# THE RESOLUTION OF POLYOMA DNA BY POLYACRYLAMIDE GEL ELECTROPHORESIS

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## 1. Introduction

Polyacrylamide gel electrophoresis is a valuable method for producing fine resolution of both single and double stranded RNA species [1–5]. In contrast to this, all DNA molecules larger than about $10^6$ daltons tend to move with the same electrophoretic mobility [1], and no linear relationship between log molecular weight and electrophoretic mobility holds as well as it does for RNA [6, 7]. On the other hand, some degree of success has been achieved in separating small DNA fragments [8, 9].

Since the DNA of Polyoma virus is a double-stranded circular molecule, we have attempted to determine whether the difference in form between circular and linear DNA would provide a basis for separation by polyacrylamide gel electrophoresis. Two forms of the circular molecule can exist: the naturally-occurring supercoiled form, and one with a more relaxed open circular configuration resulting from one or more single-strand nicks [10] (referred to hereafter as nicked circular DNA). At present, the most effective method for separating these is by isopycnic banding in CsCl–ethidium bromide gradients [11]. This method is time-consuming, but will separate supercoiled DNA from nicked circular and from linear forms, the latter two banding together at a lower density (and hence not themselves being separated by this method). We have been able to show that supercoiled and nicked circular forms of Polyoma DNA can easily be separated from each other and from linear DNA by electrophoresis in very dilute polyacrylamide gels.

## 2. Materials and methods

Secondary mouse embryo kidney cells were infected with Polyoma virus, (strain S.P.P115), in the presence of $^3$H-methyl-thymidine, (specific activity 2 Ci/mM), and the viral DNA purified from the cells by the method of Hirt [12]. The supercoiled DNA was isolated from this DNA by CsCl–ethidium bromide gradient centrifugation in a fixed angle rotor [13]. Since supercoiled DNA tends to undergo nicking on storage, a second cycle of CsCl–ethidium bromide gradient centrifugation was performed on the supercoiled fraction immediately prior to these experiments to yield a fresh supercoiled DNA preparation and a nicked circular DNA preparation. The specific activity of these two preparations was 19,700 dpm/$\mu$g.

$^{14}$C-labelled linear DNA of a high molecular weight was prepared from the CCL2 strain of HeLa cells as described by Harley et al. [14]. This was stored at 4° in 1 × SSC and had a specific activity of 5000 dpm/$\mu$g DNA.

Rate zonal density gradient centrifugation was performed in neutral linear 5–40% glycerol gradients under

the same ionic conditions and pH as was used for electrophoresis. 0.2 ml of the DNA sample was layered onto 14.5 ml gradients and centrifuged for 15 hr at 25,000 rpm and 15° in an MSE 6 × 15 ml swing-out bucket rotor.

Polyacrylamide gel electrophoresis was performed, and the gels sliced, as described previously [14]; radioactivity was eluted from the gel slices by incubation with 0.2 ml of 1 N HCl followed after 18 hr by 0.2 ml of 2 N $NH_4OH$, this eluate was then taken up in water-absorbing scintillation fluid and counted as described previously [14].

DNase treatments were performed using various concentrations of electrophoretically purified pancreatic DNase I (Sigma). Incubations were for 5 min at room temp in 0.01 M NaCl, 0.0015 M $MgCl_2$ 0.01 M Tris-HCl, pH 7.4

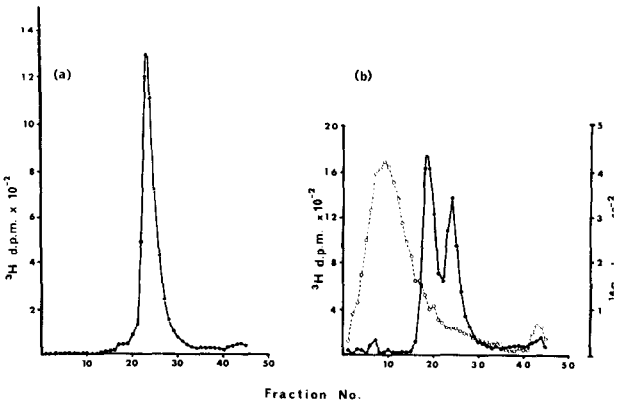


Fig. 1. Rate zonal density gradient analysis of (a) The nicked circular Polyoma DNA preparation, and (b) The supercoiled Polyoma DNA preparation pre-mixed with $^{14}C$ linear DNA. The direction of sedimentation is from right to left. (o–o–o): $^{14}C$, dpm; (•–•–•): $^{3}H$, dpm.

### 3. Results and discussion

In order to provide preliminary data on the composition of Polyoma DNA preparations, and to compare the sedimentation properties of the circular $^{3}H$ labelled DNA with those of the $^{14}C$ linear DNA, a rate zonal centrifugation analysis was performed.

About 1.5 μg of Polyoma preparations were layered on the gradients either alone or mixed with 1 μg of $^{14}C$ linear DNA. This small total quantity of DNA reduces concentration-dependent disturbances in sedimentation rate to a minimum at the centrifugation speeds used [15, 16]. In order to obtain a more meaningful comparison with the electrophoretic patterns, the ionic strength and pH of the gradient buffer was the same as that of the electrophoresis buffer. The counter-ion concentration (0.02 M) is above that level, below which substantial alterations in the sedimentation rate of nicked circular DNA occur [11].

After centrifugation the gradients were fractionated and the radioactivity determined in each fraction; the profiles from such an analysis are shown in fig. 1. The sedimentation profile of the nicked circular Polyoma DNA preparation (fig. 1a) shows a single, apparently homogeneous, peak at fractions 23–24. The method of preparation of the nicked circular species should exclude supercoiled DNA, and this is confirmed by the absence of significant quantities of the faster sedimenting (20 S) supercoiled species. On the other hand, one cannot exclude the presence of linear Polyoma DNA resulting from double-strand scission, since its sedimentation coefficient is very close to that of the nicked circular form (14.5 and 16 S, respectively [10]). To confirm that the sedimentation coefficient of the $^{3}H$ peak in fig. 1a was in the region of 16 S, the nicked circular Polyoma DNA was also co-sedimented with $^{14}C$ labelled 18 S rRNA as a marker, in buffer containing 0.15 M counter-ion concentration. The $^{3}H$ peak sedimented under these conditions just a little more slowly than 18 S RNA, consistent with a sedimentation coefficient of about 16 S.

The profile obtained from the supercoiled preparation (fig. 1b) shows two $^{3}H$ peaks in contrast with the single $^{3}H$ peak in fig. 1a. The smaller peak corresponds in position to the peak in fig. 1a, and hence shows the presence of a considerable amount of nicked circular DNA (and possibly linear Polyoma DNA), which results from gradual nicking of supercoiled DNA on storage [17]. The supercoiled DNA is itself well demonstrated on this profile as the faster sedimenting $^{3}H$ peak. In contrast the $^{14}C$ linear DNA can be seen in fig. 1b to have a much more heterogeneous sedimentation profile than the Polyoma DNA, and most of it sediments faster than the supercoiled species.

Having obtained this preliminary information on the composition of the Polyoma DNA preparations, an electrophoretic analysis was performed on gels of

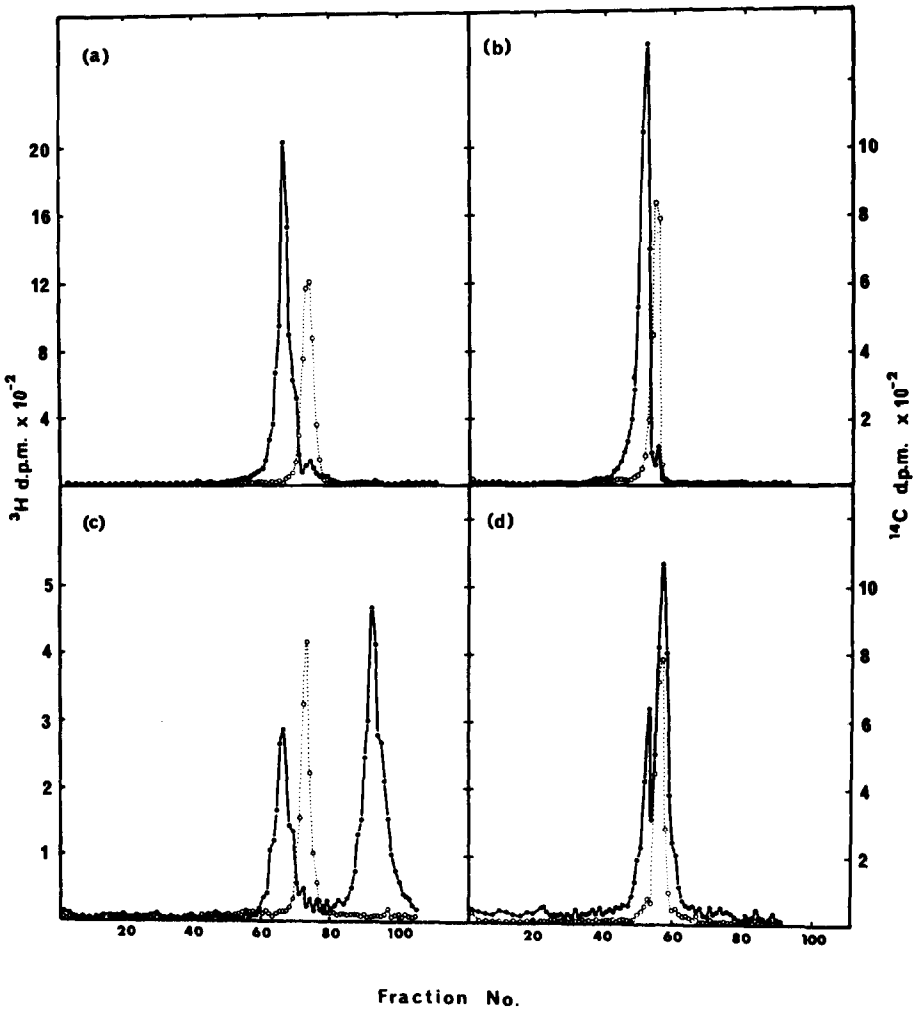


Fig. 2. Polyacrylamide gel electropherograms of the nicked circular Polyoma DNA preparation on (a) 2.0% and (b) 2.4% gels, and the supercoiled Polyoma DNA preparation on (c) 2.0% and (d) 2.4% gels. Approx. 1 μg of $^3$H Polyoma DNA was pre-mixed with 0.5 μg of $^{14}$C linear DNA in each case. Electrophoresis was for 4 hr at 12.5 V/cm of gel and 20–24°. The direction of electrophoresis is from left to right. (o–o–o): $^{14}$C, dpm; (•–•–•): $^3$H, dpm.

varying acrylamide concentration in order to determine whether differences in tertiary structure of DNA could provide a basis for electrophoretic separation. It was found (fig. 2) that such separation could be achieved on very dilute gels (2.0%), whereas on gels of only slightly higher concentration (2.4%) the separations achieved were very poor. This is of interest since a gel concentration of 2.4% will give good resolution of RNA species of comparable molecular weight to Polyoma DNA, and has been used successfully for separating low molecular weight DNA [8].

One of the most striking features of these electropherograms is the way in which the $^{14}$C linear DNA migrates at both gel concentrations as a single species. Assuming that the sedimentation profile of the $^{14}$C DNA (fig. 1) reflects a wide heterogeneity in its molecular weight and since quantitative yields of $^{14}$C are obtained from the gels, it is apparent that the electrophoretic mobility of linear DNA of this size range is independent of molecular weight.

The $^{3}H$ profiles representing the Polyoma nicked circular preparation show one predominant peak migrating more slowly than the $^{14}C$ peak at both gel concentrations.

Electrophoresis of the supercoiled preparation on the 2.0% gel shows two clearly separated peaks of $^{3}H$, (fig. 2c). Their relationship to the $^{14}C$ linear DNA is, however, quite different from that on density gradient analysis, emphasising the different principles underlying separation. The more slowly migrating $^{3}H$ peak in fig. 2c can be identified as nicked circular DNA, by comparison with fig. 2a. This, together with a comparison of relative peak heights, indicates that the faster moving $^{3}H$ peak represents the supercoiled form. Confirmation of the identity of these two species was provided by treatment of the supercoiled preparation with low concentrations of pancreatic deoxyribonuclease (10). This enzyme, introducing single-strand nicks in double helical DNA, will quantitatively convert supercoiled DNA into the more relaxed open circular configuration. If digestion is permitted to proceed further ring opening will give rise to the linear form when two nicks are sufficiently close together on adjacent strands. The supercoiled DNA preparation was therefore treated with varying concentrations of DNase and then analysed on 2.0% gels. The fast moving $^{3}H$ peak decreased in size progressively, and the slower moving $^{3}H$ peak increased in size proportionately, as the DNase concentration was increased from 0.1 to 1.0 ng per ml. This confirmed the identity of the two $^{3}H$ peaks as supercoiled and nicked circular DNA, respectively. Increasing the DNase concentration further (up to 100 ng per ml) resulted in the gradual disappearance of the nicked circular DNA and the appearance of a third $^{3}H$ peak migrating very slightly faster than the $^{14}C$ linear DNA marker (fig. 3). The appearance of a third peak is consistent with the formation of linear Polyoma DNA. Its slightly increased electrophoretic mobility relative to $^{14}C$ linear DNA either reflects a genuine separation on the basis of molecular size or is a result of a multiple single strand nicks. In view of these findings, the small $^{3}H$ peak co-electrophoresing with the $^{14}C$ linear DNA marker in fig. 2a and b suggests that a small quantity of linear Polyoma DNA is present in this preparation.

The separations illustrated in fig. 2c and d show that the electrophoretic mobility of supercoiled DNA

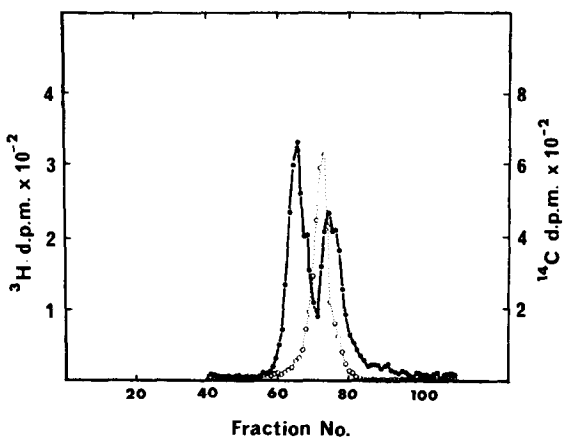


Fig. 3. Polyacrylamide gel electropherogram of 0.8 μg of the supercoiled DNA preparation treated with 10 ng per ml DNase I and mixed with untreated $^{14}C$ linear DNA. Conditions of electrophoresis as in fig. 2. (o–o–o): $^{14}C$, dpm; (•–•–•): $^{3}H$, dpm.

is much more sensitive to changes in acrylamide concentration than is linear DNA. This phenomenon might well prove to have a general application in the identification and characterisation of different structural forms of DNA.

These results show that electrophoresis through very dilute polyacrylamide gels is an easy and practical method for separating three different forms of Polyoma DNA. Since complete separation of supercoiled Polyoma DNA from linear cell DNA species can be achieved on 2.0% gels, polyacrylamide gel electrophoresis may prove of use as a preparative as well as an analytical method.

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