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Cytochemical Identification of Reserve Polysaccharides in Rumen Ciliates by Microspectrophotometry

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Abstract

Reserve polysaccharides of ophryoscolecid and isotrichid ciliate protozoa occuring widely in the bovine rumen were investigated. Iodine stain displayed reddish brown granules in the ectoplasm of ophryoscolecids or in the endoplasm of isotrichids. Moreover, the stain showed bluish purple grains in the endoplasm of ophryoscolecids and of *Isotricha* spp. Both the granules and the grains were stained intensely with periodic acid-Schiff (PAS) stain. The iodine- and PAS-positive materials disappeared after α-amylase digestion, while weakly reacted materials remained in the ectoplasm of ophryoscolecids or in the endoplasm of isotrichids after β-amylase digestion. Transmittances of iodine-positive granules were measured directly by a microspectrophotometer (Olympus AH2-STK). The minimum transmittances (λmin) of the bluish grains of ophryoscolecids and *Isotricha* spp. were 580—600 nm, suggesting that the grains are starch. Values of λmin of the reddish brown granules both of ophryoscolecids and of isotrichids were 520—530nm, which were similar to that of amylopectin of rice starch (540nm) rather than that of glycogen purified from *Neurospora crassa* (<400nm). These data suggest that the reserve polysaccharides of ophryoscolecid and isotrichid ciliates are amylopectin.

Key words: amylopectin, microspectrophotometry, reserve polysaccharide, rumen protozoa

Introduction

Reserve polysaccharides of rumen ophryoscolecid and isotrichid ciliate protozoa are considered to be amylopectin (Eadie *et al.*, 1963, Forsyth and Hirst, 1953, Wakita and Hoshino, 1980). However, the general agreement on this point was derived only from chemical analysis of polysaccharides that were extracted from the protozoa or protozoal fractions of rumen contents. There is some possibility that these polysaccharide fractions were contaminated with feed materials or other microorganisms which exist in rumen contents or inside of the bodies of ciliates. The polysaccharides were not well characterized *in situ*. Then, present authors investigated the reserve polysaccharides of rumen ciliates cytochemically.

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Materials and Methods

Source of protozoa:

Rumen contents were collected through rumen fistula from a 22-month-old steer. The contents were settled in a bottle with an air tight cap at 39°C for 1 h. The sediment and fluid at the bottom of the bottle was collected by capillary pipets. The recovered fluid contained four genera of ciliates, *Entodinium, Polyplastron, Isotricha* and *Dasytricha.*

Staining:

Paraffin sections of the ciliates were prepared after fixation with Bouin's fluid. Periodic acid-Schiff (PAS) stain and iodine stain with amylase digestion controls were performed as previously mentioned (Nakai and Ogimoto, 1983).

Microspectrophotometry (MSP):

Ciliates in 4 μm thick paraffin section,

amylopectin purified from rice starch, and glycogen from *Neurospora crassa,* of which the latter two were kindly supplied by Dr. M. Kobayashi, were stained with iodine solution (0.2% I_2, 0.4% KI (w/v) in water) (Nakai and Ogimoto, 1983). Their transmittance spectra were measured directly by a MSP (Olympus AH2-STK). Wavelength ranged from 400 to 700 nm, magnificent of the objective was 50x, and the pinhole-size was 3 μm.

Results

With PAS stain, the endoplasm of isotrichid ciliates and large grains in the endoplasm and small granules in the ectoplasm of ophryoscolecid ciliates, *Entodinium* spp. and *Polyplastron multivesiculatum*, were stained intensely (Fig. 1).

Iodine stain displayed the endoplasmic grains as bluish purple color and ectoplasmic granules as reddish brown in ophryoscolecids (Figs. 2 and 3). Of *Isotricha* spp., the large isotrichid, both small reddish brown granules and larger bluish purple grains were observed in the endoplasm, whereas only reddish brown granules were observed in the endoplasm of *Dasytricha ruminantium*, the small isotrichid (Fig. 4).

Iodine and PAS positive materials disappeared from all ciliates after α-amylase digestion (Figs. 5—7). After β-amylase digestion, however, reduced number of weakly to obviously iodine and PAS positive granules remained in the ectoplasm of ophryoscolecids and in the endoplasm of isotrichids (Figs. 8 and 9).

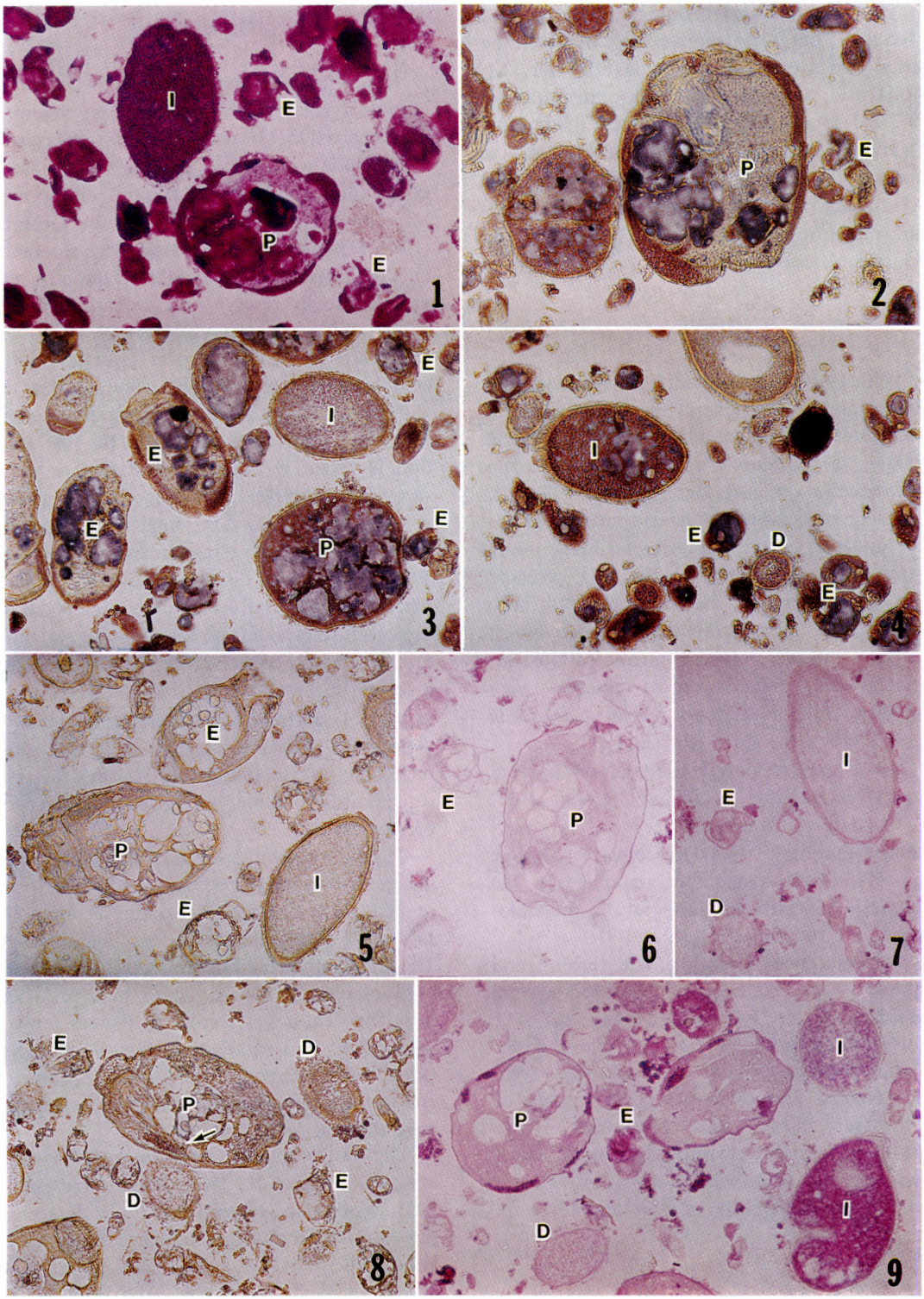
Transmittance spectra of the grains and granules of the ciliates stained with iodine were measured directly by the MSP (Fig. 10). The minimum transmittance (λ min) of bluish grains of ophryoscolecids and *Isotricha* spp. were 580—600nm. The λ min of reddish granules of ophryoscolecids and isotrichids were 520—530nm, and were much larger than that of glycogen from *N. crassa* (<400nm), and similar to that from amylopectin of rice starch (540nm).

Discussion

Kinoshita *et al.* (1984) incubated goat rumen entodiniid ciliates *in vitro* with rice starch grains, and observed that brownish granules stained with iodine stain were accumulated in the ectoplasm of the ciliates and that the granules disappeared after further incubation. Wakita and Hoshino (1989) obtained similar results in ovine entodiniid ciliates. These authors considered the brownish granules to be reserve polysaccharides of the ciliates. In the present study, we also observed iodine-stained reddish brown granules in the ectoplasm of ophryoscolecid ciliates and in the endoplasm of isotrichids. These granules were stained also with PAS stain. Since the PAS reaction is given by any polysaccharide or polysaccharide complex containing adjacent diol groups, glycogen, amylose, amylopectin, dextran, 1,4-xylan, or cellulose may be expected (Ryley, 1973). The granules disappeared after α-amylase digestion, while reduced number of weak to obvious PAS or iodine positive granules remained after β-amylase digestion. Results of α-amylase digestion suggest α-1,4-linked glucose residues in the polysaccharide molecules, and results of β-amylase digestion suggest α-1,6-glucosidic interchain linkage (Ryley, 1973). Thus the constituent of the granules is expected to be glycogen or amylopectin.

The transmission spectra of the iodine complexes of polysaccharides are considered to be related to the degree of branching in the polysaccharide (Archibald *et al.*, 1961, Ohashi, 1959). The λ min of the reddish brown granules of both ophryoscolecids and isotrichids were 520—530 nm. The λ min was similar to that of amylopectin (540 nm) rather than that of glycogen (<400 nm). Therefore, the reserve polysaccharides of ophryoscolecid and isotrichid ciliates were thought to be amylopectin.

The results coincide with previous chemical analysis (Eadie *et al.*, 1963, Forsyth and Hirst, 1953, Wakita and Hoshino, 1980). Polysaccharides purified from ovine isotrichids (Forsyth and Hirst, 1953, Oxford, 1951) and *Entodinium caudatum* (Eadie *et al.*, 1963) were identified as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amylopectin with average chain lengths of 22 and 19 glucose residues in the molecule, respectively. The λmin of the iodine complex with the latter polysaccharide was described to be 540 nm. Polysaccharides purified from entodiniids was identified as amylopectin with an average chain length of 22—25 glucose residues and with λmin of 490 nm (Wakita and Hoshino, 1980).

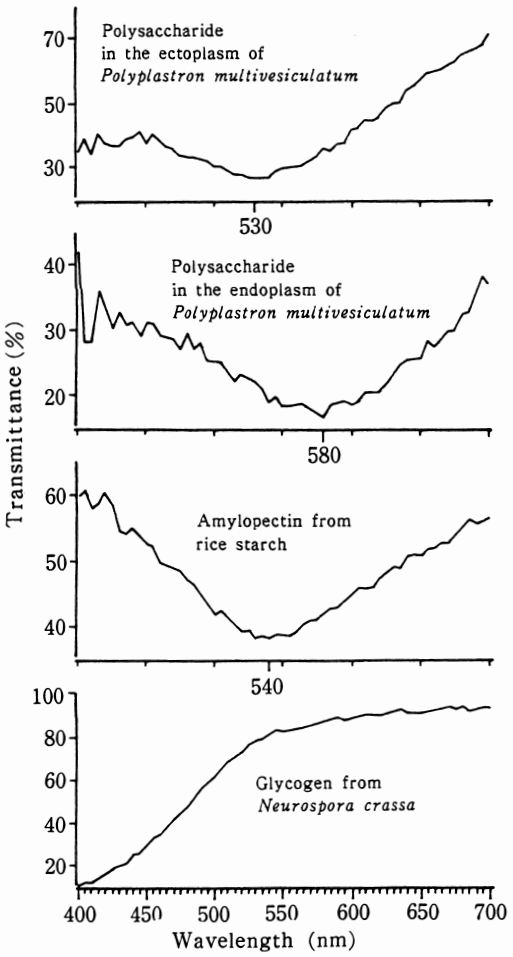

Fig. 10 Transmittance spectra of iodine stained specimens. Polysaccharides of *Polyplastron multivesiculatum*, amylopectin purified from rice starch and glycogen from *Neurospora crassa* were investigated by microspectrophotometry.

The bluish purple grains in the endoplasm of ophryoscolecids and in the endoplasm of *Isotricha* spp. had a λmin at longer wave length than that of pure amylopectin of rice starch. The grains disappeared after α- and β-amylase digestion. These results suggest that the grains may be starch which consist of α-amylose and amylopectin. The grains might be engulfed by ciliates and utilized as their energy source as mentioned by many authors (Abou Akkada and Howard, 1960, Gutierrez, 1955, Onodera and Kandatsu, 1970, Wakita and Hoshino, 1989, Yoshida and Katsuki, 1980).

The finding that no bluish purple grains were detected in the endoplasm of *Dasytricha ruminantium* may relate with the results by Gutierrez (1955) and Howard (1959), suggesting that the manner of utilization of carbohydrates in *Dasytricha ruminantium* is different from that in *Isotricha* spp.

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Figs. 1—9. Sections of rumen ciliates. × 185: *1.* The endoplasm of *Isotricha* sp. (I) and large grains in the endoplasm and small granules in the ectoplasm of *Entodinium* sp. (E) and of *Polyplastron multivesiculatum* (P) are stained intensely with PAS. *2.* and *3.* The endoplasmic grains are stained bluish purple, and the ectoplasmic granules are stained reddish brown in *Entodinium* sp. (E) and in *P. multivesiculatum* (P) with iodine stain. *4.* Bluish purple grains of *Isotricha* sp. (I), and reddish brown granules of *Isotricha* sp. (I) and *Dasytricha ruminantium* (D), are seen. Iodine stain. *5.* Iodine positive materials disappeared from the ciliates after α-amylase digestion. *6* and *7.* After α-amylase digestion, no PAS reactions are seen in the ciliates. *8.* After β-amylase digestion, iodine positive granules (arrow) remained in the ectoplasm of *P. multivesiculatum* (P). *9.* PAS positive granules also remained in the ectoplasm of *Entodinium* sp. (E) and of *P. multivesiculatum* (P) and in the endoplasm of *Isotricha* sp. (I) and *D. ruminantium* (D) after β-amylase digestion.

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