (1972) and p-phenylenediamine according to Raa (1973) for peroxidases, neo-tetrazolium according to Seligman and Rutenburg (1951, after Zawistowski 1965) for succinic dehydrogenase.

The photomicrographs from "Amplival" Carl Zeiss — Jena microscope with apochromatic optics were made by means of Exacta Varex II^a Camera.

The part of the experimental material was examined in electron microscope. Onion root tips cells, treated with phosfon-D were fixed in 2% KMnO₄ or in glutaraldehyde with postfixation in 2% KMnO₄, dehydrated in ethanol and embedded in Vestopal. Sections were stained with 8% uranyl acetate according to Reynolds (1963, after Sjöstrand 1967).

RESULTS

The experiments showed that hypoxia induced the characteristic changes in cytoplasm structure and in morphology of the mitochondria. The observations of stained material by light microscope revealed the presence of the characteristic cytoplasmic structures similar to the ER. They were formed in 90% of the cells after treatment with respiratory inhibitors as well as after the anaerobic conditions (Plate I, Fig. 1—4). The examination with cytochemical methods revealed in these structures the presence of acid proteins, RNA and lipids.

Furthermore the characteristic changes in the morphology of mitochondria was observed. The long forms of mitochondria shortened, so the spherical shapes were most common and often were slightly inflated. These changes were particularly visible *in vivo*.

The data from electron microscope showed that the treatment with respiratory inhibitors caused the visible hypertrophy of the rough ER, which was both in form of spherical bodies as well as in cisterns (Plate II, Fig. 5) and in parallel arrangements (Plate II, Fig. 6). The ends of the cisterns, very often, formed characteristic vesicular enlargements (Plate III, Fig. 7).

The action of phosfon-D caused the increase of the number of vacuoles in the cells. It has been observed, that the single, fine vacuoles or groups of them, were surrounded by rough ER and by dense cytoplasm with numerous ribosomes (Plate III, Fig. 8). However, no essential changes in ultrastructure of mitochondria and Golgi bodies were noticed.

From the data on nucleic acids and protein synthesis the relationship between the metabolic activity of cells and the changes in the protoplast's structure were studied. From the autoradiographic studies it is concluded, that inhibitors or N_2 atmosphere, do not influend the incorporation of labelled uridine. The large number of ribosomes, free or bounded to ER membranes, which have been noticed were not connected with any additional RNA synthesis. After 24 hours (the maximal time) of incubation of the tissues with respiratory inhibitors very marked drop of the nucleic acids and proteins synthesis was visible.

The lower contents of DNA, RNA and proteins in inhibitors treated tissues were confirmed by cytochemical methods. Moreover, the strong increase in the amount of lipids was observed.

After 6 hours of incubation in the anaerobic atmosphere, these changes were quite different. The level of nucleic acids and proteins synthesis and their amount in the tissues was the same as in the control. On the other hand the lipids content increased similary as in the tissues treated with inhibitors.

It can be assumed, that 6 hours of the oxygen deficiency had no essential influence on the course of the basic syntheses.

The results presented above, may indicate that there is no corelation between the protoplast's membranization (as a consequence of ER hypertrophy) and the metabolic activity of the cells. The preliminary cytochemical tests on the amount of some respiratory enzymes (succinic dehydrogenase and peroxydases), which can serve as indicators of the intensity of respiratory processes in the studied tissues showed, that the amount of succinic dehydrogenase fell down very distinctly, after treatment with 2,4-DNP. However, this enzyme was not sensitive to the action of sodium azide. No significant difference in the amount of this enzyme was observed in either N_2 atmosphere or in control tissues. On the other hand the amount of peroxydases was markedly lower after treatment with sodium azide and 2,4-DNP as well as after incubation of the tissues in the N_2 atmosphere.

These observations indicate, that the changes in the amount of respiratory enzymes examined, were similar to those observed after treatment with inhibitors.

DISCUSSION

The data show that in hypoxia characteristic ER hypertrophy occur which could be interpreted as an adaptation of the protoplast to the low O_2 conditions for instance to compensate energy requirement in the cell. Such an adaptation could occur in the active areas of the cytoplasm, rich in rough ER. Similar hypertrophy of ER was observed by many authors

in cells of various tissues, both of rich or low metabolic activity (Falck, 1962; Perner, 1966; Esau and Gill, 1971; Dereuddre, 1971, 1972; Duckett, 1972).

The cytoplasm membranization of the meristematic cells in latent buds, described by Dereuddre (1971, 1972) might be caused by reduced access of oxygen to the apical growth tips, tightly covered by cutinized and glued scales.

The membranous structures observed by Perner (1966) in radicle of *Pisum* had been formed during germination, at the stage, when the seed coat prevent proper aeration.

The cell differentiation is accompanied occasionally by the occurrence of the membranous structures (Falck, 1962; Mikulska and Rodkiewicz, 1964; Rodkiewicz and Mikulska, 1965; Esau and Gill, 1971; Duckett, 1972). Therefore it seems probable that protoplast's membranization may be connected with the synthesis of substances required for the differentiation processes. Therefore the mechanism of the ER hypertrophy is not uniform but is always connected with the energy deficit in the cell. Our results show that the main factor responsible for changes in the ergastoplasmic structures is low oxygen content. It is possible that hypoxia, can induce the changes in glycolysis pathways in order to compensate the deficiency of energy in the cell. It has been found (Nejfa ch, 1973), that in the normal cell, glycolytic enzymes are agglomerated and are located in the cytoplasm areas along the membranes. Hypertrophy of the membranes could be, therefore interpreted as an adaptation which leads to enlargement of the active surface in the condition of low oxygen. This assumption is supported by observations of Oberling and Bernhard (1961) who noticed the spherical and parallel structures in cancer cells. In which the high level of glycolysis was observed.

CONCLUSIONS

All these results allow to draw following conclusions:

1. the cytoplasm membranization can be result of the cell's hypoxia,
2. the hypoxia induced either by respiratory inhibitors or by the nitrogen atmosphere produce similar effects on the ultrastructure of the cytoplasm,
3. the changes in the membrane structures are not connected with the increase of nucleic acids and protein synthesis,
4. the membranization of the cytoplasm is accompanied by an enlargement of the lipid content,

5. preliminary enzymatic analysis suggest that respiratory processes are involved in this phenomenon.

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Wpływ niedotlenienia na strukturę protoplastu komórki roślinnej

Streszczenie

Badano wpływ niedotlenienia na strukturę protoplastu komórek merystematycznych w wierzchołkach wzrostu korzeni *Allium cepa* L. i *Tradescantia bracteata* Small. Niedotlenienie powodowano stosując inhibitory oddechowe (2,4-dwunitrofenol, azydek sodu), fosfon D oraz atmosferę beztlenową (N_2). Obserwacje przeprowadzono w mikroskopie świetlnym i elektronowym. W oparciu o metodę autoradiografii oraz specyficzne reakcje cytochemiczne określano zawartość oraz poziom syntezy kwasów nukleinowych i białek, jak również zawartość niektórych enzymów oddechowych.

Stwierdzono charakterystyczną reakcję protoplastu na niedotlenienie wyrażającą się hipertrofią membran ER szorstkiego, tworzącego układy sferyczne lub paralelne. Te charakterystyczne konfiguracje ER obserwowano zarówno w mikroskopie świetlnym, jak i elektronowym (Tabl. I—III). Stwierdzono także, że w wyniku działania inhibitorów oddechowych następuje silna wakuolizacja protoplastu. W ultrastrukturze mitochondriów i diktiosomów nie zaobserwowano istotnych różnic. Przypuszczano, że zmiany w ultrastrukturze ergastoplazmy wiążą się ze wzmożoną syntezą kwasów nukleinowych i białek. Okazało się jednak, że po długotrwałym (24 godzin) działaniu inhibitorów poziom syntez tych związków spada bardzo wyraźnie (ok. 80%). Stwierdzono natomiast wzrost zawartości fosfolipidów. Wyniki specyficznych reakcji cytochemicznych na zawartość peroksydaz oraz dehydrogenazy bursztynianowej świadczą o zahamowaniu procesów oddechowych w badanych komórkach.

Przypuszcza się, że zaobserwowana hipertrofia ER jest swoistą adaptacją komórki mającą na celu wyrównanie deficytu energetycznego wywołanego przez niedotlenienie. Jest możliwe, że niedobór ATP uzupełniany jest na drodze glikolizy, który to proces odbywa się na terenie cytoplazmy, zwłaszcza w jej strukturach błonistych.

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Meristematic cells of root tips fixed in CrAF, stained with Heidenhain Haematoxylin.
 Fig. 1. *Allium cepa* treated with phosfon-D in concentration 100 ppm during 12 hours;
 Fig. 2. *Allium cepa* kept in the nitrogen atmosphere during 6 hours; Fig. 3. *Allium cepa*
 treated with sodium azide in concentration 50 ppm during 12 hours; Fig. 4.
Tradescantia bracteata treated with 2,4-DNP in concentration 50 ppm during 12 hours.
 Enl. ca 2500 $\times$.

Root tips from meristematic cells of *Allium cepa* treated with sodium azide in concentration 100 ppm during 12 hours, fixed in 2% KMnO_4

Fig. 5. Spherical forms ER. Enl. ca 14000 $\times$; Fig. 6. Parallel forms ER; Enl. ca 10000 $\times$

Fig. 7. Spherical ER. Arrows show vacuolar extensions en the ends of the membranes. 2% KMnO_4 , sodium azide 100 ppm, 12 hours. Enl. ca 14000 $\times$; Fig. 8. Rough ER and the dense cytoplasm with many ribosomes arround the vacuola. 2,5% glutaraldehyde with postfixation in KMnO_4 , phosfon — D 100 ppm, 24 hours. Enl. ca 18000 $\times$.

Root tips from meristematic cells of *Allium cepa*.