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PAS Reaction to the Perispore of the Fern Spores

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シダ類胞子の周皮の PAS 染色

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概 要

シダ類の胞子の PAS 染色を11種類で試みた。ヒメシダでは胞子壁の最外層にある周皮はタペータムの崩壊後、胞子囊に出現する顆粒から形成される。この顆粒は胞子壁の表面に付着する前は PAS 染色に対して陽性であるが外膜に付着すると次第にその染色性が減少し完成した周皮では PAS 染色に対して陰性になる。このことは、周皮は単にタペータム細胞からの分泌物によって形成されるのではなく分泌または再構成された物質の性質の変化があって周皮を形成するものと思われる。完熟した胞子の周皮の PAS 染色に対する反応を観察してみると、オシダ科やチャセンシダ科にみられる異状の周皮は PAS 染色に陰性であったが、ピロシア・ルベストリスやヒトツバ、ビロードシダ、ゼンマイ等の周皮は強い陽性を示した。このことは、周皮を形成する物質は単一なものではなく、種々の物質がその形成に関与していることが推測される。この点は、シダ類全体で共通の構造をもつ外膜とは大きな相違点である。

従来、発達した外膜をもつ胞子では周皮の形成は貧弱であるといわれていたが、このこととも今回の研究で確かめられた。
PAS Reaction to the Perispore of the Fern Spores

Most fern spores have a perispore which is formed from the tapetum cells and surrounds an exospore. The perispore has various ornamentations and displays various colors from species to species. These variations in the perispore of fern spores must originate from the differences of the components of the perispore. Up to the present time, a main component of the perispore is considered to be polysaccharides, however any critical histochemical researches have not been done on the fern perispores.

In order to clarify the substances contained in a perispore the PAS reaction was tried on the perispore of several fern species in the present study.

Methods and materials

Spores were collected from fresh fronds and stored in the desiccator until the beginning of this study. For the section, matured spores were embedded in 1% agar and fixed in the FAA fluid, and dehydrated in the following series; 30%, 50%, 70%, 85%, 95%, and 100% ethanol, and ethanol and GMA (glycol methacrylate) mixture (1:1). These specimens were embedded in GMA monomer mixture, and polymerized at a temperature of 60°C for three hours, and sectioned 2 microns with a glass knife. PAS reaction (periodic acid Schiff) was employed for the staining of the sections in the following manner; 1% periodic acid (10 min.), wash in running water (10 min.), Schiff's reagent (15 min.), 0.5% sodium metabisulfite (three immersions, 3 min. each time), wash in running water (5 min.), and dry and apply cover slip. Young fronds with sporangia were fixed in the FAA fluid to study the process of perispore formation, and the sections of sporangia were made by the same procedure as mentioned above. Eleven species, as described in the following Results section, were chosen for the present study because their spores have been studied morphologically and ontogenetically.

Results

*Thelypteris palustris* Schott

All the tapetum cells in a sporangium break down simultaneously after the
formation of the tetrads and then many granules appear around the tetrads. These granules are stained faintly red with the PAS reaction (Plate 1, A). Deposited on the surface of an exospore, these granules change to the brown or blackish brown cristate perispore. This colored perispore becomes negative for the PAS reaction. In the previous paper (1982) I noted in this species that the PAS positive granules scattered in a sporangium were not stained with Giemsa (Plate 1, D), but after the deposition on the surface of the exospore these granules displayed a strong reaction to Giemsa solution and gradually stained dark blue (Plate 1, E, F).

*Pyrrhrosia rupesris* Ching

The developmental process of the perispore is shown in Plate 2, A–D. The most interesting fact is that individual spore is surrounded by a tapetum membrane during most of the stages in the development of the perispore. The secretion from the tapetum cells is deposited on the surface of an exospore gradually, and then a very thick and orderly perispore is formed. During the last stage of the perispore formation, the tapetum membrane dissolves to envelop each spore. The secreted substances are stained faintly red with the PAS reaction and the developed perispores are stained strongly purple red (Plate 2, E).

*Pyrrhrosia linearifolia* Ching and *P. lingua* Farwell

After the separation of each tetrad, the tapetum cells disintegrate and change to membranes containing many spherical bodies. A membrane envelops each individual spore, putting several spherical bodies between the exospore and the outer membrane (Plate 2, F, G, H). The spherical body is larger in *P. lingua* than in *P. linearifolia*. Lugardon (1981) demonstrated that these granules display the same fine sculpture as does the exospore, and are homologous to spermatophyte Ubish bodies.

The granules of two species are not stained with the PAS reaction the same as the exospore; on the contrary the outer membrane is stained red clearly as the perispore of *P. rupesris*. A similar feature is observed in the case of Giemsa staining; that is, the outer membrane is stained dark blue as in many fern perispores, while the granules are stained pale blue like the exospore. From these facts it seems that the outer membrane of both species is a perispore and the granules are substances related to sporopollenin which compose the exospore.

*Pteris vittata* L.

The perispore of this species is a very thin hyaline membrane which adheres closely to the well sculptured exospore, therefore it is very difficult to locate this perispore under the light microscope. This perispore is only visible around spores treated with NaClO, or in a section stained with Giemsa (Plate 1, A, Plate 3, D). The perispore of this species responds faintly to the PAS reaction. It can not be
decided from the present study whether the weak response to the PAS reaction is caused by the thin section or the components of the perispore.

*Gonocormus minutus* v.d. Bosch

The spore of this species has a thin and transparent exospore but no perispore. Many granules are observed on the surface of the exospore by SEM (Plate 3. B, C). These granules display the same reaction to the PAS staining and Giemsa solution as the granules of *Pyrrosia linearifolia* and *P. lingua* as mentioned above. From these facts it seems that these granules are not perisporous but exosporous. The endospore or cell wall of a gametophyte formed in a sporangium of this species is stained strongly purple with PAS reaction (Plate 3. A).

*Osmunda japonica* Thunb.

The perispore of the genus *Osmunda* is not visible under the light microscope. However Lugardon (1978) demonstrated using TEM that in *O. regalis* the perispore adheres to the surface of an exospore and its thickness is usually under 0.1μm and forms many spinulose projections. In the present study, the outer part of the sporoderma is stained strongly purple with the PAS (Plate 1. I) and this part agrees with the perispore found in *O. regalis*. Therefore, it seems that the perispore makes micro-ornamentation on the exospore in this species.

*Ceratopteris thalictroides* Brongn

Sahashi (1979) reported that the spore of this species has a filamentous or lanate layer around the outer part of the spore which should be equal to the perispore. In the present study a thin layer dyed with the PAS staining is located around the well-sculptured exospore (Plate 1. J, K).

*Cheilanthes kuhnii* Milde var. *brandtii* Tagawa

The perispore of a matured spore is fibrous and pale brown. Although the inner part of the perispore is stained strongly with the PAS reaction (Plate 1. L), the outer part is stained faintly. The density of perisporous fibers seems to account for the different intensity of staining between the outer and inner parts.

*Cryptinus veitchii* Copel.

The perispore is blackish brown granulate and approximately 2μm thick. The form and size of the granules of the perispore varies and papillose or spinose processes are densely scattered on the granulate perispore. In the sections of the matured spores, the perispore is not stained with the PAS reaction (Plate 3. D, E)

*Asplenium unilaterale* Lam.

The perispore is blackish brown, membranous and sometimes slightly separated and cavate in the elevated portions forming the crest. In a section the winged perispore is not dyed with the PAS reaction (Plate 1. M).
Lepisorus thunbergianus Ching

The spore of this species has no perispore, but has a well-sculptured exospore. In section, there are not any parts stained with the PAS reaction in the sporoderma of this species (Plate 1. O).

Discussion

The PAS reaction depends on a selective oxidation by periodic acid which attacks 1, 2 amino alcohol, oxidizing the adjacent group to aldehydes and breaking the carbon chain at these sites. The aldehydes then are detected in situ by their reaction with Schiff's reagent to form a reddish purple color. Structures rich in polysaccharides, mucopolysaccharides, glycoprotein or glycolipids are stained with this reaction.

From the present study it may be said that the PAS reaction for the fern sporoderma is useful in detecting the thin perispore surrounding the exospore. This is difficult to find with the light microscope as is the perispore of Osmunda japonica and Ceratopteris thalictroides. The degree of the staining with the PAS reaction differs between species. The thin perispore of Osmunda japonica and the thick one of Pyrosta rupestris show the most positive reaction to the PAS staining as well as the endospore (contained callose) in a spore of Gonocormus minutus. However, other perispores of matured spores are stained either faintly or not at all with the PAS reaction. A typical winged perispore in Thelypteris and Asplenium is not stained with the PAS reaction. Therefore these spores seem to have few polysaccharides and allied substances in the perispore, although the PAS positive substances are related to the formation of the perispore at the early stage of the developmental process as shown in Thelypteris palustris. These facts seem to show that various substances are contained in the perispore of fern spores. This is very different from the exospore which usually shows a constant reaction to the PAS reaction and Giemsa staining among the different species.

References

Sahashi, N. 1979. Spore morphology of *Ceratopteris thalictroides*, Japanese Journal of
Palynology 24: 15-25.

**Plate 1** A-F. *Thelypteris palustris* Schott
A. The PAS positive granules in a sporangium.
B. A photographe of the same stage as Plate A by the interference microscope.
C. A photographe of a matured spore stained with the PAS by the inter-
ference microscope.
D. The PAS positive granules are negative to the Giemsa staining (arrows).
E. The perispore in the early developmental stage (Giemsa staining).
F. A matured spore dyed with Giemsa, perispore stained dark blue, and
exospore stained pale blue.
G. A section of a spore in *Pteris vittata* L. (PAS staining)
An endospore is stained dark blue with Giemsa staining.
I. A section of a spore in *Osmunda japonica* Thunb.
The outer part of the sporoderm (arrow) is stained with the PAS.
J, K. *Ceratopteris thalictroides* Brongn, sections of spores, arrows show a
thin perispore stained with the PAS.
L. A section of a matured spore in *Cheilanthes kuhnii* var. *brandtii* Tag-
gawa. The arrow shows the part stained with the PAS reaction.
M. A section of a matured spore in *Asplenium unilaterale* Lam.
N. A section of a matured spore in *Thelypteris laxa* Ching.
The brown perispore is not stained with the PAS.
O. A section of a spore in *Lepisorus thunbergianus* Ching.
   c: exospore p: perispore Scales show 10μm

**Plate 2** A-D. *Pyrrosia rupestris* Ching, the developmental process of the perispore
(stained with Giemsa).
E. A section of a matured spore stained with the PAS.
F. A section of a matured spore stained with the PAS in *Pyrrosia linea-
rifolia* Ching.
G, H. *Pyrrosia lingua* Farwell.
G. A section of a matured spore stained with Giemsa.
H. A section of a spore stained with the PAS.
   c: exospore p: perispore Scales show 10μm.

A. A section of a spore, the endospore (arrow) is strongly stained with the PAS

B, C. Microphotographs by SEM, many glanules (not stained with the PAS) are scattered on the exospore.

D, E. *Cryptinus veitchii* Copel.

D. A matured spore.

E. A section of a matured spore, a blackish brown perispore is not stained with the PAS.

F, G. *Pteris vittata* L.

F. The distal face of a matured spore.

G. A section of a spore stained with Giemsa.

:e: exospore  :p: perispore  Scales show 10μm