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Hydrolytic enzymes in spermatogenesis in *Marchantia polymorpha*

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The present paper gives the results of investigations on the activity of two hydrolases: esterase and acid phosphatase in the process of spermatogenesis in *Marchantia polymorpha*.

These investigations were stimulated by the results obtained by Maravolo et al. (1967) concerning the presence of esterases during sexual development in *Marchantia polymorpha*. Maravolo et al. found that antheridia are the sites of the increased esterase activity. The authors did not register, however, any changes in the activity of esterase in the antheridia during their development and maturity.

The fact that there was no change in the enzymatic activity within a tissue subject to the intensive process of cells differentiation, which appears during spermatogenesis, seemed surprising. It was considered necessary to analyse more carefully the activity of enzymes in reference to various stages of spermatogenesis. The particular attention was drawn to the period of "callose stage" in the development of gametes (Górska-Brylass 1969), during which the spermatogenous cells are temporarily isolated with a layer of callose. This period, as author tried to prove, plays an important role in the process of the differentiation of gametes.

Marchantia polymorpha is the material particularly suitable for the attempts like this because on the cross section of one antheridium the spermatogenous cells, in the same stage of nucleus development, appear in groups. Limits for appearance of such groups are most frequently drawn with straight lines which form regions of almost geometrical shapes. The antheridium containing the precisely "drawn" zones of cells in various stages of development forms quite unique material for various studies of cell properties in different periods of their development.

METHODS

The antheridiophores of *Marchantia polymorpha* were cut into 30-micron sections with a freezing microtome and then incubated in the adequate substrates required by the procedure of enzymatic methods.

Acid phosphatase was detected by using standard coupling azo dye

method. Two methods for demonstration of esterase were used .i.e. Holt's indigogenic method and the α-naptyl acetate method. (All the methods after Pearse 1961).

Control sections were treated with the substrat containing the addition of 0.01 M NaF as inhibitor of acid phosphatase and esterase.

After the enzymatic reaction the sections were stained with the mixture of carbolic fuchsin and 1% neutral red in relation 1:15. This way of staining the nuclei was found to be most suitable for this purpose. Both the methods of detecting esterase gave the same results. Holt's indigogenic method was considered preferable, however, because of the necessity for stimultanous staining of cells nuclei. The blue colour of indigo indicating the site of esterase activity contrasts well with the nuclei dyed in red.

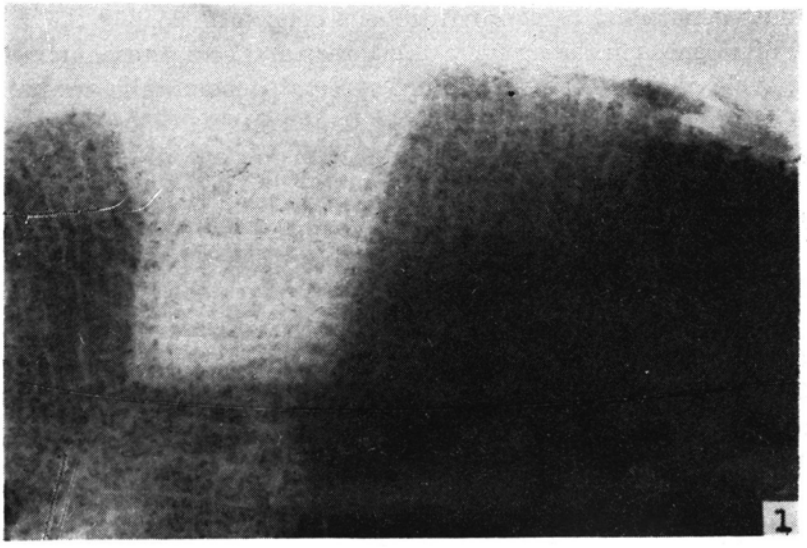
RESULTS AND DISCUSSION

Marchantia polymorpha proved to be the exeptional material on which, by using the histochemical methods for detecting enzymes, it was possible to obtain the necessary data not only about the site of the activity of enzymes but also the data concerning the degree of intensity of this activity.

On the cross sections of the anteridium containing the zones of cells in various stages of development the difference in the intensity of colour for the particular zones is noticeable. In the case of indoxyl acetate method there appear the zones intensively blue, light-blue, the zones in which indigo granules appear singly and the colourless zones. The limits for the colours differentiated in this way correspond to the limits of the zones for the particular stages of development in spermatogenous cells (Fig. 1). Acid phosphatase investigated on various stages of development of spermatogenous tissue did not show the increased activity in relation to other tissues of antheridiphore. But here as well the various zones of developing cells differed in the intensity of colour. This colour depended on the density of dark brown granules being the product of enzymatic reaction. In these conditions the difference in the activity of enzymes in various stage of development of spermatogenous cells was possible to be noticed.

The obtained results confirmed the data received by Maravolo et al. (1967) showing that antheridia are the sites of the increased esterase activity. This increased activity was not found to refer, however, to the whole cycle of development of spermatogenous cells. The final stages of spermatogenesis show the decrease and then the disappearance of the esterase activity.

Plate I



	A	B	C	D	E	F	G	H
esterase	+++	++	++	+	0	0	0	+0
acid phosphatase		0	0	0	++	++	++	+++

Fig. 1. Fragment of antheridium in *M. polymorpha* after indoxyl esterase reaction. The light square shows the zone full of spermatids in the period of their transformation into spermatozoids. The adjacent regions consist of the spermatogenous cells in their earlier stages of development. 800×

Fig. 2. The activity of enzymes in the life cycle of the last generation of spermatogenous cells in *Marchantia polymorpha*.

In addition, the more precise observation showed the fluctuation of esterase activity and acid phosphatase activity to be difficult to register in the first generations of cells, whereas this fluctuation was very clear in the last generation of spermatogenous cells (Fig. 1).

The differences in the activity of esterase and acid phosphatase during the life cycle of the last generation of spermatogenous cells are presented in Fig. 2. They are marked according to the intensity of colour in the particular zones of development of cells with the marks +++ ++ + 0.

Schematic drawings A-H in Fig. 2, show the succesive stages of development leading to the formation of the spermatozoid. The drawings E-G show the "callose stage" of this cycle. (Callose in walls marked with a thick black line).

In the life cycle of the last generation of spermatogenous cells the gradual decrease of esterase activity in the first stages of mitosis (Fig. 2, A-D) and its complete disappearance in the period of cytokinesis which produces spermatids (Fig. 2, E) have been observed. In the period of transformation of spermatid into spermatozoid it is possible to notice again a small point of esterase activity on one of the corners of spermatid. Its position corresponds to the position of blepharoplast. The brief presence of the point of esterase activity in the differentiating spermatid is presented in the Plate by the mark +0.

In the period of cytokinesis the simultaneous increase of acid phosphatase activity has been observed. It is still greater in the following stages of the differentiation of spematids (Fig. 2, E-H). The nature spermatozoids showed the high phosphatase activity and no esterase activity.

In the differentiating spermatogenous cells of fern — *Adiantum cuneatum* the author noticed the similar process i.e. the decrease of the high esterase activity at the beginning of development and its complete disapearance in the mature spermatozoids, (data non-published).

It should be pointed out that the moment of the "replacement" of the enzyme i.e. the disappearance of esterase activity with simultaneous increase of acid phosphatase activity coincides with the beginning of the "callose stage". This phenomenon may be one more evidence showing that "callose stage" is metabolically important in the process of the differentiation of gametes.

SUMMARY

The activity of two hydrolases: esterase and acid phosphatase in various stages of spermatogenesis in *Marchantia polymorpha* was investigated. It has been stated that the early generations of spermatogenous cells show the increased esterase activity. In the life cycle of the last generation of spermatogenous cells the gradual decrease of esterase activity in the first stages of mitosis and its complete disapearance in the period of cytokinesis which produces spermatids have been observed. In the period of cytokinesis the simultaneous increase of acid phosphatase activity has been observed. It is still greater in the following stages of the differentiation of spermatids. The mature spermatozoids showed the high acid phosphatase activity and no esterase activity.

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Enzymy hydrolityczne w spermatogenezie u Marchantia polymorpha

Streszczenie

Badano aktywność dwu hydrolaz: esterazy oraz kwaśnej fosfatazy w różnych etapach spermatogenezy u *Marchantia polymorpha*. Ustalono, że wzmożona aktywność esterazy charakteryzuje wczesne generacje komórek spermatogenicznych. W cyklu życiowym ostatniej generacji komórek spermatogenicznych obserwuje się stopniowy spadek aktywności esterazy w pierwszych fazach mitozy, aż do zupełnego jej zaniku w okresie cytokinezy prowadzącej do powstania spermatyd. W okresie cytokinezy zaznacza się jednocześnie wzrost aktywności kwaśnej fosfatazy, która w następnych etapach różnicowania się spermatyd stale wzrasta. Dojrzałe spermatozoidy wykazywały wysoką aktywność kwaśnej fosfatazy oraz zupełny zanik aktywności esterazy.