VISCOELASTIC ANALYSIS OF HIGH MOLECULAR WEIGHT, ALKALI-DENATURED DNA FROM MOUSE 3T3 CELLS

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ABSTRACT Alkaline lysates of mouse 3T3 cells showed viscoelastic properties characteristic of very large molecules of single-stranded DNA. The viscoelastic retardation time and the sensitivity to low doses of nitrogen mustard and of X-irradiation suggest a molecular weight in excess of 10^{10} daltons. Contact-inhibited cells yielded larger single strands than actively growing cells.

We have recently obtained alkaline lysates of mouse 3T3 cells which exhibit viscoelastic properties characteristic of very high molecular weight material. Although there is as yet no assurance that the molecules being studied are isolated single strands (Cleaver, 1974; Simpson, et al., 1973; Jolley and Ormerod, 1974) or even are pure DNA, the retardation time and sensitivity to very low doses of nitrogen mustard and of X-irradiation all indicate an extensively strand-separated structure with a molecular weight in excess of 10^{10} daltons.

Mammalian cells lysed at pH > 12 release high molecular weight components which degrade over a period of several hours (Lett et al., 1970; Elkind and Kamper, 1970). With mouse cells we have found that if the pH of lysis and prolonged incubation is decreased to 11.7, stability is considerably enhanced (Fig. 1); i.e., retardation times are measurable even after 72 h, although the amount of viscoelastic recoil decreases with time. On the other hand, if the temperature is raised to 40° or the pH to 12.4, corresponding to an increase of fivefold in OH^- concentration, the viscoelasticity of these solutions decreases until none remains after overnight incubation.

Fig. 1 suggests a strikingly larger molecular weight of the single-stranded DNA obtained from contact-inhibited cells. The exact retardation times obtained for a given series of experiments depended on the precise growth characteristics of the cells being examined, which could vary somewhat from month to month. A significant difference was always observed between actively-growing and contact-inhibited cells, however, which could be due to a lower rate of replication and transcription in the contact-inhibited cells, or possibly an increased repair capacity or decreased nuclease level.

To determine if pH 11.7 is sufficiently alkaline to produce totally single-stranded

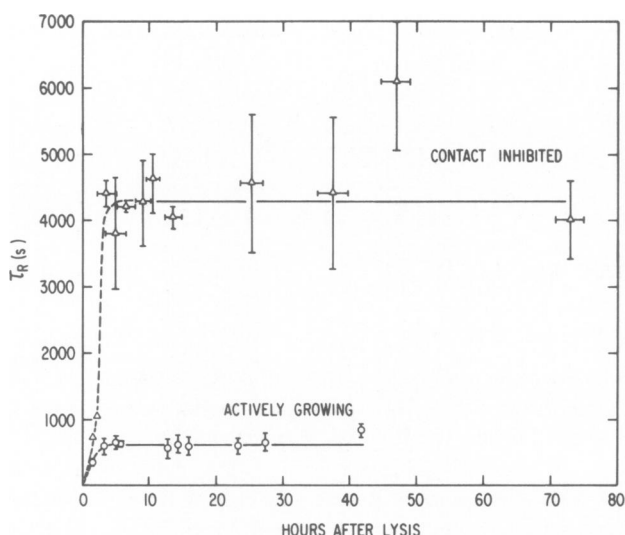DNA, the melting temperature of calf thymus DNA was examined as a function of pH. Studier (1965) has shown that for viral DNA in 1 M Na^+, pH 11.5 is high enough to insure strand separation. Elson and Record (1974) have shown that for T2 DNA in 80% formamide strand separation is complete 15° above the T_m. Fig. 2 indicates that at pH 11.7 we are at least 20° above the T_m. Taken together, these facts suggest that the experimental conditions described in Fig. 1 should be adequate to accomplish strand separation.

Studies using low concentrations of the cross-linking agent nitrogen mustard ["HN$_2$," $(ClCH_2CH_2)_2NCH_3$] and low doses of X-irradiation tended to confirm the existence of single-stranded material. When actively-growing 3T3 cells were incubated with 0.4 μM HN$_2$ for 30 min at 37°, conditions which allow greater than 90% survival
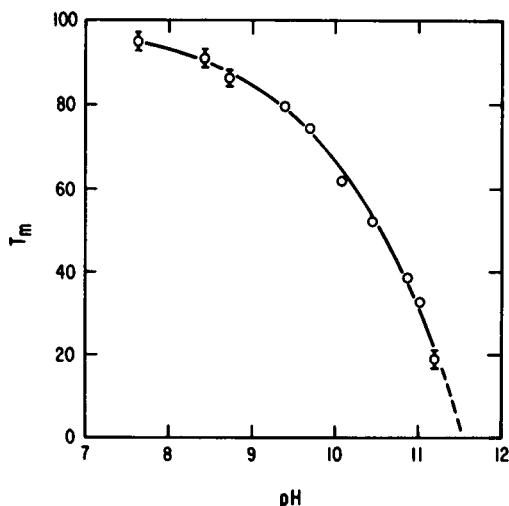
FIGURE 2 Melting temperature of calf thymus DNA as a function of pH in 1 M Na$^+$. Commercial calf thymus DNA (General Biochemicals Div., Mogul Corp., Chagrin Falls, Ohio) was dissolved in 1 M NaCl, 10 mM Na$_2$HPO$_4$, and various amounts of alkaline-EDTA-detergent solutions were added at 0° to obtain the indicated pH's. Melting curves were obtained using a recording spectrophotometer.

of Chinese hamster cells (Sakamoto and Elkind, 1969) and which should produce less than one cross-link per 10^{10} daltons of DNA (Elkind, personal communication), the retardation time of the lysates increased fivefold. This increase could be explained as the result of a covalent linkage binding two opposing DNA strands together, preventing their separation under denaturing conditions.

The effect of irradiation was more complex. Fig. 3 shows that low doses of X-rays increased the retardation time of lysates to twice that of the control, while higher doses sharply reduced τ until after 1 krad very little viscoelasticity remained. These doses were all in the biologically significant range; 100 rads should not be responsible for more than one or two breaks per 10^{10} daltons of DNA (Blok and Loman, 1973). Conceivably, the increase in τ at the two lowest doses could be due to an opening up of a circular or compacted structure or possibly to radiation-induced cross-linking (Blok and Loman, 1973). An alternative explanation for the effects of low X-ray doses and HN$_2$ treatment is an alteration of DNA metabolic processes resembling the onset of contact inhibition.

Although the experiments with HN$_2$ and X-irradiation did not prove that the DNA was exclusively single-stranded, they nevertheless emphasized the large size of the material being examined. Attempts to obtain a molecular weight estimate from retardation time relied on the viral DNA sedimentation data of Studier (1965), which were converted to [η] using the equation of Scheraga and Mandelkern (1953). The resultant empirical relationship, [η] = 5.63 × 10^{-3}M$^{0.77}$, when substituted into the equation of Zimm (1956), yielded the desired relation $M = 2.72 \times 10^8 \tau^{0.565}$. If it is assumed
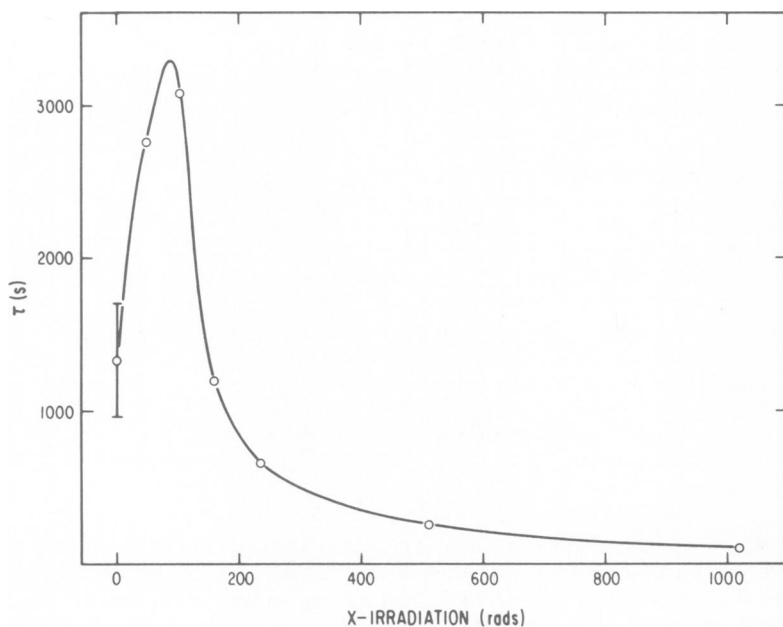
that the molecules being examined in contact-inhibited 3T3 lysates are linear, single-stranded, random coils, the molecular weight calculated using this equation is 3–4 × 10^{10} daltons.

The amount of DNA in the larger mouse chromosomes is about 2×10^{11} daltons, suggesting either that single-stranded DNA exists intracellularly in units smaller than entire chromosome lengths, or that some fragmentation took place before our measurements could be made. We feel, however, that too much uncertainty still surrounds these preliminary results to permit drawing any firm conclusions. In any case, viscoelastometry seems to be useful for characterization of DNA of this size. Even if the structure being examined is not exclusively single stranded, the viscoelastic properties respond to modification of DNA resulting from treatment of mammalian cells with low doses of drugs and radiation.

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