PURIFICATION AND SOME POSSIBLE FUNCTIONS OF INTERNAL PROTEINS FROM COLIPHAGE T2

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Received January 31, 1967

During the process of infection of Escherichia coli with bacteriophage T2, about 7% of the total phage protein is injected into the bacterial cell together with the phage DNA (Hershey, 1955). This protein is attached to the bacteriophage DNA and may be dissociated by raising the concentration of NaCl or $MgCl_2$ in the medium (Minagawa, 1961).

The function of the internal protein(s) is not known. Kellenberger (1961), suggested that they might serve as "condensation principles", bringing about the folding of DNA during phage maturation. Another possibility to be considered is that the internal proteins might play a role in regulating the transcription of phage DNA (Chaproniere-Rickenberg, Mahler and Fraser, 1964).

This communication deals with an improved procedure for the isolation and purification of the internal proteins. It will be shown that the purified internal proteins (a) modify the structure of phage DNA as reflected by changes in thermal denaturation profiles (b) inhibit DNA dependent RNA polymerase.

E. coli B was grown with aeration at 37° in glycerol-lactate medium (Hershey and Chase, 1952) and infected with T2 phages at a multiplicity of infection of 5. Chloroform was added to facilitate complete lysis, followed by centrifugation at 5,000xg for 10 min. The supernatant was brought to pH 3.9 by adding 1N HCl (approximately 40 ml per liter of lysate) and kept at 4° for 24-36 hours. The precipitate which contained

the phages was collected by decantation, dialyzed against 0.01M Tris-HCl buffer (pH 7.2) for 72 hours and purified by 5 cycles of low and high speed centrifugations (7000xg for 10 min and 13,000xg for 1 hours, respectively). The phages (0.5 - $2x10^{13}$ plaque forming units/ml in 0.01M Tris-HCl buffer, pH 7.2) were then added to an equal volume of 9M glycerol and osmotically shocked by rapid dilution into 50 volumes of distilled water. The suspension was concentrated by polyethelene glycol (m w 40,000), dialyzed against 0.01M Tris-HCl buffer, pH 7.2,for 24 hours and layered on a top of 20% sucrose in 0.01M Tris-HCl buffer, pH 7.2. Centrifugations at 15,000xg for 2 hours brought about the sedimentation of the "ghosts" which were discarded. The supernatant containing internal proteins, bound to DNA, was dialyzed against 0.001 M Tris-HCl buffer, pH 7.2, containing 0.3M $MgCl_2$, to dissociate the complex and to remove polyamines and small peptides. DNA was sedimented by centrifugation at 100,000xg for 5 hours and the supernatant containing

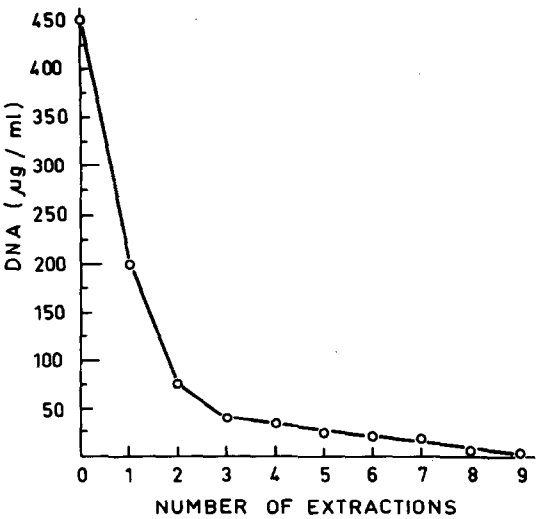

Fig. 1 Extraction of DNA from internal proteins. Internal proteins 320 µg in 2.4 ml 0.01M Tris-HCl pH 7.2, were gently shaken with an equal volume of isoamyl alcohol-chloroform (1:24) for 20 min at room temperature. After centrifugation at 10,000xg for 10 min, the DNA content of the supernatant fluid was determined spectrophotometrically and by the diphenylamine method.

the internal proteins was concentrated by polyethelene glycol to a concentration of 1 mg/ml (Lowry et al, 1951) and dialyzed against 0.01M Tris-HCl buffer, pH 7.2. The proteins thus obtained, still contained DNA which was not dissociated by 0.3M $MgCl_2$ and was not degraded by DNase. To remove this bound DNA, the proteins were repeatedly extracted with equal volumes of isoamyl alcohol-chloroform (1:24, vol/vol). This treatment resulted in a decrease of the DNA content to less than 0.5% (Fig. 1), as determined by the diphenylamine method (Dische, 1930). These purified proteins were dissolved in 1/10xSSC pH 7.0 (0.015 M sodium chloride in 0.0015M sodium citrate). Thermal denaturation studies were carried out in a Hitachi Perkin Elmer spectrophotometer in stoppered quartz cells (10 mm light path). Phage DNA was prepared by incubating T2 phages (10^{12} plaque forming units/ml, in SSC) at 37° with pronase (1 mg/ml), sucrose (final concentration 27%) and sodium dodecyl sulfate (final concentration 0.2%) for 7 hours (Berns and Thomas, 1965). Proteins were removed by three subsequent deproteinization with isoamyl alcohol-chloroform and the resulting protein-free DNA was dialyzed against 1/10xSSC pH 7.0 for 10 hours. Samples containing DNA (45 µg/ml) in 1/10xSSC were heated with various amounts of purified internal proteins. It is evident from Fig. 2 that internal proteins caused a change in

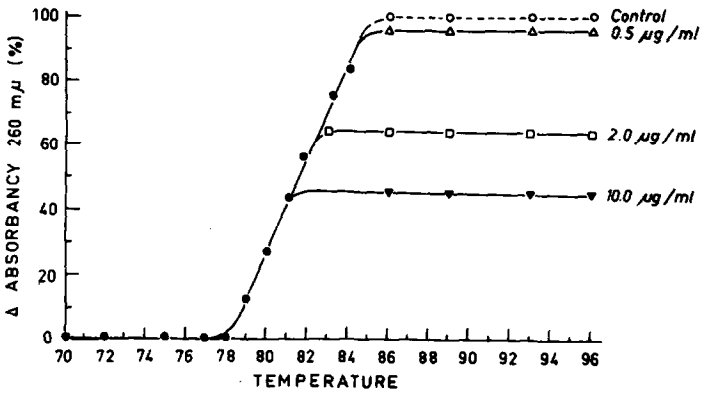

Fig. 2 Effect of internal proteins on thermal denaturation profiles. T2 DNA, (45 µg/ml) in 1/10xSSC was heated in the presence of internal proteins at various concentrations and the absorbancy measured at 260 mµ. Control, DNA only, O- - -O; DNA and internal proteins, _____ .

the denaturation profiles of the DNA. Internal proteins, 0.5, 2.0 and 10 µg/ml reduced the hypochromicity by 4, 35 and 54% respectively, when the optical density, at 260 mµ, of the native DNA was 0.800 and that of the denatured control 1.020.

RNA polymerase was prepared according to Chamberlin and Berg (1962) to the second ammonium sulphate step. This preparation was stored at -20° in a solution containing 0.01M Tris-acetate buffer, pH 7.3; 0.01M magnesium acetate; 0.022 M ammonium chloride; 0.001M mercaptoethanol in 50% glycerol. This preparation was stable for at least one month and was dependent on DNA. The addition of internal proteins to a system containing RNA polymerase, T2 DNA and triphosphates, lead to a significant inhibition of RNA synthesis. Internal proteins, 20, 10, 5 and 1 µg inhibited RNA synthesis by 59, 49, 38 and 27% respectively (Fig. 3). The internal proteins - DNA ratio in the intact phage is approximately 1:20, similar to the ratio used in our experiments.

Our results indicate that internal proteins from T2 phages have at least two functions. On one hand they modify the structure of the DNA and cause "condensation". On the other hand they seem to play a role in regulating DNA transcription. It has already been suggested by Kellenberger (1961), that internal proteins are the "condensation principles". This suggestion is supported by the findings of Chaproniere-Rickenberg, Mahler and Fraser (1964), who reported that internal proteins affected the viscosity of DNA. Contrary to our findings, these authors could not detect any effect of internal proteins on the thermal denaturation profiles. The different results may be due to differences in the preparation of the internal proteins and the DNA. Our DNA preparation was free of internal proteins unlike that used by Chaproniere-Rickenberg et al (1964), who isolated their DNA by phenol extraction. This procedure does not remove all the internal proteins (Mandell and Hershey, 1960; Bachrach and Friedmann, unpublished results).

Internal proteins are rich in lysine and arginine (Coval, Müller and Van Vunakis, 1960; Bachrach and Friedmann, unpublished results) and it is therefore not surprising that they associate with DNA and stabilize it against thermal denaturation, in analogy to the action of other polycationic compounds (Tsuboi, Matsuo and Ts'o, 1966; Ohba, 1966).

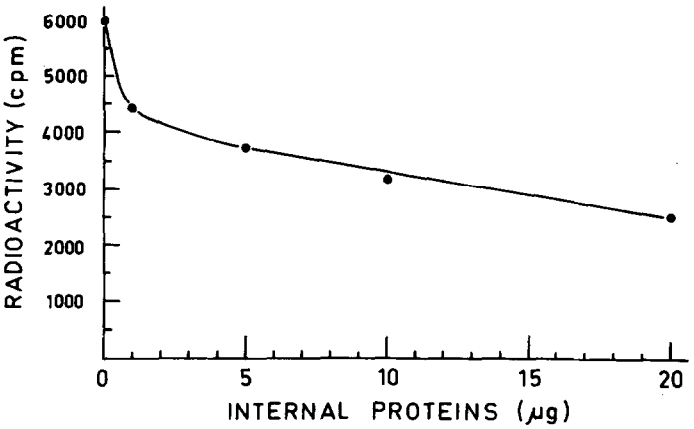

Fig. 3 Inhibition of RNA polymerase by internal proteins. The reaction mixture consisted of: 25 μmoles KCl; 12.5 μmoles Tris-HCl buffer, pH 7.5; 5 μmoles Mg acetate; 1 μmole $MnCl_2$; 0.2 μmole of each triphosphates, CTP, GTP and UTP; 0.025 μC ^{14}C ATP (1.4 μg/μC) 120 μg phosphoenol pyruvate (PEP); 20 μg PEPkinase; 20 μg T2 DNA in a final vol of 0.525 ml. This reaction mixture was incubated at 37° with internal proteins (0.2 ml) for 30 min, followed by the addition of RNA polymerase (0.2 ml). After further incubation at 37° for 30 min, 5 ml of 5% trichloroacetic acid (TCA) was added to stop the reaction. Samples were kept in an ice bath for at least 30 min and the precipitates were then collected on Millipore filters. After washing with 25 ml chilled 5% TCA, the filters were mounted on planchets and the radioactivity determined in a Nuclear Chicago gas flow counter.

Inhibition of DNA dependent RNA polymerase by basic proteins, such as histones, has been described in mammalian systems (Huang and Bonner, 1962; Bonner and Huang 1963; Allfrey, Littau, and Mirsky, 1963). Internal proteins seem to play the role of histones in viral systems - both appear to control transcription. It is not clear as yet whether internal proteins are bound to DNA at specific sites and regulate the reading of specific functions. It is, however tempting to speculate that they might control the transcription of late function genes at early times after infection. Although there is some experimental

support in favor of this assumption (Bachrach and Friedmann, unpublished results), final conclusion awaits further evidence.

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