The Composition of Ribonucleic Acids Isolated from Chloroplasts of *Lamium album* and *Symphytum officinale*

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The purpose of this investigation was to analyze the nucleic acids of chloroplasts of some plant species to find, eventually, any indication of a species-specificity and of a difference between the RNA of the chloroplasts and that of the corresponding cytoplasm (microsomes).

Chloroplasts of *Lamium album* and *Symphytum officinale* were isolated and fragmented and these preparations were freed from protein by boiling at pH 8. The RNA of the solutions thus obtained was precipitated by means of acid ethanol and was purified by repeated redissolutions and precipitations in combination with centrifugations. After acid hydrolysis the preparations were analyzed quantitatively with respect to guanine, adenine, cytidylic and uridylic acids by means of paper chromatography and spectrophotometry in U.V.

Similar analyses were performed on preparations of sap of fragmented leaves freed from chloroplasts and nuclei.

The analytical data show a characteristic difference between *Lamium album* and *Symphytum officinale* with respect to the molar ratios of the components of the RNA of their chloroplasts. The data obtained for the chloroplasts also differed from those of the cytoplasm of the same species.

From data available by analytical techniques ribonucleic acid has been estimated to be present in chloroplasts and to form up to 2 % of their dry weight ^{2,3,5,6,10}. Histochemical techniques suggest that both DNA and RNA are to be found in chloroplasts ^{1,2,7}. There is some ambiguity, however, concerning the presence of DNA since highly polymerized RNA may be responsible for the colouring of the plastids by methyl green ⁴.

In purified preparations of chloroplasts no trace of DNA was detected but enough RNA could be obtained for analysis regarding its purine and pyrimidine bases.

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MATERIAL AND METHODS

In the autumn (September 1953) nucleoproteins were isolated from purified preparations of chloroplasts from several plant species. After treatment with acid ethanol most of this material was stored at -15°C for further investigations during the winter season. However, when this frozen material was investigated with respect to the composition of its RNA it was found that the material had obviously hydrolyzed and no reproducible data could be obtained. Thus the results reported here are from preliminary experiments made on fresh material immediately after the isolation of the chloroplasts.

Chloroplasts from different plants were found to vary considerably in their stability during the purification procedures. Species with the most stable chloroplasts were selected for further work. All preparative work was carried out in the cold room at about $+4^{\circ}\text{C}$. Detached leaves were fragmented in a domestic mincing machine and the mass was pressed through cloth. The sap, about 700 ml from each preparation, was diluted immediately with 1/3 volume of 2 M sucrose solution. The suspension was freed from coarse material by filtration through a thin layer of glass wool and by a few minutes centrifugation at low speed. The sap was then centrifuged for 30 min at about 2 000 r. p. m. (radius approximately 10 cm). The green upper layers of the pellets were resuspended in about 50 volumes of cold 0.5 M sucrose solution and centrifuged until the supernatant liquid was clean. This washing was repeated once. During the purification samples were withdrawn occasionally for inspection under the microscope. In this way the chloroplast fragmentation process was observed carefully. Great care was taken to free the suspension from whole and fragmented nuclei. These were detected by the carmine-acetic acid method of Belling and Heitz⁹. It was found that nuclei could be removed by repeated washings and centrifugations as stated by Weier and Stocking¹² or by centrifugation of a chloroplast suspension superimposed on a 2 M sucrose solution in which case nuclei sedimented also through this concentrated solution of sucrose but most of the chloroplasts formed an intermediary layer above the layer of 2 M sucrose. Treatment with the ion exchange resin Amberlite IR 120 (Rohm & Haas, Philadelphia), also removed nuclei effectively.

To obtain an effective extraction of nucleoprotein from the chloroplasts it was found necessary to disintegrate the chloroplasts thoroughly. This was achieved by resuspending the pellet of purified chloroplasts in distilled water (about 30 ml per preparation) to which a few drops of xylene had been added and shaking with glass beads over night in the cold room. By ordinary microscopy and electron microscopy it was found that the fragmentation was very effective. Only small parts of chloroplasts could be seen in the electron micrographs.

The pH of the suspension was raised to 8 by adding some sodium hydroxide solution, traces of sodium chloride was also added after which the solution was kept in boiling water for 10 min. During this procedure there was a fall of pH of 0.4–0.8 units. The precipitate was removed by 45 min centrifugation. The clear supernatant fluid was diluted with an equal volume of ethanol and this mixture was acidified with HCl. The precipitated nucleic acid was sedimented in the centrifuge and then dissolved in distilled water to which a few drops of

Fig. 1. Absorption in U.V. light of extracts from filter paper Whatman No. 1 before and after washing the paper with 0.1 N HCl. Circular pieces of the paper 2.5 cm in diameter were cut out and extracted each with 5 ml 0.1 N HCl. Heavy lines show average values of 10 measurements at each wave-length. The lined areas include all measurements; a) nontreated papers; b) papers washed in HCl and distilled water.

NaOH had been added. The solution was cleared by centrifugation for 1 h at 3 500 r. p. m. Such precipitation and redissolution of the nucleic acid was twice repeated. The preparation was then only slightly tinged with dark-coloured material.

The alcohol was evaporated from the precipitated nucleic acid in an evacuated vessel. 0.6 ml of 1 N HCl was added to 3 mg of the dry preparation and hydrolysis was performed at 100°C for 1 h. As shown by Smith and Markham¹¹ this hydrolysis gives guanine, adenine, and the nucleotides cytidylic acid and uridylic acid. The hydrolysis products were analyzed quantitatively by means of paper chromatography as recommended by Smith and Markham¹¹.

The paper was, however, first investigated with respect to its content of substances absorbing in ultraviolet light and extractable with 0.1 N HCl. For this purpose a series of 10 spots 2.5 cm in diameter were cut out from papers which had not been treated with any solvent. These paper samples were extracted with 5 ml 0.1 N HCl each for about 10 h and the absorption of the extracts in the ultraviolet region was measured. As shown in Fig. 1 the filter paper was rather heterogeneous with respect to its content of U.V.-absorbing material and could be freed from this material by washing with hydrochloric acid. Therefore papers to be used for chromatography were first treated with 0.1 N HCl over night and were then washed with water and dried.

Since a very acid solvent was used in the chromatographical analysis there would be an inherent tendency for the solvent front to remove most of the disturbing material from those areas of the paper where spots were located at the end of the run. However, the best results were obtained on papers which had been washed previously with hydrochloric acid and water.

A mixture which was 0.8 N with respect to HCl and contained 70 % by volume of *tert.* butanol, the remainder being water, was used as solvent during chromatography. Ascending fronts were run on paper Whatman No. 1 in glass vessels. Each spot contained 0.2 ml of the hydrolysate mentioned above. A length of flow of 20–25 cm was obtained after about 30 h at 18–20°C. The papers were dried at room temperature over a ventilating fan. By examining the fluorescence in ultraviolet light the spots could be located easily. The occupied parts of the paper were cut out and the hydrolysis products were extracted from these paper pieces by means of 0.1 N HCl over night in the cold.

Table 1. Ultraviolet light absorption of hydrolysis products of rinonucleic acid from chloroplasts of *Lamium album*.

Preparation	Guanine		Adenine		Cytidyl. acid		Uridyl. acid	
	Extinction at 250 m μ	R_F	Extinction at 260 m μ	R_F	Extinction at 280 m μ	R_F	Extinction at 260 m μ	R_F
1	0.372	0.19	0.392	0.32	0.283	0.49	0.201	0.76
2	0.382	0.24	0.378	0.37	0.279	0.54	0.190	0.80
3	0.388	0.24	0.387	0.34	0.284	0.54	0.196	0.77
Concentration,* average, Mol. $\cdot 10^{-3}$	36.3 $\pm$ 1.0		29.7 $\pm$ 0.8		29.6 $\pm$ 0.6		19.8 $\pm$ 0.6	

* Absorbancy coefficients according to Smith and Markham¹¹ and Ploesser and Loring⁸ were used: guanine 10.6×10^3 at 250 m μ
 adenine 13.0×10^3 „ 260 „
 cytidylic acid 12.72×10^3 at 280 m μ
 uridylic acid 9.89×10^3 „ 260 „

room. The absorption in ultraviolet light of the four samples was measured in a Beckman spectrophotometer model DU against similar extracts of paper pieces cut out near the respective spots.

The R_F -values obtained for the four products were in good agreement with those reported by Smith and Markham and so were the wave-lengths of maximum absorption of the four curves.

In two separate runs the hydrolysate was investigated for amino acids by spraying with ninhydrin. No trace of amino acids was found.

Table 2. Composition of acid hydrolysates of ribonucleic acid from chloroplasts and the corresponding cytoplasms of *Lamium album* and *Symphytum officinale*.
 Ratio of molar concentrations, adenine = 1.

Source of RNA	Guanine	Adenine	Cytidylic acid	Uridylic acid
<i>L. album</i> , chloropl.	1.30	1.00	1.00	0.67
<i>L. album</i> , cytopl.	1.17	1.00	0.75	0.35
<i>S. officinale</i> , chloropl.	1.26	1.00	0.76	0.65
<i>S. officinale</i> , cytopl.	1.43	1.00	0.85	0.55

RESULTS

In Table 1 values are collected from chromatograms of 3 preparations of nucleic acid isolated from chloroplasts of *Lamium album* and in Table 2 the molar ratios of guanine, adenine, cytidylic acid and uridylic acid in hydrolysates from nucleic acid preparations from chloroplasts and cytoplasm of *Lamium album* and *Symphytum officinale* are recorded.

There is a clear difference between the two kinds of chloroplasts with respect to cytidylic acid. There is also, within each species, a difference between the RNA from the chloroplasts and that from the cytoplasm. The values for the cytoplasmic RNA were obtained from only one analysis each, those for the chloroplasts represent average data each from three experiments (cf. Table 1).

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