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Nitrogen and phosphorus compounds in the aleurone grains of *Iris pseudoacorus* endosperm and *Pisum sativum* cotyledons

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Abstract

Aleurone grains from *Iris pseudoacorus* endosperm and *Pisum sativum* cotyledons were isolated partly according to Tombs's method (1967). Nitrogen compounds content was determined in them by Kjeldahl's micromethod, and in the particular fractions after Thiman and Laloraya (1960). Mainly protein N was detected in the aleurone grains, constituting 14.8 and 15.2 per cent of the dry mass of pea and *Iris* seeds, respectively. Moreover, phosphorus compounds were fractionated according to Holden and Pirie (1955). Analyses demonstrated the presence in aleurone grains of inorganic P, acid-soluble organophosphorus compounds, phospholipids and RNA.

INTRODUCTION

Recently aleurone grains, known also as protein bodies, arouse an increasing interest. Beside a number of electron microscope studies several investigations have been undertaken to establish the chemical composition of these structures (Morton, Palk and Raison, 1964; Tombs, 1967; Mitsuda et al., 1969; Morris et al., 1970; Prokof'ev et al., 1967; Koshiyama, 1972).

Aleurone grains differ in their structure. In *Leguminosae* they are simple, and compound — containing of one or more globoids in *Gramineae*, or consisting, beside globoids of crystalloid in *Cucurbitaceae* (Olszewska, 1971). The main component of all these types of aleurone grains is protein, and in the globoids phytin (Prokof'ev et al., 1967; Sobolev et al., 1968; Suvorov et al., 1970; Swift and O'Brien, 1972; Morton, Palk and Raison, 1964; Lui and Altschul, 1967; Lott et al., 1971).

The question arises whether aleurone grains are only containers of storage proteins, and in some grains also of phytin, or whether they are

an active cell organelle with a definite physiological function. To elucidate this problem investigations of the fine structure of aleurone grains should be supplemented by biochemical analyses.

In this connection the isolation of aleurone grains from *Iris pseudoacorus* endosperm and *Pisum sativum* cotylendons was attempted in order to carry out preliminary biochemical analyses.

MATERIAL AND METHODS

Mature seeds of *Iris pseudoacorus* and *Pisum sativum* var. Łagiewnicki were used in the experiments. The seed coat and embryos were removed and the cotyledons and endosperm were ground.

Isolation of aleurone grains. The grains were isolated in a sucrose density gradient, partly according to Tombs's method (1967). The ground Iris endosperm (200 mg) and pea cotyledons (500 mg) were purified of lipids in the cold in a mortar with six 10-ml portions of acetone for 10 min. The delipidated material was treated with 5 ml of 20 per cent sucrose in 50 mM citrate buffer pH 5 and ground. The homogenate was left to stand in a refrigerator for 12 hs. Then it was centrifuged in a sucrose density gradient for 30 min at 22 300 x g in a centrifuge with cooling (Janetzki type K 70). After centrifugation 1.5—3-ml portions of the supernatant were drawn with a syringe. However, since the grains were not localized strictly in one zone but stretched to other zones of the supernatant, the whole supernatant was filtered first through a Schott G2 filter, and then a Schott G3 filter in order to remove the remaining impurities. The filtrate with aleurone grains was centrifuged for 30 min at 12 200 x g. Then the grains were rapidly washed with distilled water and inspected under a light microscope before using them for further studies.

Chemical analyses. Nitrogen content was determined in the isolated aleurone grains by Kjeldahl's micromethod. Nitrogen compounds were fractionated after Thimann and Laloraya (1960) and Thimann and Loos (1957), phosphorus compounds according to Holden (1952), and phosphorus in the particular fractions was determined by the method of Holden and Pirie (1955). The analyses were repeated in three separately isolated preparations. The results in the case of phosphorus fractions were completely reproducible, therefore in table 3 only one result is given for each fraction.

RESULTS AND DISCUSSION

The isolated aleurone grains examined in a light microscope were intact and deprived of impurities (Photos 1 and 2). The yield of aleurone grains from pea cotyledons was much higher than from Iris endosperm

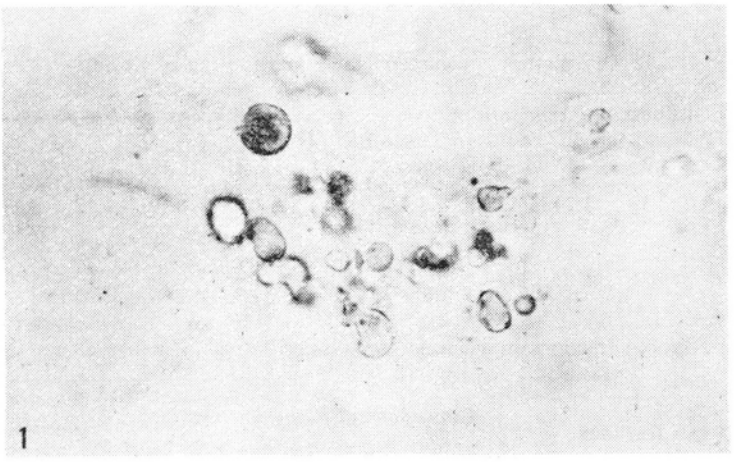

Photo. 1. *Iris pseudoacorus* — aleurone grains

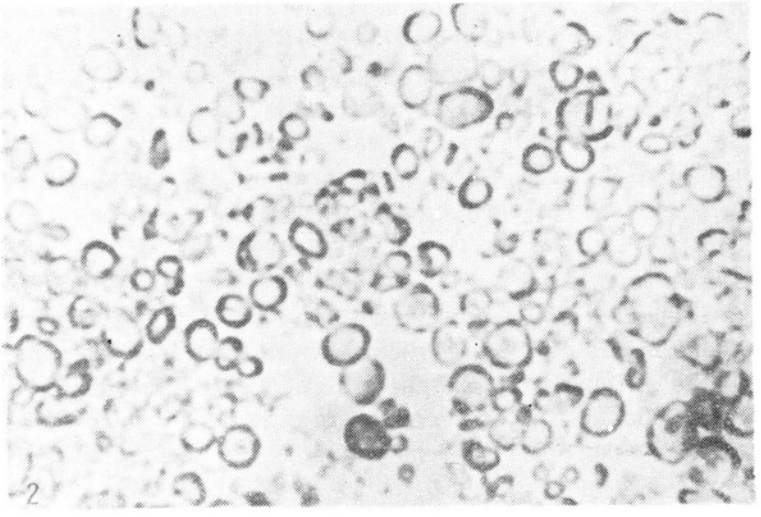

Photo. 2. *Pisum sativum* — aleurone grains

(Table 1). This may be explained by the fact that Iris deposits less storage protein than pea. Cytochemical investigations demonstrated that in ripe endosperm of *I. pseudoacorus* large quantities of lipids and hemicellulose are stored (G a b a r a, M o d r z e j e w s k i, 1971).

Analysis for nitrogen showed that protein N occurs in the largest amounts in aleurone grains constituting 14.8 and 15.2 per cent of total N in pea and Iris, respectively (Table 2). These values are rather high as compared with literature data. A similar amount of nondialysing nitrogen

Table 1

Dry matter of aleurone grains (mg/100 mg of tissue)

Cotyledons of *Pisum sativum*						Endosperm of *Iris pseudoacorus*					
1	2	3	4	5	avg.	1	2	3	4	5	avg.
0.96	1.18	1.18	1.24	1.01	1.11	0.13	0.15	0.10	0.12	0.11	0.12

Table 2

Nitrogen fractions in aleurone grains (mg/100 mg of matter of grains)

Nitrogen fractions	Cotyledons of *Pisum sativum*				Endosperm of *Iris pseudoacorus*			
	1	2	3	avg.	1	2	3	avg.
Acid-soluble	—	—	—	—	—	—	—	—
Nucleic acids	0.49	0.54	0.55	0.53	—	—	—	—
Proteins	16.00	14.90	13.50	14.80	15.10	14.90	15.80	15.20
Total N	16.49	15.44	14.05	15.33	15.10	14.90	15.80	15.20
Protein N×6,25	100.00	93.12	84.38	92.50	94.37	93.13	98.75	95.41

in samples of aleurone grains from *Vicia faba* was detected by Morris et al. (1970) — 14.1 per cent of dry mass. Other authors report lower values. Altschul et al. (1964) noted 10.7 per cent nitrogen in the aleurone grains of *Arachis hypogaea*, Graham et al. (1963) 12.6 per cent in aleurone grains from wheat, Saio and Watanabe (1966) 11.2 per cent in grains from soybean. These lower values reported may be due to the difference in protein content in aleurone grains of various plant species, or to the presence of impurities in the aleurone grain preparations. The protein content converted to the amount of nitrogen with the use of the coefficient 6.25 was 92.5 per cent of dry mass of pea aleurone grains and 95.4 per cent in the case of *Iris*. Koshiyama (1972) using the coefficient 5.8 calculated 73.6 per cent protein in soybean aleurone grains. Mitsuda et al. (1969) found 60 per cent of protein in rice aleurone and Graham et al. (1962) 85 per cent in wheat aleurone. All these authors, however, agree that protein is the main component of aleurone grains.

Neither in pea nor Iris aleurone grains could acid-soluble N be detected. In pea, nucleic acid N was revealed in these structures in contrast to Iris.

It is known from literature data that, beside protein, aleurone grains contain phosphorus compounds. Therefore this element was determined in them also with the aim of elucidating the controversy concerning the

Table 3

Phosphorus fractions in aleurone grains (mg/g of dry matter of grins)

Phosphorus fractions	Cotyledons of *Pisum sativum*	Endosperm of *Iris pseudoacorus*
Acid-soluble:		
orthophosphates	0.1	0.8
bound organic P	1.0	0.2
Phospholipids	0.2	p.3
RNA	0.4	0.3
DNA	—	—
Total P	1.7	1.5

occurrence of RNA in aleurone grains. In pea and Iris aleurone the presence of inorganic phosphorus, acid-soluble organic compounds, phospholipids and RNA was detected (Table 3). Morris et al. (1970) demonstrated that as much as 86 per cent of total phosphorus is in the acid-soluble form and phytates constitute 74 per cent of the latter. Large quantities of acid-soluble P have been also detected by Prokof'ev et al. (1967) in the aleurone grains of *Ricinus* sp. In the initial phases of development of pea aleurone grains, a greater amount of inorganic P was noted, whereas later there was more phytin. In the present results a difference in the acid-soluble fraction is noticeable. Pea aleurone grains contain more organophosphorus compounds, while in the grains from Iris inorganic P compounds prevail. This may be explained by the difference in the structure of aleurone grains between pea and Iris. The former are simple without any inclusions whereas in the latter a number of types of grains may be distinguished, both simple and compound, containing, as shown by cytochemical investigations (Gabara, Modrzejewski, 1971), from 1 to 20 globoids. These authors found within the globoids of Iris aleurone grains large amounts of inorganic phosphates. Hence analysis for phosphorus content revealed larger amounts of inorganic P in the grains from Iris than in those from pea. The pea and Iris aleurone grains contain phosphlipids. These compounds have also been detected in aleurone grains of soybean (Tombs, 1967), rice (Mitsuda et al., 1969), *Ricinus* sp. (Prokof'ev et al., 1967; Suvorov et al., 1970) and wheat (Morton, Palk, Raison, 1964). In the present study ribonucleic acid phosphorus has been detected. RNA has been also found by other authors (Tombs, 1967; Mitsuda et al., 1969; Morton et al., 1964; Morris et al., 1970; Prokof'ev et al., 1967; Yakoyama et al., 1972a, b). It is possible that aleurone grains are distended endoplasmic reticulum cavities, hence it is probable that ribosomes are present on their surface and that would be the reason for the presence of RNA. On the other

hand, it is not probable that ribosomes would be included in the grains. Electron microscopic studies will answer the question whether ribosomes are present in aleurone grain preparations. Yakoyama et al. (1972a) detected RNA in the aleurone grains of soybean and in further investigations by column chromatography on Sephadex G-200 and MAK they found a similarity between the RNA of aleurone grains and that of the ribosomal fraction. In view of this these authors suggest that the system of protein synthesis is contained in the protein bodies. According to expectations, it was found that in the period of storage protein deposition in the aleurone grains of *Ricinus*, the RNA level also rises (Prokof'ev et al., 1967). Morton, Raison and Smeaton (1964) demonstrated that aminoacyl-RNA of synthetase and sRNA associated with amino acid incorporation into storage proteins of wheat is localized in preparations of protein bodies. Morton and Raison (1963) revealed ^{14}C-labelled amino acid incorporation into aleurone grains of wheat. Cytochemical investigations (Gabara et al., unpublished) similarly showed that ^{14}C-leucine is incorporated above all into the Iris aleurone grains. These facts seem to indicate that aleurone grains are the site of storage or protein synthesis in the course of endosperm development.

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Związki azotu i fosforu w ziarnach aleuronowych bielma Iris pseudoacorus i liścieni Pisum sativum

Streszczenie

Ziarna aleuronowe z bielma *Iris pseudoacorus* i liścieni *Pisum sativum* izolowano częściowo w oparciu o metodę Tombsa (1967). Oznaczono w nich zawartość związków azotu mikrometodą Kjeldahla frakcjonowanych w oparciu o metodę Thimanna i Laloraya (1960). W ziarnach aleuronowych wykryto głównie N białkowy, który stanowi 14,8% suchej masy ziaren u grochu i 15,2% u irysa. Ponadto frakcjonowano związki fosforu metodą Holden (1952), a fosfor poszczególnych frakcji oznaczono według Holden i Pirie (1955). Analizy wykazały obecność w ziarnach aleuronowych P nieorganicznego, kwasorozpuszczalnych związków organicznych, fosfolipidów i RNA.