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There is strong evidence for cytoplasmic inheritance of chloroplasts in higher plants. This genetic autonomy, together with the demonstration of protein synthesis [1,2] and the presence of ribosomes [3,4,5] in chloroplasts, has generated considerable interest in the nucleic acids associated with chloroplasts.

Many investigators have reported the occurrence of chloroplast nucleic acids [6], but there is considerable quantitative disagreement in these results. This is undoubtedly in part due to differences in the material analyzed, and the analytical methods employed. These methods include cytochemical procedures, which depend upon the specific staining of RNA* and DNA in the chloroplasts of intact cells, and the chemical analysis of RNA and DNA extracted from chloroplasts isolated in isotonic aqueous media. A more recent technique is the incorporation of H^3-thymidine into Spirogyra [7] or tobacco leaves [8], and intracellular localization by means of autoradiography. Electron microscopy indicates the presence of DNA in Chlamydomonas chloroplasts [9]. Sedimentation studies in $CsCl_2$ density gradients indicate that DNA extracted from higher plant chloroplasts [10], and algal chloroplasts [10,11], differs in density from that of the nucleus.

The amounts of nucleic acid reported in chloroplasts using cytochemical procedures are at the lower limit of the methods' sensitivity[6]. This lack of sensitivity is circumvented by direct chemical analysis of polynucleotide

extracted from isolated chloroplasts. However, in this procedure, artifacts are encountered, owing to contamination of the plastids by nucleic acids from other cell components during isolation. This contamination has been elegantly avoided for chloroplasts DNA by Baltus and Brachet[12], whose results were confirmed by Gibor and Izawa[13]. These workers removed the nucleus of Acetabularia, prior to chloroplast isolation and analysis. Additional errors in these experiments may also arise by the loss of nucleic acids from chloroplasts either by leakage[14], or enzymic degradation, particularly when aqueous isolation procedures are employed. However, these difficulties are largely avoided if plastids are isolated by the non-aqueous method. This paper reports the chemical estimation of RNA and DNA in spinach chloroplasts isolated by a non-aqueous density technique.

Chloroplasts were isolated by modifications of methods by Heber[14]. Polynucleotides were estimated by the Smillie-Krotkov modifications[15] of the Schmidt-Thannhauser procedures. The purity of the chloroplasts was checked by phase microscopy after staining with methyl green, and an electron micrograph taken by one of us (R.B.P.) of a similar preparation has been published by Heber.[16] This micrograph, together with enzymic checks by Heber, also demonstrates the purity of the preparations.

One hundred milligram samples of non-aqueous chloroplasts were extracted with methanol, cold trichloracetic acid, ethanol and ethanol-either mixtures, and the insoluble residues were either subjected to mild alkaline hydrolysis for RNA nucleotides, or strong acid hydrolysis for total nucleotide estimations. The results are summarized as follows:

Method of Analysis / Treatment	Milligrams polynucleotide per 100 mg. non aqueous chloroplasts			
	UV absorption 260 mu	diphenylamine test for deoxyribose	orcinol test for ribose	phosphate
1. Strong acid hydrolysate (5% perchloric acid, 15 min, 90°C.)	7.95 (DNA + RNA)	0.40 (DNA)	7.80 (DNA)	8.10 (DNA + RNA)
2. Mild alkaline hydrolysate: (0.3NKOH, 16 hr. 34°C.)	7.49 (RNA)	0	7.10 (RNA)	7.62 (RNA)
3. Residue from 2. after strong acid hydrolysis as in 1.	0.795 (DNA)	0.37 (DNA)	0	0.41 (DNA)

The confirmation of values obtained by analysis of deoxyribose and phosphate is good. However, the high value obtained from UV absorption at 2600 Å for DNA suggests interference by some hydrolysis products other than nucleotides. This emphasizes the necessity of working with several complimentary analytical methods in such a study.

These data show that the chloroplasts prepared by the non-aqueous technique contain 0.5% DNA, and 7.5% RNA by weight. An objection to these data might be that a considerable portion of the plastid lipid was extracted during the isolation procedure, yielding high nucleic acid contents on a weight basis. That this is not the case is shown by other experiments[17], in which non-polar solvents were shown to extract not more than 5% of the chloroplast mass.

The value obtained for the DNA content of non-aqueous chloroplasts is in good agreement with previous reports for aqueous chloroplasts. The value obtained for the RNA content is considerably higher than that reported previously (2-4%), and this difference is probably due to the application of the non-aqueous technique for the isolation of chloroplasts. It is likely that RNA is leached from chloroplasts isolated from aqueous cellular homogenates, whereas this is minimized by using non-aqueous techniques. In addition, the action of the chloroplast RNAse[18] is inhibited by operation in a non-polar medium.

In order to localize the polynucleotides within the chloroplasts, stroma and grana fractions were prepared[17] and analyzed using the same procedures. The polynucleotides were found to be in the stroma fraction and easily leached out of the chloroplasts by means of aqueous buffers.

In summary, the DNA content of spinach chloroplasts prepared in non-aqueous media is similar to that of chloroplasts prepared in aqueous buffers. However, the RNA content of non-aqueous chloroplasts is higher than that of aqueously prepared chloroplasts by a factor of two. The RNA is localized in the stroma phase of the chloroplast and is not bound to the quantum conversion apparatus. It is suggested that the low RNA content of aqueous chloroplasts is due to leaching[6] of the polynucleotide during chloroplast isolation.

* Abbreviations used in this paper:

RNA - Ribonucleic acid

DNA - Deoxyribonucleic acid

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