Short Communication

The Constancy of the Buoyant Density of Chloroplast and Mitochondrial Deoxyribonucleic Acids in a Range of Higher Plants

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A previous report (4) gave buoyant density values for chloroplast and mitochondrial DNAs prepared from spinach, lettuce, broad bean, and sweet pea by the DNase technique, which takes advantage of the property of intact organelles to resist exogenous DNase action (3). With these higher plants, the buoyant densities of the chloroplast and mitochondrial DNAs fell into two distinct groups, with values centered around 1.697 and 1.706 g·cm^{-3}, respectively. It was felt, however, that this constancy could not be interpreted as significant since the densities of the nuclear DNAs of these plants were all in the range 1.694 to 1.695 g·cm^{-3}.

In this report, we wish to present density values of chloroplast and mitochondrial DNAs from higher plants with nuclear DNA ranging in density from 1.691 to 1.702 g·cm^{-3}.

Chloroplast and mitochondria were prepared as previously described (4). The chloroplast fraction was the 500 to 2,000g pellet (500–2,500g with onion) from the leaf homogenate, and the mitochondrial fraction was the 2,000 to 10,000g pellet (2,500–10,000g with onion). The organelle preparations, suspended in the homogenization medium containing an excess of magnesium, were incubated with DNase (50 µg/ml) for 1 hr at 0 C. This treatment digested all the DNA present except that contained by the intact organelles. The action of the enzyme was stopped by the addition of excess EDTA, pH 7.4, and the organelles were collected by centrifugation. DNA was prepared by lysing the pellet with a detergent solution containing 1% (w/v) triisopropylnaphthalene sulfonate, 6% (w/v) p-aminosalicylate, 50 mM NaCl, 10 mM tris (pH 7.4), 10 mM EDTA (pH 7.4), and 6% (v/v) n-butanol. The presence of the EDTA inhibits any DNase activity and also greatly increases the yield of DNA, particularly from older leaf tissue. The salt concentration was increased to 0.5 M, and an equal volume of chloroform-3-methylbutanol-1 (24:1, v/v) was added. The mixture was shaken vigorously for 10 min and centrifuged at 2,500g for 10 min, and the top aqueous layer was removed. This initial deproteinization with chloroform increases the yield of DNA from some tissues, at the same time decreasing the amount of RNA released into the aqueous phase. With certain plants such as artichoke, this procedure yields DNA containing only traces of RNA, whereas varying amounts of RNA are recovered from other tissues. The aqueous phase was further deproteinized by shaking with an equal volume of phenol mixture (phenol saturated with 10 mM tris, pH 7.4, and containing 10% (v/v) m-cresol and 0.5% (w/v) 8-hydroxyquinoline). After centrifugation at 2,500g for 10 min, the top aqueous layer was removed and re-extracted with the phenol mixture. The DNA was precipitated from this aqueous phase by the addition of 2 volumes of ethanol and storage at 0 to 4 C overnight. The precipitate was recovered by centrifugation, dissolved in 0.1 × SSC[2] and the solution was then made up to 1 × SSC. The DNA was purified by digestion of the preparation with 50 µg/ml of deoxyribonuclease-free RNase for 30 min at 37 C and was followed by digestion of the preparation with 400 µg/ml of pronase for 3 hr. The DNA was recovered by centrifugation for 18 hr at 39,000 rpm in a Spinco 40.2 rotor at 15 C, and dissolved in 0.1 × SSC. Samples containing approximately 1 to 4 µg of DNA were made up to a density of 1.720 g·cm^{-3} with CsCl, and centrifuged to equilibrium at 44,770 rpm at 25 C for 20 hr.
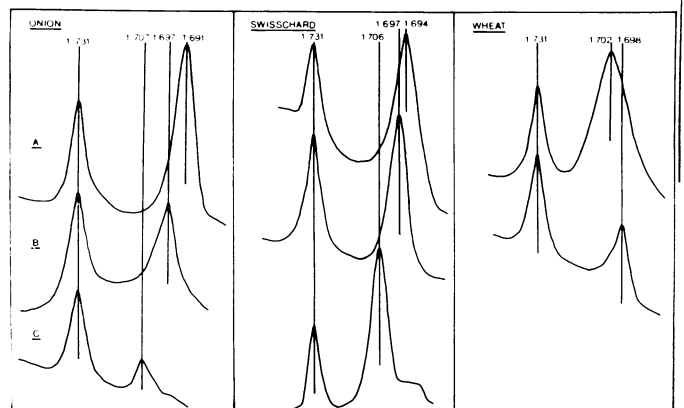

FIG. 1. Microdensitometer tracings of ultraviolet photographs of cell organelle DNAs. The marker DNA from *M. lysodeikticus* has a buoyant density of 1.731 g/cm^{-3}. A: Nuclear DNAs from onion, 1.691; swiss chard, 1.694; and wheat, 1.702; B: chloroplast DNAs from onion, 1.697; swiss chard, 1.697; and wheat, 1.698; C: mitochondrial DNAs from onion, 1.707; and swiss chard, 1.706.

Micrococcus lysodeikticus DNA was included as an internal marker in each sample. Since the CsCl density gradient produced by centrifugation is not linear, the separation of two components varies depending on their position in the analytical cell, which in turn depends on the accuracy of the initial CsCl concentration. The centrifugation was calibrated, therefore, by determining the separation of two DNAs of known densities,

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[2] Abbreviations: SSC: standard saline citrate (0.15 M NaCl, 0.015 M sodium citrate, pH 7.2).

Table I. *Buoyant Densities of Plant Organelle DNAs*

Tissue	Buoyant Density		
	Nuclear	Chloroplast	Mitochondrial
		$g \cdot cm^{-3}$	
Onion (5 preparations)	1.691	1.6964	1.7064
		1.6964	1.7069
		1.6963	...
		1.6965	1.7060
		1.6966	1.7058
		1.6964 ± 0.0001	1.7063 ± 0.0005
Spinach	1.694	1.6959	...
Swiss chard (8 preparations)	1.694	1.6965	1.7057
		1.6968	1.7052
		1.6965	1.7056
		1.6963	1.7053
		1.6961	1.7051
		1.6962	1.7051
		1.6967	1.7051
		1.6969	1.7054
		1.6965 ± 0.0003	1.7053 ± 0.0002
Tobacco	1.695	1.6972	...
Wheat (3 preparations)	1.702	1.6976	...
		1.6982	...
		1.6979	...
		1.6979 ± 0.0003	...

M. lysodeikticus 1.731 and *Xenopus* 1.699, when equilibrated at different positions in the analytical cell. The distance between the *M. lysodeikticus* marker and an unknown DNA was converted to a difference in density using the relevant density differ-ence-separation factor from the calibration curve (density difference divided by the separation of the two known markers plotted against the position of the *M. lysodeikticus* DNA in the cell).

When organelle DNAs were examined in the analytical ultracentrifuge, they all showed a single component (Fig. 1). Such results contrast with the recent report by Bard and Gordon (1) that chloroplasts contain two distinct DNAs, with densities of 1.696 and 1.706 $g \cdot cm^{-3}$. However, in those studies (1) no attempt was made to remove mitochondria from the chloroplast preparation by an initial differential centrifugation. The chloroplasts were isolated from a relatively large region of a zonal sucrose density gradient which also, probably, contained the bulk of the mitochondria.

The results obtained from a series of preparations are given in Table I, which shows the high reproducibility of the analytical method when used under these conditions. In fact, the accuracy of the determination of buoyant density is such that the chloroplast DNAs from onion (1.6964) and swiss chard (1.6965) are significantly different at the 0.1% level (Student's t test) from wheat chloroplast DNA (1.6979). Similarly, onion mitochondrial DNA (1.7063) differs significantly (1% level) from swiss chard DNA (1.7053).

The constancy of the density of chloroplast DNAs at 1.697 ± 0.001 in higher plants with nuclear DNA varying in density from 1.691 to 1.702 $g \cdot cm^{-3}$ supports the hypothesis put forward by Baxter and Kirk (2) that chloroplast DNA in all higher plants may have a rather constant base composition in the region of 37 to 38% guanine–cytosine. The more limited data on mitochondrial DNA suggest a similar constancy.

LITERATURE CITED

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