SPECIFIC EFFECT OF T2 INTERNAL PROTEINS ON THE VISCOSITY OF T2 DNA

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SUMMARY: Internal proteins from T2 coliphages have been purified by a new method, involving osmotic shock and chromatography on hydroxylapatite columns. Purified T2 internal proteins interacted specifically with T2 DNA causing folding of the DNA as reflected by significant changes in its viscosity. Internal proteins from T4 phages, similarly affected T4 DNA.

Despite extensive research the mechanism of phage DNA condensation and its packaging during phage morphogenesis has not been resolved. It is well known that the negatively charged phosphate groups of the DNA repel each other; hence, the folding of the DNA can be accomplished only when at least part of these charges are neutralized. It has indeed been demonstrated that purified T-even coliphages contain substantial amounts of cations, polyamines (putrescine and spermidine) (1-2) and basic proteins-internal proteins, which are bound to the viral DNA (3-4) and serve as neutralizing agents.

Baxter-Gabbard and Fraser (5) have recently shown that cations and polyamines reduce the viscosity of T2 DNA, but this effect was found to be non-specific and polyamines acted as surrogate cations. It was tempting to speculate that internal proteins, which are associated with T2 DNA, exert a specific effect on T2 DNA and may thus explain the condensation of phage DNA. Recent studies in our laboratory indicated that internal proteins formed after infection with T4 phage, interact with T4 DNA and not with the DNA of E. coli, T2, and ϕx174 coliphages (6). These binding experiments led to the suggestion that internal proteins are specific folding agents for phage DNA, but final conclusion awaited physical evidence for a specific conformative change in the DNA.

In this communication we report the specific effect of internal proteins of T2 on the degree of folding of the corresponding viral DNA as reflected by viscosity measurements.

MATERIALS AND METHODS:

T2 phages (10^{12} plaque forming units) were suspended in 5 M NaCl solution and osmotically shocked by rapid dilution into 50 volumes of distilled water. Internal proteins were thus released from the disrupted particles along with phage DNA. Thereafter, the salt concentration was adjusted to 1 M, and the suspension stirred for 1 hour followed by centrifugation at 100,000xg for 5 hours. The supernatant fluid was then loaded onto hydroxylapatite columns (2.5x50cm), containing 20g hydroxylapatite (Bio Gel HTP Bio Rad Lab. Richmond, California), equilibrated with 1mM sodium phosphate buffer, pH 6.8. Columns were subsequently washed with 1 to 80mM sodium phosphate buffer to elute free proteins. DNA-internal protein complexes were next eluted from the column by washing with 0.45 M sodium phosphate buffer, pH 6.8. Internal proteins were recovered from the complex by repeated deproteinization with isoamylalcohol-chloroform (1:24), finally dissolved in 0.1xSSC (15mM NaCl and 1.5mM sodium citrate) and stored at -20°.

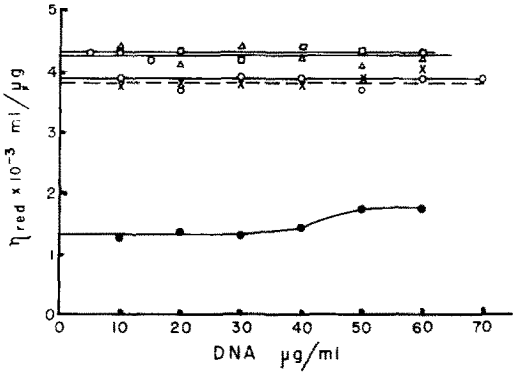


Figure 1. Effect of internal proteins on the reduced viscosity of DNA. Viscosities were measured at 25°C in an Ostwald-Cannon capillary viscometer. Solutions were prepared in 0.1xSSC. Purified T2 DNA-control, 0———0; T2 DNA and T2 internal proteins (25µg/ml), ●———●; T2 DNA and T4 internal proteins (15µg/ml), X-----X; purified E. coli DNA, □———□; E. coli DNA and T2 internal proteins (15µg/ml), Δ———Δ.

DNA from various phages were prepared according to Berns and Thomas (7). No special precaution was taken to avoid breakage and shearing of the DNA during its isolation and handling. Viscosities were determined at 25°C in an Ostwald-Cannon capillary viscometer. This simple procedure was preferred over the more accurate method described by Zimm and Crothers (8), as we were mainly interested in the specificity of the interaction and not in the absolute values. For the same reason, no effort has been made to overcome the non-Newtonian behavior of the DNA solution by extrapolation to zero hydrodynamic shear rate. Intrinsic viscosities, [η] were determined, as usual, by extrapolation to zero DNA concentration of the reduced viscosity, η_{red} = (specific viscosity - 1)/c, versus DNA concentration plots.

RESULTS:

The effect of T2 internal proteins on the reduced viscosity of T2 DNA has been examined. It may be seen (Figure 1) that internal proteins of T2 phage significantly reduced the viscosity of T2 DNA. In control experiments the effect of T2 and T4 internal proteins on T2 and E. coli B DNA was tested. Figure 1 shows that T2 internal proteins did not affect the viscosity of E. coli DNA, neither did internal proteins from T4 phages affect the viscosity of T2 DNA (Figure 1). On

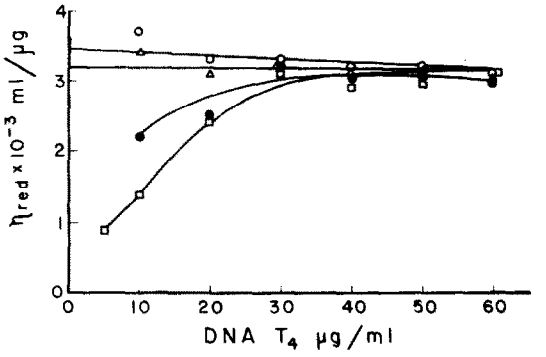
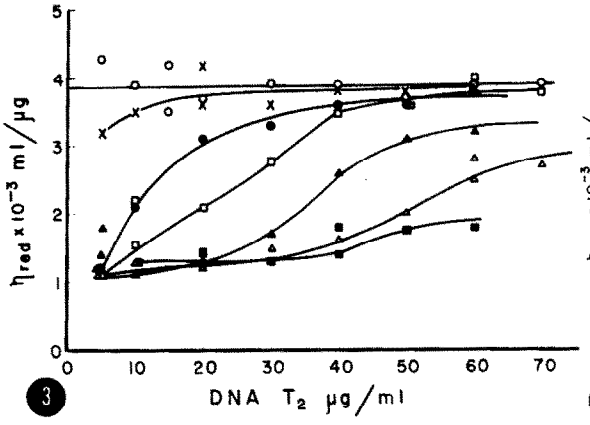


Figure 2. Effect of internal proteins from various phages on the reduced viscosity of T4 DNA. Purified T4 DNA-control, O——O; T4 DNA and T2 internal proteins (15µg/ml), Δ——Δ; T4 DNA and T4 internal proteins (5µg/ml), □——□; T4 DNA and T4 internal proteins (2.5µg/ml), ●——●.

the other hand, T4 internal proteins considerably reduced the viscosity of T4 DNA, the magnitude of the effect being dependent on the protein/DNA ratio (Figure 2). A similar concentration dependence was also noticed when T2 DNA was mixed with T2 internal proteins at various proportions (Figure 3). Moreover, above a certain concentration, T2 internal proteins had no further effect on the η_{red} of T2 DNA (Figure 3). This finding may be explained by a limited capacity of the DNA to bind internal proteins. To test this hypothesis, reduced viscosities were plotted against weight ratios of internal-protein/DNA. Figure 4 shows that at internal-protein/DNA ratios > 0.25, the reduced viscosities resembled [η] and was practically the same even when the internal-protein/DNA weight ratio increased.

DISCUSSION:

The effect of internal proteins on the viscosity of phage DNA was first studied by Chaproniere-Rickenberg et al. (9) who reported a non-specific reduction in viscosity. The effect of internal proteins on the T_m of DNA has also been



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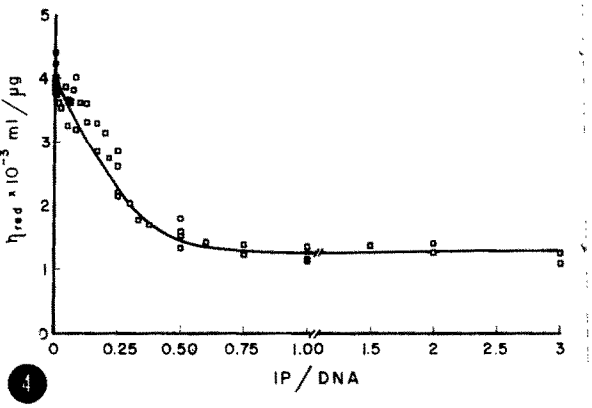

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Figure 3. Effect of T2 internal proteins on the reduced viscosity of T2 DNA. Purified T2 DNA-control, O——O; T2 DNA and T2 internal proteins → 0.25µg/ml; X——X; 2.5µg/ml, ●——●; 5µg/ml □——□; 10µg/ml, ▲——▲; 15µg/ml, Δ——Δ; 25µg/ml, ■——■.

Figure 4. Dependence of reduced viscosity of T2 DNA on the weight ratio of T2 internal proteins and T2 DNA. The weight ratio IP/DNA is expressed as internal proteins (µg/ml) per DNA (µg/ml).

described by Bachrach and Friedmann (10). In the above mentioned experiments crude preparations of internal proteins have been employed. Since contaminating proteins may have caused a non-specific effect, the use of pure internal proteins appeared most desirable. In the present study we used a new procedure for the purification of phage internal proteins and showed that purified T2 internal proteins specifically reduced the viscosity of T2 DNA. These results conform with previous findings in our laboratory regarding the specific cosedimentation of internal proteins with homologous DNA (6).

Experiments depicted in Figure 4 show that 50% of the maximal decrease in DNA viscosity is caused by internal proteins at a concentration ratio internal-proteins/DNA of 0.12. In the intact phage this ratio is approximately 0.05 (11). It thus appears that the condensation of phage DNA, in vivo, is not mediated only by internal proteins. Since cations (such as Mg++) and polyamines may have a synergistic effect, smaller amounts of internal proteins might be sufficient for the specific folding of DNA in vitro. In addition, it is most likely that at least part of the internal proteins used in the in vitro experiment underwent denaturation during the purification procedure.

It is not clear as yet how internal proteins interact specifically with homologous DNA. Previous studies (6), however, exclude the possible function of glucose residues of T-even phage DNA in this specific binding, so that the binding is apparently related to specific base sequences in the respective DNA molecules.

Work is now in progress to elucidate the mechanism of this specific protein-DNA interaction.

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