HIGHLY POLYMEROUS NUCLEAR DNA FROM CALF THYMUS ISOLATED BY GEL FILTRATION IN 4 M GUANIDINE HYDROCHLORIDE

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1. Introduction

It has previously been shown that concentrated solutions of guanidine hydrochloride (GHCl) in an SSC medium (0.15 M NaCl; 0.015 M sodium citrate) at room temperature do not cause irreversible denaturation [1] to DNA but severely denature proteins [2]. Denatured protein components of cell nuclei, viruses, and bacterial particles are soluble in this medium. By separating DNA and protein components by gel filtration [3], followed by an additional purification (if necessary) on a methylated albumin column (MAC) [4], highly polymerous, pure DNA can be obtained. In this paper, certain physico-chemical characteristics of nuclear thymus DNA isolated by gel filtration in 4 M GHCl are compared with the properties of DNA prepared according to Kay [5].

2. Materials and methods

Calf thymus nuclei were prepared at $-10°$ from quick-frozen tissue ($-70°$) using a modification of the Welsh's method. The tissue suspension in basic medium I (0.44 M sucrose; 40% glycerol; 0.039 M sodium glycerophosphate; 0.00958 M citric acid, and 0.00938 M sodium citrate at pH 6.1) was purified by layering and repeated centrifugation in media II and III (at 800 and 1,100 *g* for 20 and 30 min) which differ from medium I in glycerol (50%) and sucrose (0.58 M) concentrations, respectively. The resulting nuclear pellet in medium III was freed from a small number of aggregates by centrifugation for 5 min at 100 *g*, and stored at $-70°$.

The nuclear suspension was diluted with tris buffer at pH 8.0 (0.05 M tris; 0.01 EDTA in SSC) and the concentration of GHCl adjusted to 4 M by addition of the solid. (The absorbance of 4 M GHCl was $A_{1\,cm}^{260\,nm} = 0.2$.) The lysed nuclei were kept overnight at $-10°$ and then fractionated at room temperature on Sephadex G-150 (Pharmacia, Uppsala, Sweden).

Sedimentation analysis of DNA (10 to 12 μg/ml in SSC) was performed in Spinco Model E analytical ultracentrifuge at 42040 rpm. The average sedimentation coefficients $s_{20,w}$ were evaluated. The sedimentation of DNA in CsCl gradient was carried out according to Meselson et al. [7]. DNA (2–3 μg/ml) was centrifuged at 44,770 rpm and 20° for 24 hr. *Streptomyces chrisomallus* DNA at density 1.730 g/cm^3 was used as a standard.

The absorption melting curves of DNA were measured by the continuous method [8] in SSC. The concentration of DNA in quartz stoppered cells was 15 μg/ml.

Protein contaminants in DNA were determined by the method of Lowry et al. [9].

DNA samples for electron microscopy were prepared by the modified method of Kleinschmidt and Zahn [10]. DNA solution in 4 M GHCl was diluted with 1 M ammonium acetate buffer at pH 7.2 containing 1 mM EDTA. Samples of DNA (1 μg/ml) were spread onto the clean surface of distilled water, cytochrome *c* was added to give 0.01% protein immediately before spreading the film. After gentle compression, samples were picked up on carbon-coated parlodione films supported on electron microscope grids.

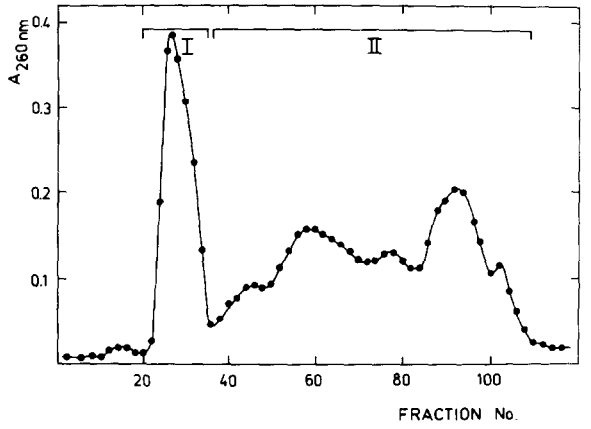

Fig. 1. Gel filtration of 3 ml of a lysate of calf thymus cell nuclei at an A_{260nm} = 3.0 on a column of Sephadex G-150 (2 X 100 cm). Flow rate 1 ml per cm^2 of column cross section. The absorbance of the fractions (3 ml) was measured at 260 nm. (I) DNA; (II) proteins (every other fraction is recorded).

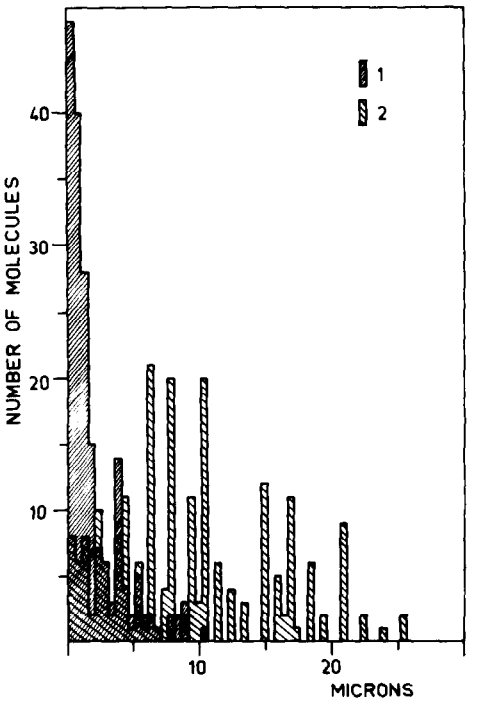

Fig. 2. Frequency distribution of contour lengths of DNA molecules from calf thymus cell nuclei. (1) DNA isolated by the method of Kay [5], 183 molecules measured; (2) DNA isolated by gel filtration, peak I (cf. fig. 1), 212 molecules measured.

3. Results and discussion

Elution peak I (fig. 1) contains DNA. The average sedimentation coefficient $s_{20,w}$ of peak I DNA, after dialysis against SSC, lies within the range 29–32 $s_{20,w}$ corresponding to an average molecular weight of DNA 20 X 10^6 daltons [11]. The quantity of protein contaminants in DNA peak I is about 2%. Peaks II contain proteins. After additional purification of DNA on a methylated albumin column, the sedimentation coefficient does not change, but the concentration of protein contaminants falls to less than 1% [9] (A_{260nm}/A_{280nm} = 1.90; A_{260nm}/A_{230nm} = 2.26). The T_m value of purified native DNA is 87.3°. The sedimentation analysis of 30 $s_{20,w}$ DNA in a CsCl gradient revealed a main asymmetric peak at a density of 1.699 g/cm^3 in addition to two discrete satellite bands at 1.707 and 1.713 g/cm^3. Both the optical and sedimentation methods used, which are sensitive to the presence of denatured DNA, indicate that this DNA is native in character.

The distribution curves of frequencies of contour lengths of (fig. 2) DNA molecules from peak I (fig. 1) show roughly two maxima, between 6 and 10 μm, and 15 and 21 μm, corresponding to a DNA of molecular weight [12] 12–20 and 30–40 X 10^6 daltons, respectively. The maximum frequencies of DNA molecules, prepared by the method of Kay [5] and limited to the shortest lengths, indicate the presence of its degradation product. Specific degradation by 4 M GHCl of thymus DNA, isolated by gel filtration, also cannot be excluded. (Gel filtration of phage T_4 DNA in 4 M GHCl yielded practically intact DNA ($s_{20,w}$ = 60).) If longer molecular units, of nuclear DNA existed in vivo they should be linked together by bonds labile in 4 M GHCl. Nevertheless, relatively large (30 S) intermediates of its replicative form [13] have been found in mammalian DNA. On the other hand, as obvious from fig. 3, even the very long molecules of nuclear DNA form a continuous polynucleotide chain without apparent aggregation.

The results obtained permit us to conclude that 'low-molecular weight' DNA form calf thymus nuclei [6, 14] and similarly, DNA isolated by the stan-

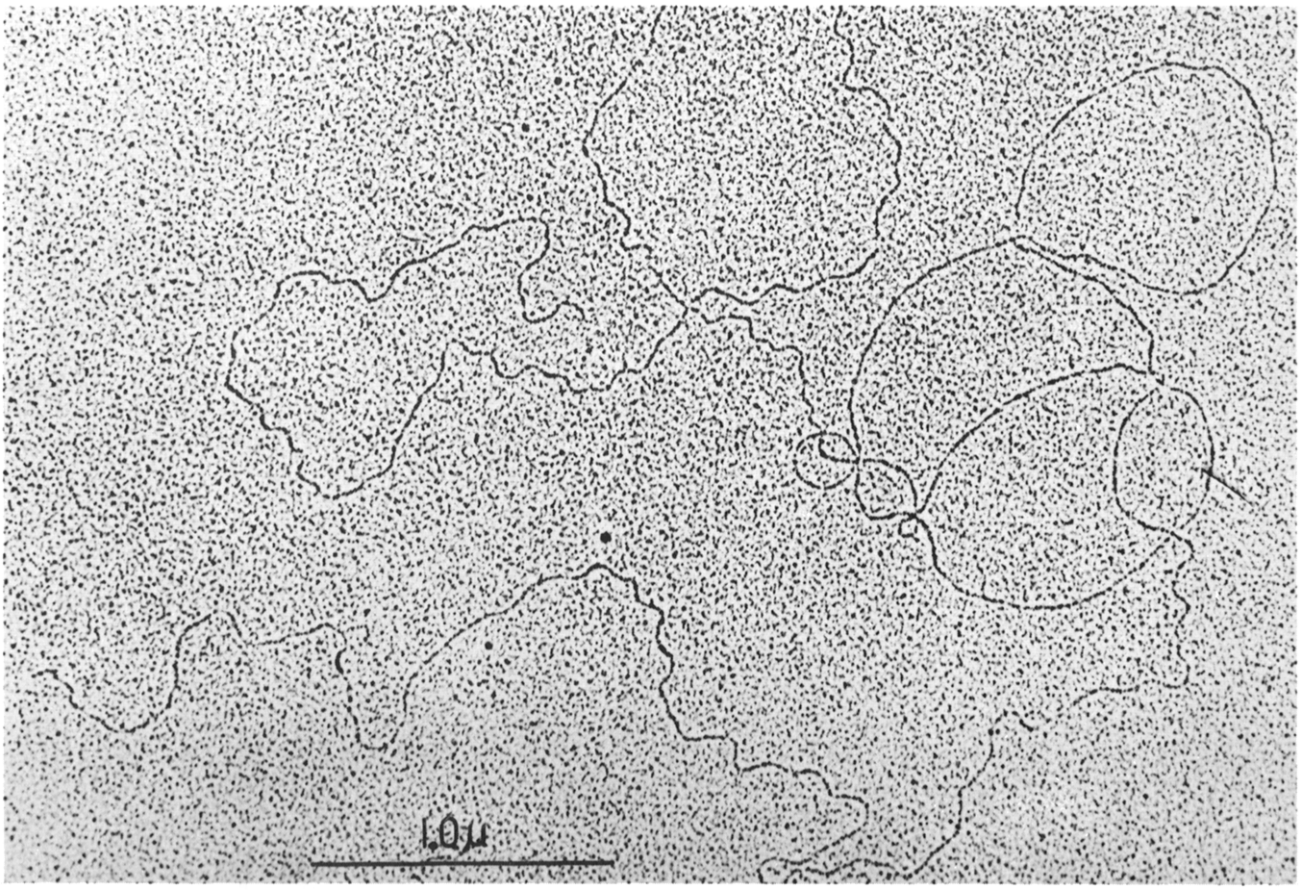

Fig. 3. Electron micrograph of DNA from peak I (cf. fig. 1). The rotating samples were shadowed by Au/Pd and photographed in TESLA electron microscope. The contour lengths of the molecules were measured by a map ruler.

dard procedure, represent a product of partial enzymatic [15] or mechanic degradation.

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