6. CHLOROPLAST THYLAKOID MEMBRANES IN THE INTINE OF RAGWEED POLLEN GRAINS AS A POSSIBLE CONSTITUENT IN THE BIOSYNTHESIS OF ITS EXTREME ALLERGENIC ANTIGENS

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Abstract

During our experimental Transmission Electron Microscope (TEM) investigations on the partially dissolved ragweed pollen grains chloroplasts were found in the intine. Till this time chloroplasts or other kind of plastids were not observed in the pollen wall. On the surface of the thylakoid membranes different kinds of molecular organizations were observed which may be important in the biosynthesis of antigens. But as an alternative hypothesis it must be mentioned, that the products of assimilation are factors of extreme allergenic character of ragweed pollen grains. The accumulation of extreme allergenic antigens, mainly antigen E in the wall (intine) of ragweed pollen grains have been described by several authors. Direct contact come with the intine of the pollen grains and the mucosa of the human respiratory organs. In consequence of the body temperature and the moist surface of the respiratory passages pollen tube development begins and the antigens enter the human cells.

Key words: Ragweed pollen, experimental ultrastructure, allergenic antigens.

Introduction

The extreme allergenic character of the antigens of ragweed pollen grains was pointed out, in the first place, in aeropalynological papers. GRATER and STEMEN (1967) emphasized, that among the numerous pollen forms the antigenic agents of ragweed have been the most intensive studied. Based on the paper of O'ROURKE (1996), *Ambrosia*, with 45 species is the most important aeroallergen in North America. Two species (*Ambrosia trifida*, and *A. elatior*) have 52 identifiable antigens. Of these, two are acidic proteins and major antigens (E and K). The pollen wall (intine) localization of antigenic and allergenic proteins in the ragweed pollen grains was established also by several authors (KNOX and HESLOP-HARRISON, 1970, 1971, KNOX, HESLOP-HARRISON and REED, 1970, KNOX, WILLING and ASHFORD, 1972). TEM results of *Ambrosia* pollen grains were published by PAYNE and SKVARLA (1970), but acetolysed material was used, so the intine and the protoplasm were degraded, and the ultrastructure of the ectexine was described.

During our ultrastructure studies on partially dissolved and degraded pollen grains, we established in the partially dissolved pollen grains of *Platanus hybrida* with 50% glycerine, that the organelles were in an excellent preservation (KEDVES, PÁRDUTZ and

TÓTH, 1999). We have started several experiment on fresh ragweed pollen grains and the results of the partially, dissolved pollen grains are the most surprising.

Materials and Methods

Fresh ragweed pollen grains were dissolved in 50% glycerine at 30 °C, for 30 days the washed material was postfixed in OsO_4 embedded in Araldite. The investigations were made on a Tesla BS-540 (resolution 6-7 Å) and Opton EM-902 (resolution 2-3 Å) instrument.

Results and Discussion

At the general survey picture (Plate 6.1.) the characteristic *Compositae* type outer wall ectexine, (ect) with spinac (sp), on the apertural area (aa), the oncus (on), the endexine (en), the intine (in) with some chloroplasts (chl) are well shown. The organelles of the protoplasm are also well illustrated, the characteristic plasma membrane (pm) may be emphasized. The chloroplast of the intine is connected with the plasma membrane (Plate 6.1., plate 6.2., figs. 1,2). The highly magnified pictures of the chloroplasts were taken with Opton instrument, illustrate the characteristic thylakoid membranes (Plate 6.2., figs. 1,2) with the molecular organization (Plate 6.3.). There are granules of different size on the membranes.

This organization, together with chemistry and function, were described in detail in the cell biology or molecular cell biology handbooks, (cf. AVERS, 1986). The freze-fractured picture from the thylakoid membranes of STAEHELIN, CARTER and MCDONNEL, was published in 1980. Four different membrane faces were distinguished with the superficial molecular systems. The function of these was described by BOGORAD (1981); e.g., ADP + P $\rightarrow$ ATP, and further functions were established. From the point of view of the allergenic character of ragweed pollen grains, it is important, that the place of the above mentioned functions is the intine, which is also the place of the accumulation of the antigens.

Plate 6.1.

Ambrosia artemisiifolia L. General survey TEM picture from the partially dissolved pollen grains. The picture was taken with an instrument of 6-7 Å of resolution. Experiment No: 1045. Negative no: 7542, 9.000x.
Abbreviations: aa = apertural area, on = oncus, sp = spinae, ect = ectexine, en = endexine, in = intine, chl = chloroplast, pm = plasma membrane.

Plate 6.2.

1,2. *Ambrosia artemisiifolia* L. TEM pictures from the chloroplast from the intine were taken with an instrument of 2-3 Å. Detail from the chloroplast, the plasma membrane and the protoplasm. 1. Negative no: 7865, 80.000x, 2. Negative no: 7866, 150.000x.

Plate 6.3.

Ambrosia artemisiifolia L. Detail of the thylakoid membranes. The electron dense granular system, which may be identified as the superficial molecular structures of STAEHELIN, CARTER and MCDONNEL 1980, after AVERS (1986, p. 388) are well shown. Negative no: 7867, 400.000x.

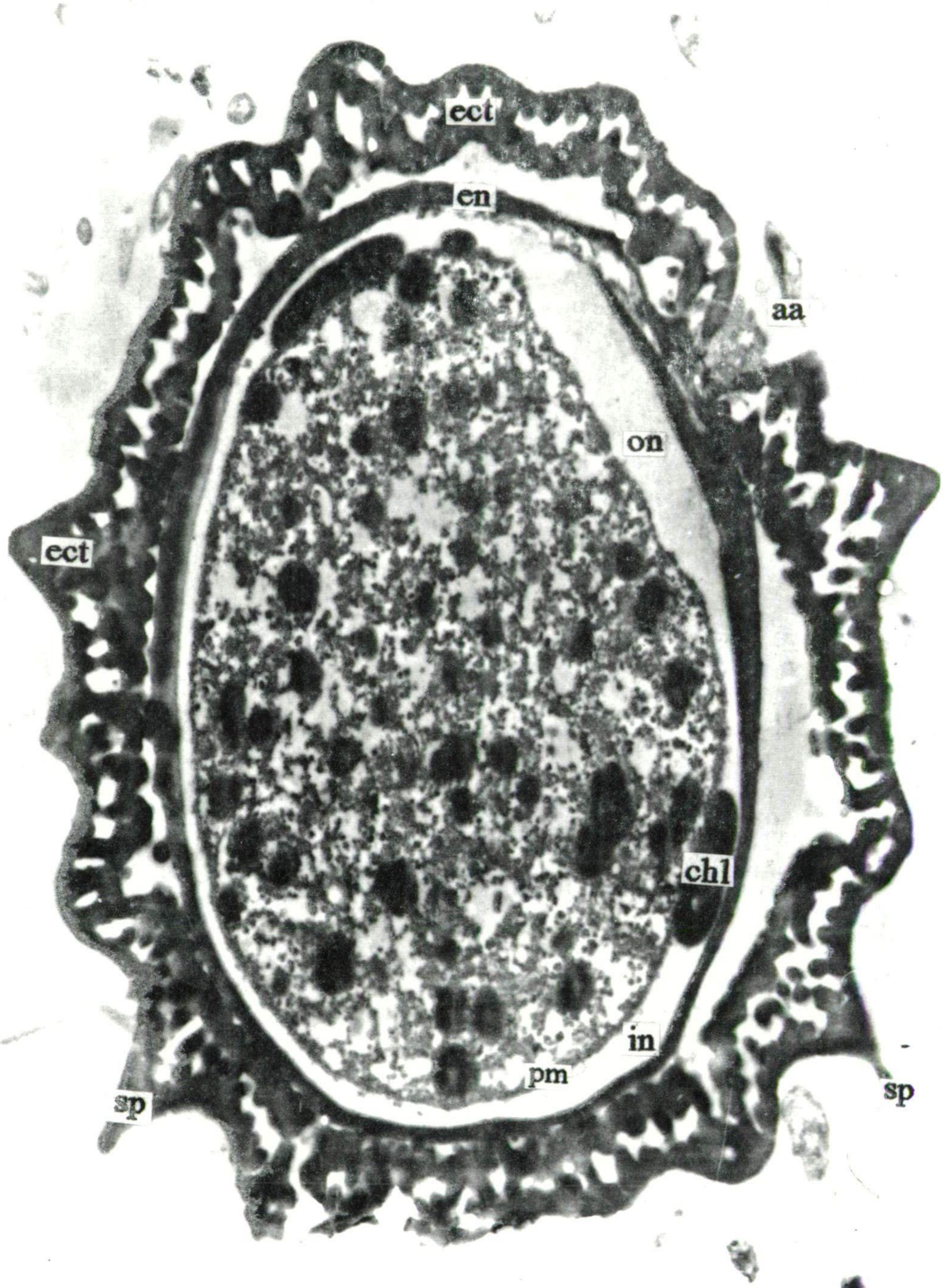

Plate 6.1.

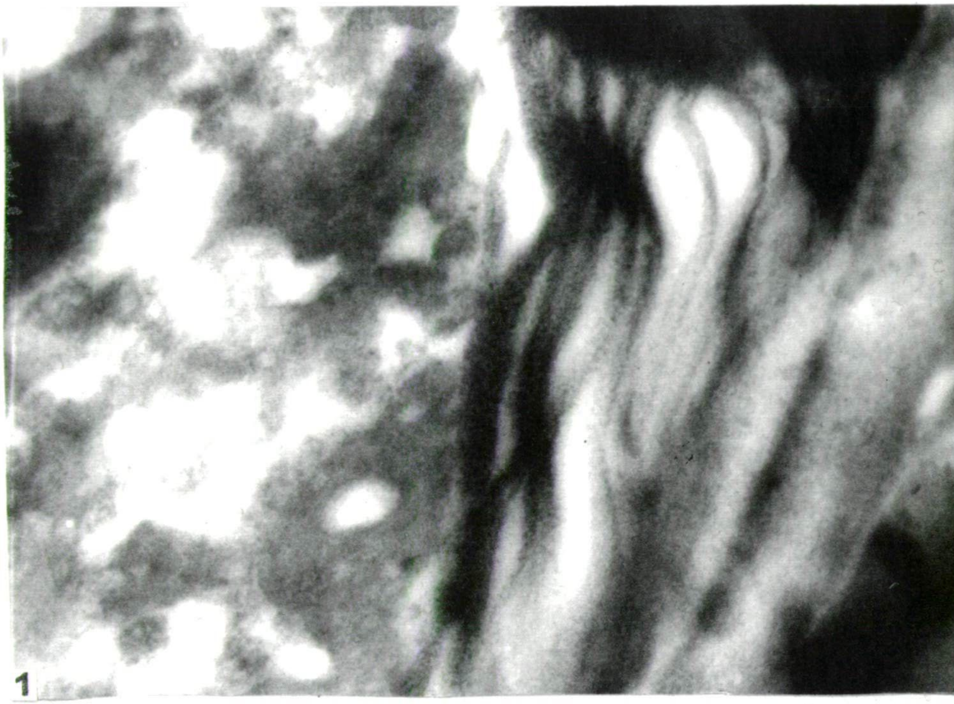

Plate 6.2.

Plate 6.3.

Conclusions

Taking into consideration the complex function of the thylakoid membranes it may be presumed, that these are in connection to the biosynthesis of the peculiar antigens of ragweed pollen grains. In addition we should like to point out that the products of the chloroplasts in the intine are factors of the reinforcement effect of the antigens. Finally, it is hoped that the experimental palynological method will be useful to better understand the allergenic effect of the pollen grains.

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