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Hydroxyapatite chromatography of deoxyribonucleic acids from *Euglena gracilis*.

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Summary. — The chromatographic behavior of native *Euglena gracilis* DNA's on hydroxyapatite columns has been investigated. Total nucleic acid preparations can be fractionated by column chromatography to yield nuclear DNA and organelle (mitochondrial + chloroplast) DNA. A batch procedure permits the rapid separation of organelle DNA from total nucleic acid preparations.

Chromatography of DNA preparations from heat-bleached mutants permits the separation of mitochondrial DNA from nuclear DNA. Chromatography of DNA preparations from chloroplast-enriched fractions of *Euglena* permits the separation of chloroplast DNA, and in addition, of a satellite DNA previously described (ρ = 1.700 g/cm^3).

Some physical and chemical properties of the *Euglena* DNA's, as obtained by chromatography on hydroxyapatite, are reported.

INTRODUCTION.

Though mostly used for the separation of native and denatured DNA [1, 2], hydroxyapatite (HA) columns have a considerable discriminatory power towards different native DNA structures [3, 4]. Not taking into consideration the case in which the secondary (single-stranded DNA from Φ X 174 phage) or the tertiary structure (twisted circular DNA from polyoma virus) are grossly different and cause a different chromatographic behavior [3, 4], it is becoming increasingly evident that differences in nucleotide sequences in otherwise similar DNA's (the similarity concerning the double-strandedness, the molecular weight, the linear, or open circular, configuration, and also the nucleotide composition) may be sufficient to determine different elution molarities. Recent work [5-7] suggests that DNA's containing short repetitive sequences like satellite DNA's may, in general, show particular elution molarities.

A particularly striking case is that of mitochondrial DNA's from wild-type and cytoplasmic « petite » mutant yeast (*Saccharomyces cerevisiae*) cells [8-10]. These DNA's, which are characterized by very low GC contents (ranging from 18 p. cent in wild-type cells to only 4 p. cent in some cytoplasmic « petite » mutants), are eluted at higher phosphate molarities compared to yeast nuclear DNA's or bacterial DNA's. The higher elution molarities of yeast mitochondrial DNA's are due to the presence in these DNA's of long AT-rich stretches [10] containing both alternating dAT : dAT and non-alternating dA : dT structures [11]. In fact, poly (dAT : dAT) and poly (dA : dT) and the dAT-rich satellite DNA from *Cancer pagurus*, elute at slightly and much higher molarities, respectively, than bacterial DNA's [10]; finally experiments on enzymatically degraded yeast mitochondrial DNA's have shown that GC-rich fragments elute at a lower phosphate molarity compared to the AT-rich fragments [12, 13].

The results with yeast mitochondrial DNA prompted a similar kind of experiments with *Euglena gracilis* chloroplast and mitochondrial DNA which both are low in their GC contents (24 p. cent and 32 p. cent, respectively) [14]. These DNA's might share some structural features with the mitochondrial DNA's from yeast and display, therefore, a similar chromatographic behavior.

From a practical point of view, the separation of organellar from nuclear DNA in the case of *Euglena* is complicated by the fact that the former only represents 1-2 p. cent of the total cellular DNA, a level about 10 times lower than that of yeast mitochondrial DNA. If successful, however, the method would lend itself to scale up the preparation of organellar DNA in a relatively simple and inexpensive way, a point of considerable im-

portance in view of the current interest in the biological function of both DNA's [15].

In the following we report the chromatographic separation of both *Euglena* chloroplast and mitochondrial DNA using either total cellular nucleic acids or organellar DNA's enriched samples as starting materials. In addition, the procedure used permits the separation of nuclear DNA and of a satellite DNA.

This work has been presented at the Colloque de la Société de Chimie Biologique sur les Acides Désoxyribonucléiques des Eucaryotes (Strasbourg, March 16-18, 1972).

MATERIALS AND METHODS.

1) *Cultivation of cells.*

Euglena gracilis, Klebs (Z-strain ; Culture Collection of Algae, Indiana University, N° 753) was grown autotrophically, harvested and stored as reported earlier [16]. *Euglena gracilis*, heat-bleached mutant, was grown heterotrophically on Euglena Broth (Difco) either in light or in dark. The mutant was isolated in Evanston and grown for more than 12 months without showing any greening capacity. No chloroplast DNA (ρ = 1.685 g/cm^3) could be detected in this mutant.

2) *Purification of chloroplast and mitochondria.*

Chloroplasts were isolated from wild-type cells as reported [16], and purified by flotation in a Ludox gradient [17]. Mitochondria were isolated from bleached cells which were broken using a French Press at 2 000 psi, in 0.25 M sucrose, 0.01 M tris HCl, pH 7.9, 0.25 M KCl, 0.004 M $MgCl_2$ and 0.005 M β-mercaptoethanol. The crude mitochondria were obtained by differential centrifugation according to Krawiec and Eisenstadt [18].

3) *Nucleic acid extraction.*

Total nucleic acid, 60 g (wet packed) wild-type cells were resuspended in 0.15 M NaCl containing 2.5 p. cent sodium dodecylsulfate (SDS) (150 ml). The mixture was adjusted to 1 M NaCl with NaCl crystals and stirred overnight at 4°C. The suspension was centrifuged for 20 min at 27,000 × g (Sorvall SS-34) and the supernatant precipitated with 1 volume of cold ethanol. The precipitate was recovered by spinning at 27,000 × g, for 20 min (Sorvall SS-34), redissolved in 0.01 M NaCl (50 ml), adjusted to 1.5 p. cent SDS, 0.15 M NaCl and stirred for 30 min. After adjustement of NaCl to 1 M and repetition of stirring overnight at 4°C, the suspension was again centrifuged at 27,000 × g, for 20 min and the supernatant precipitated with 1 volume of ethanol. The recovered precipitate was dissolved in 0.15 M NaCl (15 ml) and the solution extracted with chloroform-isoamyl alcohol (24:1 ; v/v). The chloroform extraction was repeated 3 times and the final aqueous phase was dialyzed against 0.01 M sodium phosphate buffer and used for hydroxyapatite chromatography (total A_{260} units : 1021). In some cases the nucleic acid extract was treated with ribonuclease as specified below (Section 4).

4) *DNA from chloroplasts and mitochondria.*

The purified organelles from 50 g of either green or bleached cells were resuspended in 10 ml of 0.1 M tris-HCl (pH 7.5), 2.5 p. cent SDS, 0.01 M EDTA, and heated for 10 min to 60°. The suspension was adjusted to 1 M $NaClO_4$ and extracted twice with 2 volumes of chloroform-isoamyl alcohol (24:1 ; v/v). The final aqueous layer was digested with RNAase (ribonuclease B, bovine pancreas, type VII, Sigma) reextracted and dialyzed overnight against 0.1 M NaCl-0.05 M sodium phosphate buffer, pH 6.8, 0.1 M EDTA. The dialyzate was purified on a methylated serum albumin-kieselguhr (MAK) [19] column. The DNA peak was pooled and dialyzed against 0.1 × standard saline citrate (SSC : 0.15 M NaCl, 0.015 M citrate, pH 7.5). 1.2 to 1.5 A_{260} units were recovered from green cells ; 10-15 A_{260} units from bleached mutant. The degree of contamination of chloroplast DNA by nuclear DNA varied from practically nothing to about 60 p. cent. The mitochondrial DNA preparation used here contained 90 to 95 p. cent of nuclear DNA.

5) *Chromatography on hydroxyapatite.*

Column and batch procedures were patterned after the experiments described elsewhere on yeast mitochondrial DNA [10].

6) *Ultracentrifugation experiments.*

Sedimentation velocity experiments and analytical caesium chloride density-gradient experiments on native and denatured DNA were done as described elsewhere [10]. Preparative caesium chloride density gradient experiments were also performed as described elsewhere [6].

Denaturation - renaturation experiments were performed as follows : DNA samples (2-3 µg in 0.5 ml) in 0.05 M NaCl were denatured by heating at 100° for 10 min, followed by quenching in an ice bath. Denatured samples were renatured by adjusting the solvent to 2 × SSC with 10 × SSC and by keeping them for 4 hours at 64°.

7) *Base composition of DNA.*

This was determined by enzymically degrading DNA to nucleosides and by analyzing the latter

on either Bio Gel P-2 columns according to Piperno and Bernardi [20] or Aminex A6 columns as described by Thiery, Ehrlich and Bernardi [21].

bed, except that no RNA peak was evident : some low-eluting material, probably formed by residual, degraded RNA, was found in these preparations.

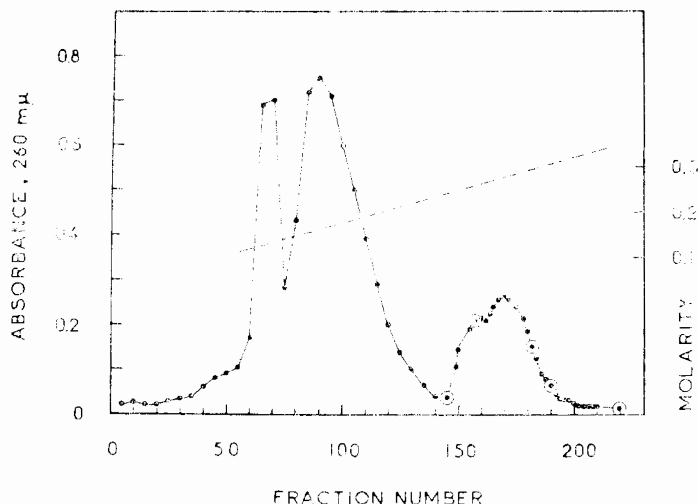


FIG. 1. — Chromatography of a total nucleic acid extract of *Euglena gracilis.*

150 ml of nucleic acid solution in 0.01 M sodium phosphate buffer (150 A_{260} units) were loaded on a 1.08 × 60 cm hydroxyapatite column equilibrated with the same buffer. 160 ml of this buffer were used to wash the column and a 0.01 to 0.5 M gradient of sodium phosphate buffer, pH 6.8 (1200 ml) was applied. 3.4 ml fraction were collected. Flow rate was 34 ml/hr. Circles indicate the limits of the fractions which were pooled together.

RESULTS.

Figure 1 shows the hydroxyapatite chromatogram of a total nucleic acid extract from *Euglena gracilis.* The first biphasic peak is formed by RNA which is well separated from the second major peak representing DNA. 21.7 A_{260} units were recovered under the DNA peak, which is approximately 15 p. cent of the total A_{260} input. This roughly corresponds to the DNA : RNA ratio found in *Euglena* wild-type cells [22]. The bulk of the DNA eluted at a phosphate concentration of 0.28 M. The fractions under the DNA peak were pooled in four DNA samples *a, b, c, d,* which, after concentration and dialysis, were analyzed for their buoyant densities in neutral CsCl. The four profiles are shown in figure 2 and though the gradients were overloaded in case *a, b,* and *c,* no satellite DNA was detectable. It was only in sample *d* that a component of lower buoyant density (ρ = 1.685 g/cm³) showed up in addition to the main nuclear DNA (ρ = 1.707 g/cm³). The 1.685 g/cm³ buoyant density is typical for *Euglena* chloroplast DNA [23, 24, 25]. The mitochondrial DNA with a reported density of 1.690 g/cm³ [26, 27] could not be discerned in any of these profiles. The satellite peak represented 15 p. cent of the nuclear peak in profile *d* and somewhat less than 1 p. cent of the total DNA.

The chromatographic behavior of total nucleic acid preparations which had undergone RNase treatment was exactly the same as that just descri-

The fact that the organelle DNA is eluted at the highest molarity in the chromatogram is very

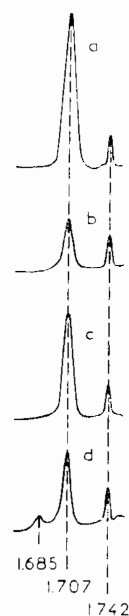


FIG. 2. — Scannings of pooled DNA fractions from the hydroxyapatite chromatogram of figure 1.

a) 147-156 (280 nm) ; *b*) 157-181 (280 nm) ; *c*) 182-190 (280 nm) ; *d*) 191-220.

Samples were centrifuged at 44,000 rev/min for 20 hr at 25°C in a neutral CsCl density gradient. DNA from phage 2 C (1.742 g/cm³) was used as a density marker. Scannings were done at 265 nm, unless otherwise indicated by a number in brackets after the fraction number.

favorable for isolating this component using a batch procedure (10). Knowing from the first experiment that the bulk of nuclear DNA was eluted with 0.28 M sodium phosphate buffer, 50 ml of hydroxyapatite, previously equilibrated with 0.28 M phosphate, were suspended in a total volume of 100 ml of 0.28 M phosphate containing 871 A_{260} units of total nucleic acid. The suspension was gently stirred overnight at room temperature. The HA crystals were washed three times with 50 ml-volumes of 0.28 M phosphate and layered on a 2 × 60 cm hydroxyapatite column equilibrated with the same buffer. The column was washed with 80 ml of 0.28 M buffer and eluted with a linear 0.28 to 0.48 M (1,920 ml) molarity gradient of phosphate. The flow rate was 68.5 ml/h. Fractions of 5.7 ml were collected. Under these conditions, 3.25 A_{260} units were eluted (chromatogram not shown). Three DNA samples were obtained by pooling fractions eluted between the following phosphate molarities : *a*, 0.28-0.29 ; *b*, 0.29-0.30 ; *c*, 0.30-0.32. The recovery in *a*, *b*, *c* were 1.07, 0.62, 1.56, A_{260} units, respectively. The pooled fractions were concentrated by rotary evaporation, dialyzed

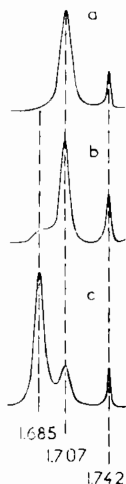


FIG. 3. — Scannings of pooled DNA fractions from the batch experiment (see Text).

a) 40-54 ; *b*) 55-68 ; *c*) 69-92. The molarity of the sodium phosphate buffer were 0.28 at fraction 40, 0.29 at fraction 55, 0.30 a (fraction 69. Centrifugation conditions in neutral CsCl density gradients as given in figure 2.

against 0.1 M NaCl and aliquots were analyzed by ultracentrifugation in CsCl density gradients (fig. 3). Sample *a* contained only nuclear DNA (1.707 g/cm³) ; sample *b* and *c* contained, in addition, a lighter DNA (1.685 g/cm³) at levels of about 5-10 p. cent, and 70-80 p. cent, respectively. The amount of this lighter DNA, corresponding to organelle DNA (see below), extracted by the batch method is equivalent to the amount of organelle DNA found in the chromatogram of figure 1. This indicates that the batch method gives the same yield as column chromatography, and is in essence feasible for large-scale production of organellar DNA from *Euglena*.

The purification of chloroplast DNA from *Euglena* cells is complicated by the presence of mitochondrial DNA, since the fraction eluting at high molarity might contain the mitochondrial DNAas

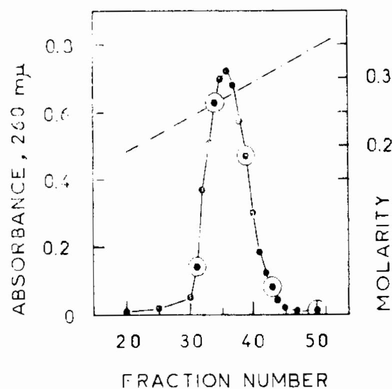


FIG. 4. — Chromatography of DNA from a mitochondria-enriched fraction from a bleached mutant of *Euglena gracilis*. 25 ml of DNA solution in 0.1 M sodium phosphate buffer (25.2 A_{260} units) were loaded on a 1.08 × 60 cm hydroxyapatite column equilibrated with the same buffer. The column was washed with 30 ml of this buffer and a gradient of 0.1 to 0.5 M sodium phosphate (800 ml) was applied. 4.5 ml fractions were collected. Flow rate was 28 ml/h. A_{260} recovery was 92 p. cent. Circles indicate the limits of the fractions which were pooled together.

well. The profile *c* (fig. 3) dose not reveal any component at 1.690 g/cm³, which is the reported buoyant density of *Euglena* mitochondrial DNA. Therefore, either the mitochondrial DNA eluted at a different molarity or the two satellites did not band sufficiently apart.

To clarify this point, we chromatographed DNA extracted from a mitochondria-enriched fraction obtained from a heat-bleached mutant. Figure 4 shows the chromatogram which was again separated into four fractions, *a*, *b*, *c* and *d*. The density profiles of these fractions are shown in figure 5. Again, it is only the last fraction (buffer concentration 0.30-0.35 M) which contains the satellite, though still heavily contaminated with nuclear DNA. The buoyant density in neutral CsCl of the mitochondrial DNA was 1.688 g/cm³, slightly

below the reported values (26, 27). Identical density values (1.688 g/cm³) were obtained, however, for mitochondrial DNA isolated from a bleached mutant (18). The proximity of the densities of chloroplast and mitochondrial DNA probably prevented the resolution of mitochondrial from chloroplast DNA in profile *c* (fig. 3). A denaturation-

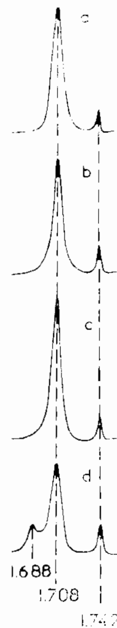


Fig. 5. — Scannings of pooled DNA fractions from the hydroxyapatite chromatogram of figure 4.

a) 31-33 ; *b*) 34-38 ; *c*) 39-41 ; *d*) 42-50. Centrifugation conditions in neutral CsCl are given in figure 2.

renaturation experiment with sample *c* (fig. 3) and sample *d* (fig. 5) confirms this assumption ; the respective profiles are displayed in figure 6. The DNA fraction from the wild-type denatures as one broad peak (profile *c*) (ρ = 1.700 g/cm³) with a shoulder at 1.720 g/cm³. After renaturation, however, a sharp major peak (1.687 g/cm³) and an evident shoulder (1.696 g/cm³) appears, in addition to the minor nuclear DNA peak (1.717 g/cm³)-profile *c'*). The satellite from the bleached mutant shows, after denaturation (profile *d*), a shoulder of density ρ = 1.700 g/cm³ and a major nuclear DNA peak (ρ = 1.720 g/cm³) ; after renaturation (profile *d'*) a sharp peak (ρ = 1.696 g/cm³) replaces the broad shoulder of profile *d* and a major nuclear DNA peak (ρ = 1.717 g/cm³). Comparing profile *c'* with *d'* one readily sees that the shoulder in the former matches the renatured satellite (mitochondrial DNA) of the bleached mutant. The conclusion is that both chloroplast and mitochondrial DNA elute from hydroxyapatite at approximately the same buffer molarity, and therefore the satellite DNA from the wild-type is a mixture of chloroplast and mitochondrial DNA. Organelle

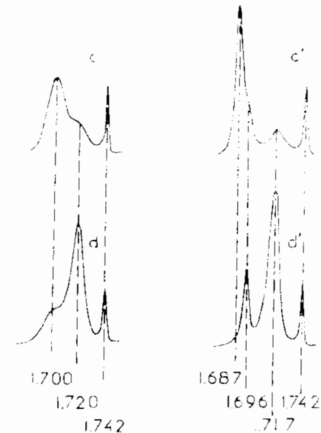


Fig. 6. — Scannings of heat-denatured and renatured DNA from fraction (*c*) of the batch experiment (see figure 3) and fraction (*d*) from chromatogram of the bleached mutant DNA (see figure 4).

c and *c'* are the denatured and renatured DNA, respectively, from fraction (*c*) ; *d* and *d'* are the denatured and renatured DNA from fraction (*d*), respectively. Centrifugation conditions in neutral CsCl as given in figure 2.

DNA's should be prepared therefore from bleached mutants for mitochondrial DNA and from chloroplast enriched fractions for chloroplast DNA.

In subsequent work, we investigated the chromatographic purification of chloroplast-enriched fractions. In the course of these experiments some

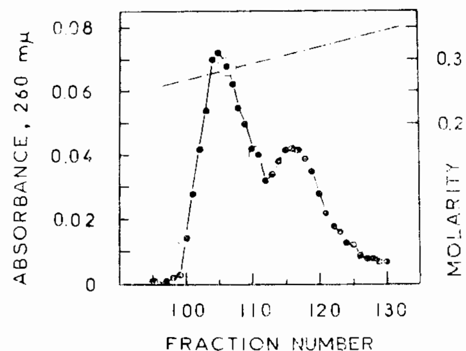


Fig. 7. — Chromatography of DNA from a chloroplast-enriched fraction of *Euglena gracilis*.

7 ml of DNA solution in 0.01 M sodium phosphate buffer (5.7 A_{260} units) were loaded on a 1.08 × 60 cm hydroxyapatite column equilibrated with the same buffer. The column was washed with 30 ml of this buffer and 0.01 to 0.5 M gradient (1000 ml) was applied. 4.6 ml fractions were collected. Flow rate was 28 ml/hr. A_{260} recovery was 82 p. cent. Circles indicate the limits of the fractions which were pooled together.

novel information was obtained on the satellite DNA which was recently described to occur in chloroplast-enriched fractions from *Euglena* [17].

Two different chloroplast DNA preparations were used, the first one still heavily contaminated by nuclear DNA, the second one with no significant amount of nuclear DNA (ρ = 1.707 g cm^{-3}). Figure 7 shows the chromatogram obtained with the first preparation ; the elution profile shows two distinct peaks which correspond to nuclear DNA an dchloroplast DNA, respectively. In fact, this profile almost mimics the buoyant density profile of the starting material (fig. 8, *a*), confirming thereby that the column is quite efficient in separating the two types of DNA. This is confirmed by the buoyant density analysis of the five fractions *b*, *c*, *d*, *e*, *f*, which is in essence gave a

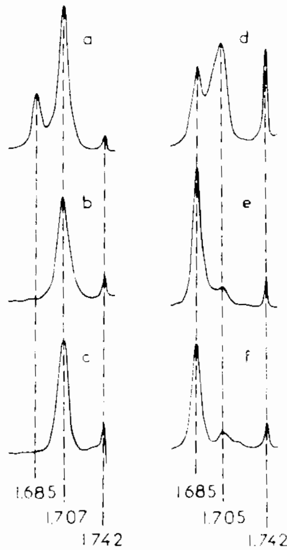


FIG. 8. — Scannings of pooled DNA fractions from the chromatogram of figure 7. The scanning of the starting material is shown in profile (*a*).

b) 100-106 ; *c*) 107-110 ; *d*) 111-114 ; *e*) 115-117 ; *f*) 118-127. Centrifugation conditions in neutral CsCl density gradients as given in figure 2.

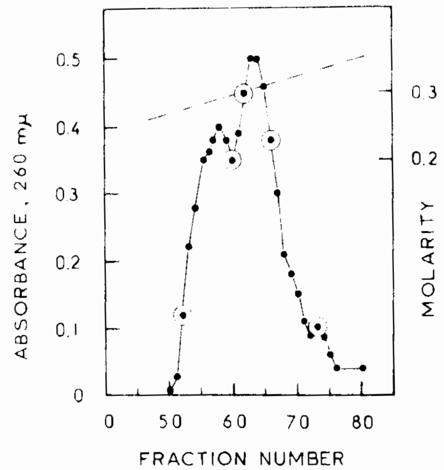


FIG. 10. — Chromatography of DNA from chloroplast fraction of *Euglena gracilis*.

4.6 ml of DNA solution in 0.1 M sodium phosphate buffer (3.6 A_{260} units) were loaded on a 1.08 × 60 cm hydroxyapatite column equilibrated with the same buffer (30 ml) and a 0.1 to 0.5 M gradient (800 ml) was applied. 5.5. ml fractions were collected. Flow rate was 33 ml/hr. A_{260} units recovery was 95 p. cent. Circles indicate the limits of the fractions which were pooled together.

similar result as the first chromatogram with a proportionate up shift of chloroplast DNA. Again, the early eluting fraction (*b*, *c*) contain only the nuclear DNA, the later fractions (*e*, *f*), contain mainly the lighter chloroplast DNA. The intermediate fraction (*d*) is a mixture of heavy and light DNA.

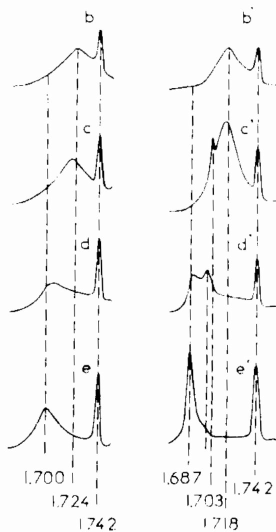
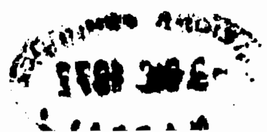


FIG. 9. — Scannings of heat-denatured *b*, *c*, *d*, *e* and renatured *b'*, *c'*, *d'*, *e*, pooled DNA fractions from the chromatogram of figure 7. Centrifugation conditions in neutral CsCl as given in figure 2. Pooled fractions are indicated as in figure 8.

The DNA samples were characterized by their denaturation-renaturation behavior. The results are given in figure 9. Patterns *b*, *c*, *d*, and *e* belong to the denatured DNA ; patterns *b'*, *c'*, *d'*, and *e* belong to the renatured DNA. All denatured DNA samples show the typical increase in buoyant density of approximately 15 mg. The samples differ, however, in their renaturation behaviors. As expected [28], the chloroplast DNA (*e*, *e'*) regains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almost completely the density of the native DNA (1.687 *versus* 1.185 g/cm³). The nuclear DNA (*b*, *b'*) showed after renaturation a broad peak with a mean buoyant density of 1.718 g/cm³. The profile of the two intermediate fractions reveal two

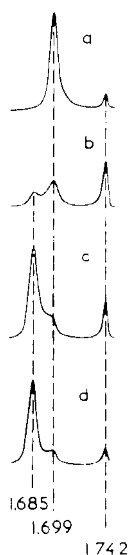

Fig. 11. — Scannings of pooled DNA fractions from the chromatogram of figure 10.

a: 52-59 ; *b*: 60-61 ; *c*: 62-65 ; *d*: 66-73. Centrifugation conditions in neutral CsCl density gradient as given in figure 2.

components after renaturations. Profile *c'* shows a sharp peak with a density of 1.703 g/cm³ and a broader components with an average density of 1.714 g/cm³. The sample *d'* contains probably some chloroplast DNA (1.688 g/cm³) and a component of density 1.700 g/cm³, probably identifiable with satellite DNA recently described [17]. The results of figure 9 suggest that the satellite DNA is eluted from hydroxyapatite at an intermediate position between nuclear and chloroplast DNA, a conclusion confirmed by the following experiments.

Table I.

Nucleoside analysis of chloroplast and satellite DNA's.

	G	T	C	A	G + C
Chloroplast DNA (a)	12.9	37.8	12.1	37.2	25.0
	12.9	37.4	12.2	37.5	25.1
Satellite DNA (b)	22.6	28.6	19.2	29.5	41.8
	21.7	28.9	19.2	30.2	40.9

(*a*) Duplicate analysis on Bio Gel-2 column.
(*b*) Duplicate analysis on Aminex A6 column.

Figure 10 shows the chromatogram obtained with the second chloroplast DNA preparation. As already mentioned, in this case (not shown) nuclear DNA (1.707 g/cm³) was not detectable, but the satellite DNA (1.700 g/cm³) was very prominent. Figure 11 shows the buoyant density profiles obtained on pooled fractions from the chromatogram. It is evident that fractions eluting at increasingly higher molarities become enriched in chloroplast DNA with concomitant decrease of satellite DNA.

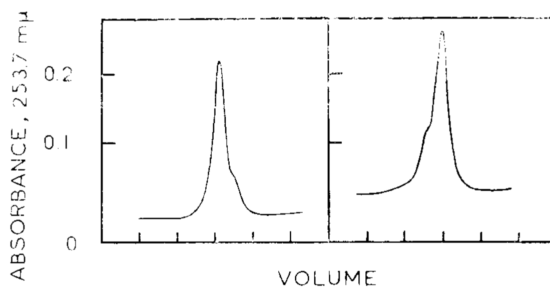

Fig. 12. — Preparative CsCl density gradient of (a) satellite DNA (2.28 A_{260} units) (b) chloroplast DNA (0.94 A_{260} units). The DNA preparations used in this experiment were obtained from the pooled *a* + *b* and *c* + *d* fractions of the chromatogram shown in figure 10, respectively. Divisions on the abscissa scales correspond to 1.93 ml. Arrows indicate the central fractions which were used for analytical gradient (fig. 13) and base analysis (table I).

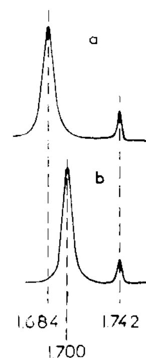

Fig. 13. — Analytical CsCl density gradient of (a) satellite DNA (b) chloroplast DNA. The DNA preparations used in this experiment originated from the central fractions of the preparative gradients shown in the previous figure.

Fractions *a* + *b* and *c* + *d* from the chromatogram of figure 10 were further purified by preparative CsCl density gradients (fig. 12). Figure 13 shows the analytical scannings of these purified fractions, corresponding to satellite (ρ = 1.700 g/cm³), and chloroplast (ρ = 1.785 g/cm³) DNA. Nucleside analysis for these products are shown in table I. The GC levels found for chloroplast and

satellite DNA were 25 p. cent and 10 p. cent, respectively, in good agreement with the buoyant density results. The sedimentation coefficients of the two DNA's were 20 S and 28 S, respectively.

DISCUSSION.

The present work establishes chromatography on hydroxyapatite as a new tool for the preparation of both mitochondrial and chloroplast DNA's from *Euglena gracilis*. In addition, a satellite DNA having a buoyant density of 1.700 g/cm^3 can also be separated from chloroplast-enriched preparations. The cellular origin of this satellite DNA will be discussed elsewhere (paper in preparation).

The practical advantages of the hydroxyapatite chromatography, particularly in its batchwise operation, have been discussed elsewhere [10] and are too obvious, especially for large-scale preparations, to be further commented upon here.

It may be interesting to remark that the four DNA's from *Euglena* investigated here, namely the nuclear DNA, the satellite DNA (ρ = 1.700 g/cm^3), mitochondrial DNA and chloroplast DNA are evidently eluted in the order of increasing AT contents. Some satellite DNA's also appear to be eluted according to their AT contents : for instance, satellite I and II from calf thymus, which are richer in GC compared with main band DNA, are eluted at a lower molarity than the latter [5-7] ; mouse satellite which is poorer in GC compared with main band DNA is eluted after the latter [6, 7]. It might be tempting to conclude therefore that AT-rich DNA's are eluted from hydroxyapatite at higher molarities than GC-rich DNA's, as they are from methylated serum albumin - kieselguhr columns [29], particularly in view of the different secondary structure reported or suggested for very AT-rich DNA's [30, 31]. Such a conclusion in all likeliness is not of general validity since the elution molarities of native DNA's from hydroxyapatite columns are probably related to structural features which do not depend upon the overall base composition in a simple way. It is possible, however, that the high elution molarity from hydroxyapatite is a property common to many AT-rich DNA's and reflects the presence in these DNA's of short alternating and non-alternating AT sequences ; investigations of other AT-rich DNA's such as the mitochondrial DNA's from *Tetrahymena pyriformis* [32] or *Physarum polycephalum* [33] might be interesting in this connection.

Acknowledgments.

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Résumé.

Nous avons étudié le comportement chromatographique des DNAs natifs d'*Euglena gracilis* sur colonnes d'hydroxyapatite. Des préparations d'acides nucléiques totaux peuvent être fractionnées sur colonne, en DNA nucléaire et DNA des organites (mitochondries et chloroplastes). Une séparation en « batch » permet d'obtenir rapidement le DNA des organites à partir de préparations d'acides nucléiques totaux.

La chromatographie de préparations de DNA de mutants étiolés par la chaleur permet de séparer le DNA mitochondrial du DNA nucléaire. La chromatographie de préparations de DNA obtenues à partir de fractions d'Euglène enrichies en chloroplastes permet de préparer le DNA des chloroplastes et, en outre, un DNA satellite déjà décrit (ρ = 1.700 g/cm^3).

Nous présentons ici quelques propriétés physiques et chimiques des DNAs d'Euglène obtenus par chromatographie sur hydroxyapatite.

Zusammenfassung.

Wir haben das Benehmen der nativen DNA von *Euglena gracilis* an Hydroxyapatitsäulen untersucht. Präparate von gesamten Nukleinsäuren können an einer Säule in Nukleus- und Organellen (Mitochondrien und Chloroplasten)-DNA fraktioniert werden. Eine « batch »-Trennung erlaubt es die Organellen-DNA aus Präparaten von gesamten Nukleinsäuren rasch zu erhalten.

Die Chromatographie von Präparaten der DNA aus durch Hitze verblassten Mutanten erlaubt die Trennung der Mitochondrien-DNA von der Nukleus-DNA. Die Chromatographie der aus Euglenfraktionen, welche an Chloroplasten angereichert wurden, erhaltenen DNA-Präparate erlaubt es die Chloroplasten-DNA zu erhalten und ausserdem eine schon beschriebene Satellit-DNA (ρ = 1.700 g/cm^3).

Wir stellen hier einige physikalischen und chemische Eigenschaften der Euglen-DNA vor, welche durch Chromatographie an Hydroxyapatit erhalten wurden.

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