Methylmercury-Induced Sedimentation Heterogeneity of T7 Bacteriophage DNA

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Single-stranded and methylmercurated T7 DNA is composed of two species as evidenced from the sedimentation pattern displayed during band-sedimentation in self-generating density gradients as well as during equilibrium-sedimentation in Cs_2SO_4 density gradients. Under the given experimental conditions, *viz.*, at pH 9.18 and in presence of 0.1 mM CH_3HgOH, the two species band in Cs_2SO_4 with a density difference of 0.008 g/ml. The ratio of the sedimentation coefficients of the two species is $s^{\circ}_{w,20}$(fast)/$s^{\circ}_{w,20}$(slow) = 1.63 at pH 9.18 and in presence of 1.6 mM CH_3HgOH. Both native and denatured T7 DNA behave as monodisperse systems in the absence of CH_3HgOH.

Introduction

We have become aware that DNA isolated from the coliphage T7 (wild-type) and denatured either by alkali, heat, or by CH_3HgOH, displays paucidispersity, if not polydispersity, when subjected to centrifugation in the presence of CH_3HgOH. This finding is of interest since T7 DNA is considered to be essentially monodisperse [1–3], and its two strands are said not to contain physical interruptions along their polynucleotide chains [2, 4–6]. It appears that the bimodal distribution of methylmercurated T7 DNA observed during centrifugation is indicative of a compositional heterogeneity in the native molecule; the polydispersity noted in one of the bands might have a bearing on the question whether or not T7 DNA contains preformed single-strand breaks [2, 4–9].

Materials and Methods

T7 phage stock (wild-type) was a gift from Dr. M. Chamberlin, Department of Biochemistry, University of California, Berkeley, who, in turn, had received it from Dr. F. W. Studier, Biology Department, Brookhaven National Laboratory, Upton, N. Y. [10]. The lineage of this phage stock has been described in the literature [11]. The phage equilibrium buoyant density in CsCl corresponds to that found for the T7(L) (Luria) strain [12]. Information with regard to the propagation of the phages and the isolation of the DNA can be found elsewhere [13]. The two techniques velocity-sedimentation and equilibrium-sedimentation used in this work have been described [13–15].

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Results and Discussion

In Figs 1 and 2, we have assembled the photoelectric scanner tracings (Beckman Model E analytical ultracentrifuge) that were obtained when T7

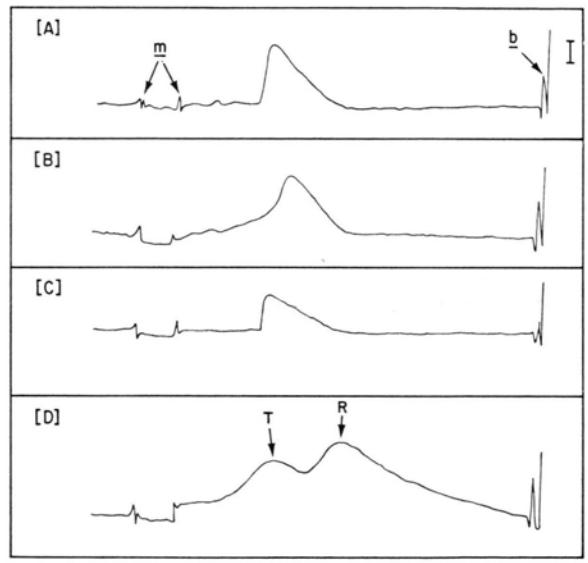

Fig. 1. Band-sedimentation of T7 DNA in self-generating density gradients. [A] Native DNA (1.0 M NaCl+0.01 M Tris buffer, pH 8.0): s°=24.6 S ($s^{\circ}_{w,20}$=30.3 S). [B] Alkali-denatured DNA (1.0 M NaCl+0.1 M NaOH, pH 13): s°=29.5 S ($s^{\circ}_{w,20}$=36.3 S). [C] Native DNA (5 mM Na_2SO_4 +5 mM sodium borate buffer+50% D_2O, pH 9.18): s°=21.4 S ($s^{\circ}_{w,20}$=31.8 S). [D] Alkali-denatured DNA in presence of 1.6 mM CH_3HgOH and at pH 9.18 (5 mM Na_2SO_4 +5 mM sodium borate buffer+50% D_2O, pH 9.18): fast peak R, s°=43.1 S ($s^{\circ}_{w,20}$=57.7 S); slow peak T, s°=26.5 S ($s^{\circ}_{w,20}$=35.5 S). Sedimentation was performed at 26,000 rpm and at 20 °C using 30 mm double-sector centerpieces. DNA concentrations in [A]–[C] about 1 μg/well; in [D] about 3 μg/well. Tracings (photoelectric scanner) were recorded between 30 and 40 min of elapsed sedimentation time. *m* denotes the menisci in the double-sector cells and *b* the cell bottom. The vertical bar on the right-hand side of [A] corresponds to 0.2 absorbance units at 260 nm. Sedimentation was performed in a Beckman Model E analytical ultracentrifuge. Rates of sedimentation are given in Svedberg units S. s°≡observed rate.

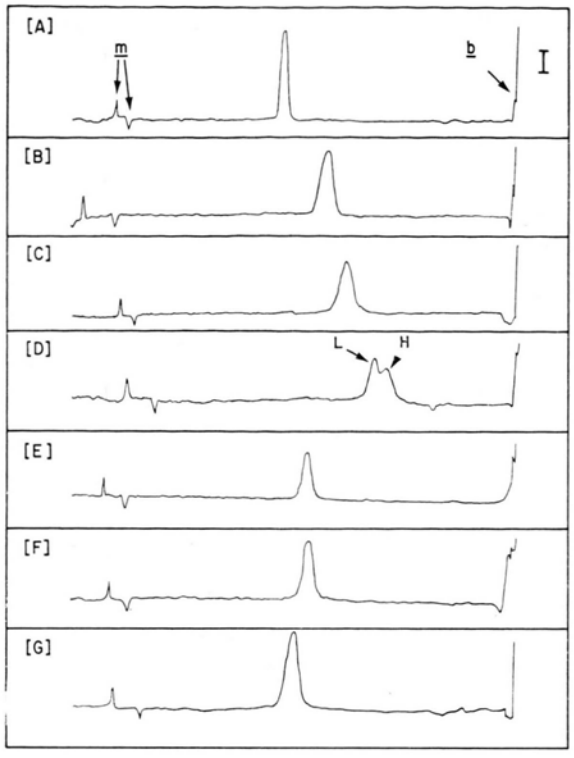

Fig. 2. Equilibrium-sedimentation of T7 DNA in Cs_2SO_4 density gradients. [A] Native DNA, pH 9.18, θ (buoyant density) = 1.426 g/ml. [B] Alkali-denatured DNA, pH 9.18 θ = 1.461 g/ml. [C] Heat-denatured DNA, pH 9.18, θ = 1.458 g/ml. [D] Alkali-denatured DNA in presence of 0.1 mM CH_3HgOH, pH 9.18, θ_L = 1.551 g/ml, θ_H = 1.559 g/ml. [E] Alkali-denatured DNA in the presence of NaOH, pH 13, θ = 1.447 g/ml. [F] Alkali-denatured DNA in presence of 0.1 mM CH_3HgOH and adjusted with NaOH to pH 13, θ = 1.448 g/ml. [G] Alkali-denatured DNA in presence of 0.1 mM CH_3HgOH and a five-fold excess of NaCN, pH 9.18, θ = 1.467 g/ml. Sedimentation was performed at 48,000 rpm and at 25 °C using 12 mm double-sector centerpieces. DNA concentrations in [A] – [G] about 1 μg/cell. *m*, menisci; *b*, cell bottom. Photo-electric scanner tracings were taken between 20 – 24 h of elapsed sedimentation time. The vertical bar on the right-hand side of [A] represents 0.2 absorbance units at 260 nm.

DNA was subjected to centrifugation under a variety of conditions. Fig. 1 contains the results of velocity-sedimentation measurements, Fig. 2 refers to equilibrium-sedimentation. The first three frames ([A], [B], [C]) in Fig. 1 depict, respectively, native DNA in 1.0 M NaCl + 0.01 M Tris buffer, pH 8.0, alkali-denatured DNA in 1.0 M NaCl + 0.1 M NaOH, pH 13, and native DNA in 5 mM Na_2SO_4 + 5 mM sodium borate buffer + 50% D_2O, pH 9.18. The conclusion to be drawn is that T7 DNA, whether in the native state ([A], [C]) or denatured by alkali ([B]), behaves essentially as a monodisperse entity, irrespective of solvent composition. In fact, taking the sedimentation pattern displayed under alkaline conditions ([B]) as a measure of single-strand breaks [2] one concludes that the DNA preparations used in this study consisted of molecules that were 80 – 90% intact. Correcting the observed rates of sedimentation, s°, where the superscript "°" indicates "vanishing" polymer concentrations, in the usual manner for the effects of solvent viscosity and solvent density yields $s^\circ_{w,20}$ values *(cf.*, legend to Fig. 1) that are in excellent agreement with published data [3]. Monodispersity is also indicated in equilibrium sedimentation as can be seen in frames [A] – [C] of Fig. 2, and the buoyant density value obtained for native T7 DNA in Cs_2SO_4 and at pH 9.18 *(cf.*, Fig. 2, [A]: θ = 1.426 g/ml) corresponds to the one listed in the literature [16] as does the buoyant density value determined in CsCl (θ = 1.710 g/ml; tracing not shown). We are, however, unaware of any published buoyant density data for either heat- or alkali-denatured T7 DNA in Cs_2SO_4 density gradients, whether at neutral or alkaline pH, and thus cannot compare our data with those possibly found by others *(cf.*, legend to Fig. 2).

In the presence of sufficiently high concentrations of CH_3HgOH, denatured T7 DNA behaves as a paucidisperse system with one species sedimenting at $s^\circ_{w,20}$ = 35.5 S (T) and the other one at $s^\circ_{w,20}$ 57.7 S (R) *(cf.*, Fig. 1, [D]). Two DNA species are also observed in equilibrium sedimentation as is shown in Fig. 2, [D]. The heavy component bands at θ_H = 1.559 g/ml and the light component at θ_L = 1.551 g/ml. Increasing the concentration of CH_3HgOH above 0.1 mM rapidly shifts the two bands towards locations of higher solvent density without greatly affecting either the relative areas under the peaks or the distance of the peaks from one another. At CH_3HgOH concentrations above 0.4 mM, the two bands become too dense to remain buoyant (for a detailed discussion on this matter see, for instance, ref. [15, 17]). That the methylmercury-induced sedimentation heterogeneity is not an irreversible process can be gathered from Fig. 2, frames [F] and [G]: removal of CH_3Hg^+ from the nucleic acid with the help of suitable complexing agents, *viz.*, OH^- or CN^-, restores the monodisperse system (the high affinity of CH_3Hg^+ to, for instance, Cl^-, but not to SO_4^{2-}, is the reason why Na_2SO_4 and Cs_2SO_4 served as solvents in the investigation).

CH_3HgOH is known to bind to the base moieties of (poly)nucleotides in the sequence $T > G \gg A, C$ at appropriate values of pH [18, 19]. Consequently, we conclude that the existence of two methylmercurated T7 DNA species reflects the existence of a biased base distribution within the DNA with one strand being richer in T and/or G than the other one. Szybalski and coworkers [20] have shown that T7 DNA contains cytosine-rich sequences on only one of the two complementary strands; our results would thus be in harmony with their findings. The other, and potentially more important point of interest is our finding that the two banding species, although comprising equal areas under the curves as expected if they are to represent separated complementary strands, differ in their widths, the heavier band being broader by a factor of about 1.7 – 2.0 (Fig. 2, [D]: $\sigma_H/\sigma_L = 1.75$ assuming Gaussian distribution). Since the T7 phage contains a single piece of double-stranded DNA [21], it is unlikely that broadening is brought about by a distribution of single-stranded whole DNA molecules that differ in their chemical composition but, rather, by a distribution of single-strand fragments. If it is assumed that the two banding species experience about the same density gradient it is easily calculated that the heavier band contains molecules that are approximately only 1/4 to 1/3 the size of the molecules found in the lighter band. Freifelder and collaborators [7 – 9] have advanced the hypothesis that T7 DNA contains single-strand breaks or exceptional bonds which are susceptible to breakage upon denaturation. While this has been challenged by others, *viz.*, [5], our results would be in agreement with the basic premise of Freifelder and collaborators' claim. Attempts will be made to isolate the two methylmercurated DNA fractions on a preparative scale in order to investigate their physical-chemical properties in further detail.

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