Phosphorus compounds, proteins, nuclease and acid phosphatase activities in isolated spinach chloroplasts

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(Received: July 15, 1971)

Abstract

This paper deals with attempts to elaborate a simple method of spinach chloroplast isolation ensuring a high proportion of intact chloroplasts. We obtained 3 preparations of isolated chloroplasts. Several preliminary analyses of the obtained chloroplast fraction were also performed. Phosphorus compounds, total protein and the enzyme activities of RNase, DNase and GPase were determined. We found: 0,36—0,59% of RNA, 0,19—0,24% of DNA, 2,1—2,9% of phospholipids and 26—28% of protein. RNase activity was very high.

INTRODUCTION

The proportion of chloroplasts obtained in the intact form depends on the physiological condition of the leaf and the method used for disintegrating the leaf tissue (Lyttleton 1962).

Taking into consideration several methods of chloroplast isolation (Whatley and Arnon — 1963 cit. by Hall, Whatley 1967; Jensen and Bossham 1966), we based on the procedure described in the paper of Odincowa, Mikulska and Turiszczewa (1970) and used for pea chloroplasts obtaining. According to Lyttleton (1962) of several plants examined spinach has proven to give the highest proportion of intact chloroplasts. Therefore we decided to adapt the procedure of Odincowa and co-workers to the isolation of chloroplast fraction from spinach leaves. Several preliminary analyses of the obtained chloroplast fraction were also performed. We determined phosphorus compounds total protein and the enzyme activities of RNase (Ribonucleate nucleotido-2-transferase-cyclizing, E.N. 2.7.7.17), DNase (Deoxyribonucleate oligonucleotidohydrolase, E.N. 3.1.4.5) GPase — the acid β -glycerophosphatase (Orthophosphoric monoester phosphohydrolase, E.N. 3.1.3.2).

MATERIAL and METHODS

Whole chloroplasts were isolated from spinach leaves (*Spinacia oleracea* cv. Matador). The plant were grown in a greenhouse by a nutrient culture technique, at room temperature and under natural light conditions. The mature leaves were harvested prior to each experiment. The leaves were washed with distilled water, shaken to remove excess water and placed in a plastic bag in a refrigerator for one to several hours to ensure their turgidity. Then we applied mainly the procedure described by Odincowa, Mikulska and Turiszczewa (1970) with some modifications. We removed the midribs from the fully turgid leaves, weighed the leaf blades and then sliced quickly into pieces about 0,5 cm square to facilitate grinding. We ground about 50 g of sliced leaf blades by hand in a precooled porcelain mortar placed on ice (0–4°C) with a solution containing 0,5 M saccharose in 0,05 M Tris-HCl buffer at pH 7,2 used in relation volumes of the solution for 1 weight unit of plant tissue. This was homogenized for 5 minutes and the slurry was squeezed through a double leyar of cheesecloth and the green juice centrifuged at 0°C for 10 minutes at $400 \times g$ to sediment leaf debris and whole cells. The green supernatant liquid was decanted and centrifuged for 10 minutes at $730 \times g$. (in the work of Odincowa and co-workers 15 minutes at $1000 \times g$. It should be emphasised that these authors isolated pea chloroplasts). In this centrifugation most of the intact chloroplasts were sedimented. The supernatant fluid was discarded, the sediment however washed with the previously mentioned solution and centrifuged at $1300 \times g$ for 15 minutes (in the work of Odincowa et al. at $3000 \times g$ for 20 minutes). In the case of spinach using this procedure we obtained „broken” chloroplasts. Then the sediment was washed again with a small quantity of Tris-HCl buffer with saccharose and centrifuged for 5 minutes. The last procedure was applied only in our experiments.

Fig. 1 and 2. Chloroplast suspension obtained by the method of Whatley and Arnon (1963).

1. The chloroplasts completely broken
2. In this suspension not numerous chloroplasts maintain their contours (single arrow). Only around one of them an intact mebrane is visible (double arrow)

Fig. 3. Chloroplast suspension in 0,5 M sucrose with 0,05 M Tris-HCl buffer at pH 7,2. Chloroplasts which are intact appear as bright, highly refractive bodies. This phenomenon is connected with the intact membrane surrounding the chloroplast, impermeable to high molecular weight soluble constituents. When these constituents are present, the chloroplast has a higher refractive index than the surrounding medium, so appears bright under phase contrast. Arrows indicate the chloroplasts with broken membranes

Fig. 1, 2 i 3

Phosphorus compounds were fractionated into four groups by the method of Holden (1952) and determined according to Holden and Pirie (1955b). Enzyme activities were measured by the methods described by Holden and Pirie (1955a,b). Determining RNase activities we used highly polymerised RNA preparation obtained from yeasts in the laboratory of Physiological Chemistry Department of Medical Academy. Highly polymerised DNA preparation needed for DNase activities determination was obtained from a calf thymus in our laboratory by routine methods. Proteins were extracted by the method of Fletcher and Osborne (1965) and determined after Lowry (Mejbaum-Katzenellenbogen, Mochnacka 1968).

RESULTS and DISCUSSION

Microscopic examination under phase contrast of a sample resuspended in Tris-HCl buffer with saccharose revealed about 60% of osmotically intact chloroplasts. The use of the phase contrast microscope to determine the condition of isolated chloroplasts has been very helpful (Figs. 1,2,3). Lyttleton's results showed 40 and 70 per cent of intact chloroplasts. According to this author in spite of the fact that spinach has proven to give the highest proportion of intact chloroplasts but even here the proportion never approaches 100 per cent. Therefore the proportion of intact chloroplasts obtained in this research may be considered as the success of a preparation.

The results of biochemical analysis are shown in tables 1,2 and 3.

We obtained 3 preparations of isolated chloroplasts. The numbers obtained for each preparation represent mean values from 3 samples.

Table 1

Phosphorus compounds in spinach chloroplasts suspension in mg/g of dry matter

P fractions	Preparation		
	1	2	3
0.2 N HClO ₄			
orthophosphate	0.78	0.26	0.20
acid-soluble bound P	0.64	0.60	0.59
Ethanol—ether (3 : 1) lipid P	0.86	1.15	1.12
N HClO ₄ R.T. overnight RNAP	0.36	0.48	0.59
N HClO ₄ 37°C overnight DNAP	0.24	0.24	0.19
Total P	2.88	2.73	2.69

The fractionation and determination of phosphorus compounds in isolated spinach chloroplasts show a remarkable level of the acid soluble phosphorus fraction which comes mainly from high amounts of acid-soluble organic phosphates. Jóźwiak and Leyko (1969) found

Table 2

Contents of nucleic acids, phospholipids and proteins in spinach chloroplasts (in per cent of dry matter)

Compounds	Preparation		
	1	2	3
RNA	0.36	0.48	0.59
DNA	0.24	0.24	0.19
Phospholipids	2.15	2.87	2.80
Protein	26.24	28.26	26.93

that the nucleotide concentration in chloroplasts is relatively high — about twice higher than in all the spinach homogenate.

The use of biochemical techniques for the detection of nucleic acids associated with chloroplasts has been seriously hampered by the difficulty in obtaining preparations free of contamination by nuclear and cytoplasmic material. Furthermore it is difficult to determine objectively the „degree of purity” of chloroplast preparations.

According to literature chloroplasts contain very small amounts of nucleic acids. The chloroplasts of *Antirrhinum majus* contain about 1% of ribonucleic acids (Ruppel, 1964 cit. by Menke 1966) and about 0,02% of deoxyribonucleic acids (Ruppel and van Wyk, 1965 cit. by Menke 1966). In this research we found 0,36—0,59% of RNA. According to Wettstein (1966) the amount of DNA represent 0,05—0,1% of the dry matter of isolated plastids and chloroplasts. In this study we found 0,19—0,24% of DNA occurring in the spinach chloroplasts. Multiplying our values for RNA-P and DNA-P fractions by 10 we obtained RNA and DNA contents.

Total phospholipid fraction was estimated on the basis of P contents in lecithin and cephalin being approximately 1/25. Multiplying our values for P lipid fraction by 25 we obtain phospholipid fraction as 2,1—2,9%. Granick (1961) noticed great amounts of lipids (containing neutral lipids, phospholipids and steroids) in the chloroplasts of higher plants (20—30 per cent as related to dry matter).

Table 3

Enzyme activity in units/fresh weight of spinach chloroplasts suspension

Enzyme	Preparation		
	1	2	3
RNase	6.89	5.65	6.38
DNase	2.84	3.57	4.52
GPase	4.11	4.34	4.24

The enzyme activities are expressed as units per g fresh suspension according to the definition of an enzyme unit by Holden and Pirie (1955a)

According to the Granick's (1961) opinion the chloroplasts of higher plants contain 35—55% of protein as related to dry matter. Menke (1961) found 45% of proteins in the spinach chloroplasts. In this research however we found 26—28% of proteins in the spinach (cv. Matador) chloroplasts.

We found very high RNase activity as compared with the activities of DNase and GPase. Szarkowski, Gołaszewski and Ombach (1967) noticed also a high RNase activity in the chloroplasts of rye and carrot attaining almost the activity of this enzyme in mitochondria.

According to Sisakian (1958) the activity of chloroplast RNase increases in the course of plant development causing a drop of RNA content. Ledoux and co-workers (1962) noticed a parallel between RNase activity and RNA content in barley seedlings.

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Zawartość związków fosforu i białek oraz aktywność nukleaz i kwaśnej fosfatazy w izolowanych chloroplastach szpinaku

Streszczenie

Podjęto próbę opracowania prostej metody izolowania chloroplastów szpinaku zapewniającej otrzymanie preparatów o dużej ilości nienaruszonych chloroplastów. Przygotowano 3 preparaty izolowanych chloroplastów. Wykonano kilka wstępnych analiz otrzymanej frakcji chloroplastów. Oznaczono zawartość związków fosforu, białko całkowite oraz aktywność enzymów RNazy, DNazy i GPazy. Stwierdzono, że zawartość RNA w chloroplastach wynosiła 0,36—0,59%, DNA 0,19—0,24%, fosfolipidów 2,1—2,9%, a białek 26—28%. Aktywność RNazy była bardzo wysoka.