CHANGES IN THE SEDIMENTATION CHARACTERISTICS OF DNA DUE TO METHYLMERCURATION

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SUMMARY

The methylmercuric ion induced denaturation of DNA has been followed by determining the rate of sedimentation of native T7 DNA at neutral pH as a function of increasing CH_3HgOH concentration in solvents of varying ionic strength. The denaturation and subsequent complexing reaction of DNA by CH_3Hg^+ appears to proceed in two steps: at first, there is a highly cooperative transition of the DNA from the native to the denatured state as indicated by an abrupt increase of $\underline{s}$ by a factor of about 3. This is followed by a second sharp transition at higher CH_3HgOH concentrations in which $\underline{s}$ increases additionally by a factor of 3 or more. The nature of this very rapidly sedimenting CH_3Hg-DNA complex is unknown at present.

Methylmercuric ions react with native DNA by causing prior denaturation. We have studied this reaction spectrophotometrically (1) and by buoyant density measurements in an ultracentrifuge (2). The salient facts of the buoyant density measurements are: a) the titration of native DNA in Cs_2SO_4 with CH_3HgOH does not affect the buoyant density of the DNA until a critical CH_3HgOH concentration is reached beyond which there is a cooperative transition to denatured DNA which now binds so much methylmercury that it becomes very dense and nonbuoyant; b) the titration of denatured DNA with CH_3HgOH produces a gradual increase in the buoyant density, indicating a gradual increase in the amount of methylmercury bound; c) the denatured CH_3Hg-DNA complex becomes nonbuoyant at the same concentration of CH_3HgOH as does the native DNA.

In an attempt to learn more about the properties of the nonbuoyant CH_3Hg-DNA complex we decided to study the methylmercury-DNA interaction with the help of sedimentation velocity experiments. Some results of this investigation are presented below.

MATERIALS AND METHODS

T7 phage were grown on E. coli B (3) and purified by differential centrifugation and by banding in CsCl (4). T7 DNA was isolated with the help of the phenol extraction method (5) and purified as described previously (6). The DNA was stored at 4° C over chloroform either in 0.05 M Na_2SO_4 + 0.005 M cacodylic acid, pH 6.8, if the sedimentation experiments were performed using 0.05 M Na_2SO_4 + 0.005 M cacodylic acid + 0.9 M Cs_2SO_4, pH 6.8, as a stabilizing medium ("high salt" bulk solution; ρ = 1.250 g/ml at 20° C) or in 0.0025 M Na_2SO_4 + 0.005 M cacodylic acid, pH 6.8, if the sedimentation was performed in 0.0025 M Na_2SO_4 + 0.005 M cacodylic acid + 0.45 M Cs_2SO_4, pH 6.8, ("medium salt' bulk solution; ρ = 1.127 g/ml at 20° C) as well as in 0.0025 M Na_2SO_4 + 0.005 M cacodylic acid + 0.16 M Cs_2SO_4, pH 6.8, ("low salt" bulk solution; ρ = 1.044 g/ml at 20° C).

Sedimentation velocity experiments were performed routinely at 20° C and at 24,000 rpm or 26,000 rpm in a Beckman Model E Ultracentrifuge. The method of band sedimentation in self-generating density gradients was used in order to determine the rate of sedimentation (7-9). The lamellar solutions contained DNA ranging from 0.5 µg/50 µl (at low concentrations of CH_3HgOH) to 11 µg/50 µl (at limiting concentrations of CH_3HgOH) due to the increased polydispersity of the DNA samples. The ionic composition of the lamellar DNA solutions was that of the DNA stock solutions described above. Both bulk and lamellar solutions contained identical DNA concentrations. All s-values, given in Svedberg units, are uncorrected for the solvent.

RESULTS AND DISCUSSION

The addition of increasing amounts of methylmercuric hydroxide to native T7 DNA does not alter the rate of sedimentation of the macromolecules until a critical CH_3HgOH concentration is reached, beyond which s suddenly increases (Fig. 1, curves A and B, and Fig. 2). At the particular salt concentration pertaining to curve A, "high salt" bulk solution, the increase in s commences at pM = 3.5 [pM = - log (CH_3HgOH)]. At pM = 3.3, s appears to reach a plateau

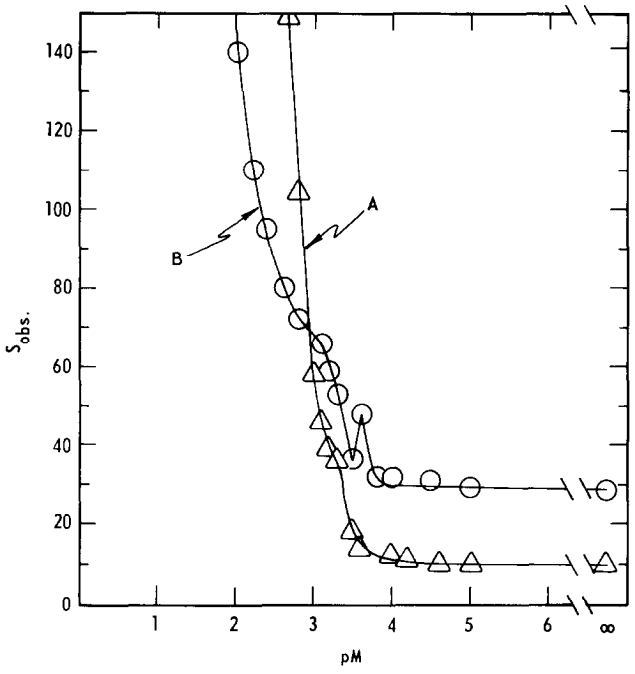

Fig. 1. Plot of the rate of sedimentation s as a function of pM. pM = - log (CH_3HgOH). Curve A: "high salt" bulk solution. Curve B: "medium salt" bulk solution (see MATERIALS AND METHODS).

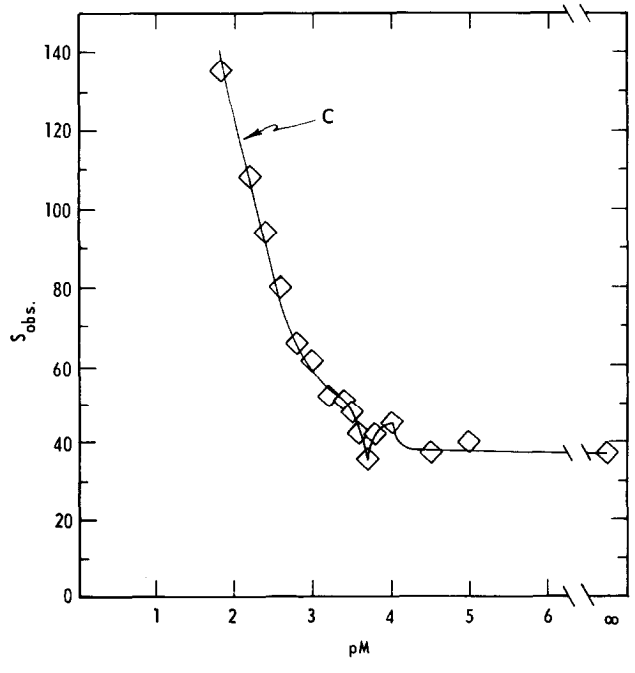

Fig. 2. Plot of the rate of sedimentation s as a function of pM. pM = - log (CH_3HgOH). Curve C: "low salt" bulk solution (see MATERIALS AND METHODS).

region after having increased from $\underline{s}$ = 12 to $\underline{s}$ = 36. However, at pM = 3.0, the rate of sedimentation increases again sharply; indeed, at pM = 2.6, $\underline{s}$ = 170.

It can be shown that removal of CH_3HgOH at pM > 3.5, for instance, by adding a sufficiently strong complexing agent such as sodium cyanide, yields DNA that sediments with a rate equal to that of native DNA whereas removal of CH_3HgOH at pM < 3.5 yields a DNA that sediments at a rate comparable to that of alkali denatured DNA. We conclude from this that native DNA does not react with CH_3HgOH until a critical concentration is reached, at which point a large amount of reaction abruptly occurs yielding denatured methylmercurated DNA. These results are thus in complete agreement with the results of our spectrophotometric and buoyant density studies in which it was shown that the interaction of methylmercuric ions with native DNA leads ultimately to single-stranded DNA (1,2).

Curve B, Fig. 1, and curve C, Fig. 2, show the dependence of $\underline{s}$ on pM at the ionic strength of the corresponding sedimentation bulk solutions. Due to the decrease in the counter ion concentration, the denaturation reaction proceeds at progressively lower CH_3HgOH concentrations. For instance, in the "medium salt" bulk solution, $\underline{s}$ increases at pM = 3.8 whereas $\underline{s}$ starts to increase at pM = 4.1 in the "low salt" bulk solution. In contrast to the sedimentation behavior of T7 DNA exhibited in the "high salt" bulk solution, the initial increase in $\underline{s}$ of T7 DNA in the "medium salt" as well as "low salt" bulk solution is followed by a sudden decrease in the rate of sedimentation with a subsequently renewed increase of $\underline{s}$ at still higher CH_3HgOH concentrations. Although the $\underline{s}$, pM-values of the various titration curves were highly reproducible and thus seem to indicate to us real differences in the sedimentation characteristics of the macromolecules, we are nevertheless aware that we may have overlooked the corresponding drop in $\underline{s}$ in the "high salt" medium.

It is reasonable to associate the initial increase in $\underline{s}$ with a breakdown of the helical structure since this lowers the radius of gyration and, consequently, enhances the flexibility of the macromolecules. The subsequent drop in $\underline{s}$ is a result of strand separation and the accompanying decrease in molecular

weight. Indeed, the sedimentation coefficient measured at pM = 3.5 (cf. curve B, Fig. 1), $\underline{s}$ = 36.5, is almost identical with that of alkali denatured DNA, $\underline{s}$ = 36.0, at the same ionic strength. Similar results were obtained by Freifelder and Davison in studying the heat denaturation of T7 DNA in the presence of formaldehyde (10), by Studier in studying the alkaline denaturation of DNA (11), and by Zimmer and Triebel in their investigations concerning the acid induced helix-coil transition of DNA (12).

The renewed increase of $\underline{s}$ at the higher CH_3HgOH concentrations is less easily explained. In part, the increase is undoubtedly due to the mass increase brought about by the addition of the methylmercuric cation to the thymidine and guanosine binding sites of single-stranded DNA since this cation has a high mass and a low specific volume (1,2). However, after saturation of all binding sites, $\underline{s}$ should reach a limiting value and cease to be a function of pM. Let us consider this somewhat more in detail: at the limit of infinitely diluted polymer, the observed sedimentation coefficient $\underline{s}$ may be related to properties of the solvent and sedimenting species by the equation $\underline{s}N\eta f = M_s(1 - \bar{V}_s\rho)$ [eqn. (I)] where M_s, $\bar{V}_s$, f are the molecular weight, partial specific volume and frictional coefficient of the solvated species, and ρ, η and N are the density and relative viscosity of the solvent and Avogadro's number (13-15). If we define with $\underline{r_b}$ the number of moles of bound methylmercuric cation per mole of DNA nucleotide and with $\underline{\Gamma}$ the preferential solvation in moles of bound water per mole DNA nucleotide, $M_s = M_3 + \underline{\Gamma}M_1 + \underline{r_b}M_x$ [eqn. (II)] where M_3 is the unhydrous molecular weight of the polymer, M_1 the molecular weight of water and M_x the molecular weight of the methylmercury group. Similarly,

$\bar{V}_s = (M_3\bar{V}_3 + \underline{\Gamma}M_1\bar{V}_1 + \underline{r_b}M_x\bar{V}_x)/(M_3 + \underline{\Gamma}M_1 + \underline{r_b}M_x)$ [eqn. (III)] where $\bar{V}_3$, $\bar{V}_1$, and $\bar{V}_x$ are, respectively, the partial specific volumes of the polymer, the water of solvation and the bound methylmercury group. Substitution of eqns. (II) and (III) into (I) yields $\underline{s}N\eta f = M_3(1 - \bar{V}_3\rho) + \underline{r_b}M_x(1 - \bar{V}_x\rho)$ [eqn. (IV)] if, at the same time, $\bar{V}_1$ is set equal to $1/\rho$. If it is further assumed that the frictional coefficient of single-stranded DNA is unaffected by methylmercuration, eqn. (IV)

permits an estimate of the increase in $\underline{s}$ due to the addition of the CH_3Hg-group to denatured DNA. We set M_3 = 441 (monomer molecular weight of the cesium salt of T7 DNA), $\bar{V}_3$ = 0.47 ml/g, $\bar{V}_x$ = 0.19 ml/g, M_x = 216, and $\underline{r_b}$ = 0.5 [that is, we assume that binding occurs only to two bases, e.g., T and G; see ref. (2)] and obtain from eqn. (IV) $\underline{s}_{(r_b=0.5)}/\underline{s}_{(r_b=0)}$ = 1.45. Curve C, Fig. 2 [$\rho \rightarrow 1/\bar{V}_1$], shows that $\underline{s}$ indeed increases by a factor of about 1.5 when going from the pM value at the point of strand separation to the pM values at the beginning of the plateau region indicating to us that the above interpretation is in all likelihood correct and that this change in $\underline{s}$ is caused by the mass increase due to methylmercuration. We are, however, at a loss to explain the dramatic increase in $\underline{s}$ at pM < 3.

Figs. 1 and 2 show that a lower CH_3HgOH concentration is needed in the "high salt" bulk solution than in the "low salt" bulk solution in order to produce the rapidly sedimenting CH_3Hg-DNA species. We expect that this trend continues if the supporting electrolyte concentration is further increased. Indeed, in our buoyant density measurements we find that, for instance, calf thymus DNA becomes nonbuoyant at pM = 3.2 ($\underline{s}$ > 0) in a buoyancy medium containing about 1.6 $\underline{M}$ Cs_2SO_4(2)

We have previously proposed (2) that at a sufficiently high CH_3HgOH concentration and at sufficiently high Cs_2SO_4 concentrations, or correspondingly low water activities, the addition of the CH_3Hg-group to DNA causes an abrupt decrease in the net hydration of the polymer. This would mean that the approximation $\bar{V}_1 = 1/\rho$ is invalid and that $\underline{s}$ will also be a function of $\underline{\Gamma}$ [eqn. (IV)]. The experimental results presented thus far seem to support this notion. It is possible that the sharp rise of $\underline{s}$ at pM < 3 reflects the onset of microgel formation brought about by the increase in hydrophobic character of the methylmercurated DNA.

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