Circular Dichroism and Ultraviolet Absorbance of Calf Thymus DNA in Presence of CH_3HgOH

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The changes that one observes upon the addition of CH_3HgOH in the circular dichroism spectrum and ultraviolet absorbance spectrum of native calf thymus DNA, dissolved in buffered (pH 6.8) solutions of Na_2SO_4 at pNa 2.0, 1.5, 1.0, and 0.0, respectively ($pNa \equiv -\log [Na^+]$), are shown to be due to denaturation brought about by the organomercurial interacting with the base moieties of the polymer. The changes are characterized by an extensive shift of both spectra to longer wavelengths, by a decrease of the rotational strength of the long-wavelength positive dichroic absorption band, and by an increase in the UV absorbance at λ_{max}. Both the hyperchromicity H^λ of calf thymus DNA and the normalized decrease of the rotational strength of its long-wavelength positive dichromic band, Ω, display cooperativity when plotted against the methylmercury concentration pM ($pM \equiv -\log [CH_3HgOH]_{added}$) at a given salt strength. Rotational strength data, evaluated by integration of the area under the positive and negative dichroic absorption bands, have been tabulated for selected values of pNa and pM. They are compared with data available from the literature. In absence of CH_3HgOH, and with varying salt strength, native calf thymus DNA exhibits alterations in the long-wavelength positive dichroic absorption band that are interpreted as representing $B \rightarrow C$ transitions in agreement with currently held views regarding their origin. Similar salt-induced alterations have been noted in the case of denatured DNA; their meaning in terms of DNA geometry remains unclear at this point in time.

Introduction

Methylmercury, for instance in the form of CH_3HgOH, is a powerful denaturant that disrupts Watson-Crick hydrogen bonding in double-stranded nucleic acids [1, 2], interferes with the bonding of the arginine-rich histones H3 and H4 by DNA in chromatin (Otsuki and Gruenwedel, to be published), and that causes, *in vitro* as well as *in vivo*, extensive chromosome damage such as pycnotic clotting, chromosome breakage and so forth [3–6].

While studying its effect on the optical properties of calf thymus chromatin (the diffuse interphase form of the chromosomes of the cell nucleus) by means of circular dichroism and ultraviolet absorbance measurements, we also investigated, for comparison reasons, its effect on the optical properties of the constituent chromosomal nucleic acid, *i.e.*, calf thymus DNA, alone. The optical properties of nucleic acids, as determined by circular dichroism (CD) or optical rotatory dispersion (ORD), are extremely sensitive to changes in solution composition [7–10] and to alterations in the polynucleotide secondary structure [7, 11, 12]. Hence, the application of CD measurements should prove useful in studying the conformational changes and solvent perturbations brought about in DNA by methylmercury and thereby also brought about in chromatin.

In this communication, we present the results of CD measurements obtained with calf thymus DNA in presence and absence of CH_3HgOH and at different levels of supporting electrolyte. UV absorption measurements accompanied circular dichroism studies in order to correlate the data on optical activity with those on absorption. The results of the chromatin-methylmercury study will be presented at a later date (Clegg and Gruenwedel, manuscript in preparation).

Experimental

Materials

Calf thymus DNA (sodium salt) was purchased from Worthington Biochemical Corporation and stored at $-20\,^\circ C$ until used. Anhydrous Na_2SO_4, analytical grade, was obtained from Mallinckrodt. It served as supporting electrolyte since it does not complex methylmercuric hydroxide. CH_3HgOH (97 + %) was purchased from Alfa Products, Ventron Corporation. Further information concerning

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the reagents used in this study can be found elsewhere [13].

A primary stock DNA solution was prepared by gradually dissolving native DNA in SSC (0.15 M NaCl, 0.015 M sodium citrate, pH 7) at 5 °C. Chloroform was added as a preservative. Experimental solutions were then prepared by dialyzing the DNA stock solution against several changes of Na_2SO_4 solutions that were constant in buffer (5 mM cacodylic acid buffer, pH 6.8) but that contained varied amounts of Na_2SO_4. Dialysis was performed at 5 °C. The experimental solutions are referred to in terms of pNa and pM. $pNa \equiv -\log[Na^+]$, as brought into the system by Na_2SO_4 and buffer, and $pM \equiv -\log[CH_3HgOH]$, with $[CH_3HgOH]$ representing the stoichiometric CH_3HgOH concentrations of the solutions. Experiments were performed at the salt levels pNa 2.0, 1.5, 1.0, and 0.0.

Methods

Circular dichroism and ultraviolet absorbance measurements. Circular dichroism was measured with the Jasco-Durrum ORD/UV/CD5 recording spectropolarimeter, equipped with a 450 W xenon lamp as light source. All measurements were performed at 25 °C and in an atmosphere of nitrogen. Spectra were recorded from 350 – 210 nm. An instrumental program varied the slit width, and it, together with the photomultiplier voltage, was recorded simultaneously with the spectral measurements. Off-scale photomultiplier voltage or off-scale pen travel was taken to stop a scan. The instrument was calibrated before and after each scan with (+)-10-camphorsulfonic acid to minimize potential errors due to instrument drift. Scans were also performed on salt solutions alone so that all spectra presented in this work are corrected for solvent effects. The primary results of the measurements are given in terms of the difference of the molar absorptivities for left- und right-handed circularly polarized light, $\varepsilon_L - \varepsilon_R$, at the frequency ν (or wavelength λ). $(\varepsilon_L - \varepsilon_R)$ values were obtained from averaged and smoothed data. The rotational strength R of a particular dichroism absorption band was calculated by using the relation [7]

$$R = \frac{3\,h\,c \times 10^3 \ln 10}{32 \times \pi^3 \times N} \int \left(\frac{\varepsilon_L - \varepsilon_R}{\nu} \right) d\nu \qquad (1)$$

where h and N are Planck's constant and Avogadro's number, respectively, and where c is the velocity of light. Integration was performed between crossover points using $\Delta\lambda = 1$ nm increments, plus their associated $(\varepsilon_L - \varepsilon_R)$ values, in the calculation. The Gauss-Legendre quadrature formula was employed [14].

Ultraviolet absorbance measurements were performed at 25 °C in the Cary/Varian Model 118 C recording spectrophotometer by scanning each sample from 350 – 210 nm. Appropriate $CH_3HgOH - Na_2SO_4$ mixtures served as optical blanks. Scans were stopped when the recorder pen went off-scale, *i. e.*, beyond 2 absorbance units.

DNA concentrations were determined by using the molar absorptivity of 6,600 l/mol DNA-P · cm at the absorption maximum. All measurements were performed in square 1 cm × 1 cm Teflon-stoppered quartz cuvettes. DNA concentrations usually amounted to 1.0 – 1.5 A^{260} units.

Denaturation experiments. DNA samples were treated with CH_3HgOH at 25 °C in the manner described in detail elsewhere [1]. The methylmercury-induced hyperchromicity of DNA is defined as the normalized absorbance increase at λ_{max} after treatment of the sample with methylmercuric hydroxide: $H^\lambda \equiv (A^\lambda_{pM} - A_0{}^\lambda)/A_0{}^\lambda$, where A^γ_{pM} is the absorbance of the sample at λ_{max} in presence of methylmercuric hydroxide at the concentration pM and where $A_0{}^\lambda$ is the absorbance of the untreated sample at λ_{max}.

Methylmercury-induced changes in the rotational strength R of a dichroic absorption band are expresses as: $\Omega \equiv (R_0 - R_{pM})/R_0$, where R_0 is the rotational strength of the untreated sample and R_{pM} is the rotational strength of the sample at the CH_3HgOH concentration pM. Lastly, $pM_{1/2}$ is defined as that pM value at which both H^λ and Ω equal one-half of their limiting (maximum) values.

Results

The spectral changes that one observes when increasing amounts of CH_3HgOH are added to native calf thymus DNA, dissolved in Na_2SO_4 of pNa 2.0 at pH 6.8, are shown in Figs 1 and 2. In Fig. 1, we have displayed the CD spectra and in Fig. 2 the UV absorbance spectra.

There exists a certain concentration range of CH_3HgOH in which no changes in either circular dichroism or ultraviolet absorbance of native DNA can be observed indicating that in this range CH_3HgOH fails to interact with the polymer. At the

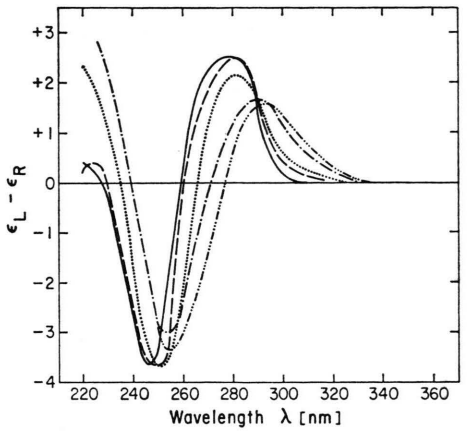

Fig. 1. CD spectra of calf thymus DNA in presence of varied concentrations of CH_3HgOH at pNa 2.0 and pH 6.8. (———) pM ∞, 5.0, 4.5; (— — —) pM 4.0; (· · · · ·) pM 3.5; (—·—·—) pM 3.0; (— · · · —) pM 2.5. pNa ≡ $-\log[Na^+]$. pM ≡ $-\log[CH_3HgOH]_{added}$.

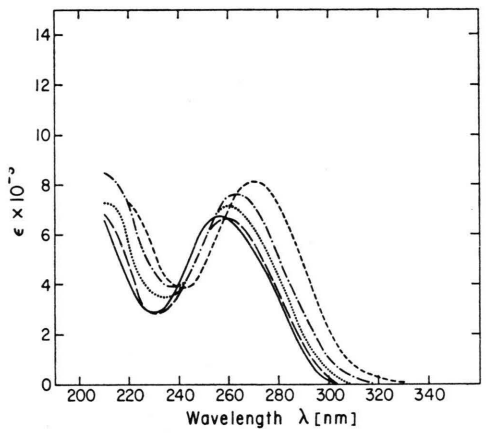

Fig. 2. UV absorbance spectra of calf thymus DNA in presence of varied concentrations of CH_3HgOH at pNa 2.0 and pH 6.8. (———) pM ∞, 5.0, 4.5; (— — —) pM 4.0; (· · · · ·) pM 3.5; (—·—·—) pM 3.0; (— — —) pM 2.0. pNa ≡ $-\log[Na^+]$. pM ≡ $-\log[CH_3HgOH]_{added}$.

salt level presented, *i. e.*, at pNa 2.0, this comprises pM values ranging from pM ∞ to pM 4.5 (for definitions of pNa and pM see Experimental). Increasing the organomercurial concentration beyond this range, however, *viz.*, pM < 4.5, leads to the sudden occurrence of spectral alterations thereby showing that now CH_3HgOH reacts with the DNA. The alterations are, with CD, characterized by a considerable shift of the positive dichroic band, located between 259 – 306 nm prior to the addition of CH_3HgOH, to longer wavelengths while at the same time the rotational strength decreases. Also the negative CD band at 230 – 259 nm experiences a red shift, albeit less dramatic, and its rotational strength is affected by methylmercury to a lesser degree and in a less clear-cut pattern (*cf.*, Fig. 1). Red shifts are noted also in the UV absorption spectrum of calf thymus DNA (*cf.*, Fig. 2) although here, at CH_3HgOH concentrations starting with pM < 4, the absorption increases with increasing organomercurial concentration rather than decreases as is seen with CD.

Similar changes in the spectral properties of calf thymus DNA are observed at the salt levels pNa 1.5, 1.0, and 0.0 although the organomercurial concentration at which the changes first become noticeable increases with increasing counterion concentration and, for instance, with CD, must be at pM < 3.5 in 0.1 M Na^+. No new information is contained in those spectra and, thus, we show here only the pNa 2.0 data.

The influence of the supporting electrolyte concentration on the CD of native calf thymus DNA in absence of CH_3HgOH is shown in Fig.3 (main figure). As has been known for some time, the area under the long-wavelength positive absorption band is extremely sensitive to changes in ionic strength and decreases with increasing salt concentration. The decrease is barely noticeable when going from pNa 2.0 to 1.5 but is considerable at pNa 0.0. It appears that the decrease of the long-wavelength positive

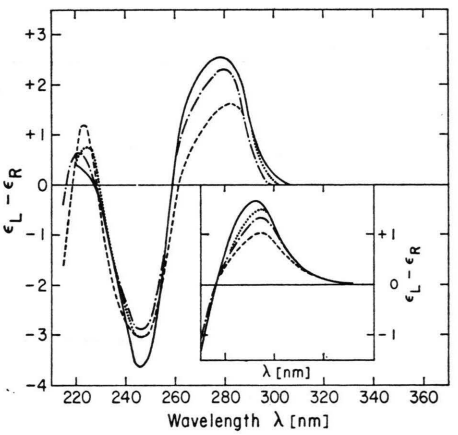

Fig. 3. CD spectra of calf thymus DNA at varied supporting electrolyte concentrations and at pH 6.8 but in absence of CH_3HgOH. (———) pNa 2.0; (· · · · ·) pNa 1.5; (—·—·—) pNa 1.0; (— — —) pNa 0.0. pNa ≡ $-\log[Na^+]$. (Inset of the figure depicts the situation at pM 2.5 with all other conditions unchanged. pM ≡ $-\log[CH_3HgOH]_{added}$.)

dichroism is compensated in part by an increase in the short-wavelength positive dichroism located between the cross-over points 220 – 230 nm. Unfortunately, the rapidly deteriorating resolving power of the instrument at wavelengths below 220 nm prevented us from exploring this matter in further detail. The rotational strength of the negative dichroic band, located betwen 230 – 259 nm, is susceptible to salt influences to a lesser degree than the strength of the positive band(s) (*cf.*, Fig. 3 and Table I). As can be seen from the inset of Fig. 3 (positioned at wavelengths corresponding to those of the main figure), a similar salt dependence exists when DNA has reacted with CH_3HgOH. The example depicts the situation at pM 2.5. For space reasons, only the long-wavelength positive band is presented. In contrast to CD, no ionic strength effects can be discerned in the UV absorption spectrum of native calf thymus DNA in absence of CH_3HgOH, and those observed at pM 2.5 amount to minor red shifts that are noticeable only in the 270 – 310 nm wavelength region (not shown). Further information on the methylmercury- and salt-induced changes in the CD of calf thymus DNA is presented in Table I. The rotational strengths of the various bands were calculated as described under Experimental.

The pronounced red shift of both the CD and UV spectra subsequent to the addition of appropriate amounts of CH_3HgOH can be reversed by adding substances that are known to complex strongly with

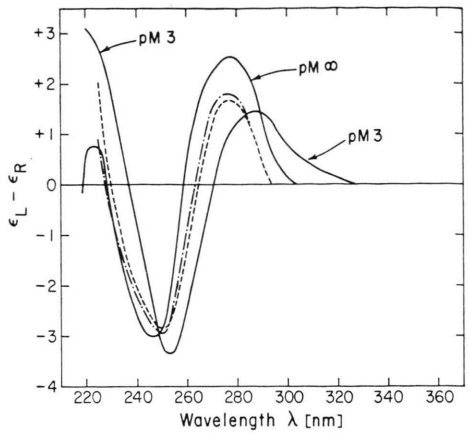

Fig. 4. CD spectra of calf thymus DNA: in absence of CH_3HgOH and at pNa 1.5 (pM ∞), in presence of 1 mM CH_3HgOH (pM 3) and at the same ionic strength, (—·—·—) in the mixture pNa 2.0/pM 3 after the addition of 0.01 M NaCN (pNa 1.7), (– – – –) at pH 12.5 and at pNa 1.5 and in absence of CH_3HgOH. pNa ≡ $-\log[Na^+]$. pM ≡ $-\log[CH_3HgOH]_{added}$.

methylmercury. This is shown for CD in Fig. 4. The curve labeled pM ∞ represents native calf thymus DNA in absence of CH_3HgOH and at the salt level of pNa 1.5. The curve labeled pM 3 is the DNA in presence of 1 mM CH_3HgOH at the same salt level while the dash- dotted curve is the CD spectrum of DNA originally in a pNa 2.0/pM 3 medium to which, however, a ten-fold excess of NaCN has been added (which increased the counterion concentration to

Table I. Circular dichroism parameters of calf thymus DNA in Na_2SO_4 as a function of added CH_3HgOH.

pNa [a]	pM [a]	λ of $\Delta\varepsilon_{max}$ [b] [nm]	λ of $\Delta\varepsilon_{min}$ [c] [nm]	λ of c. p. [d] [nm]	$R\times10^{40}$ [e] [c. g. s.]	$R\times10^{40}$ [f] [c. g. s.]
2.0	∞	278	246	259	+5.6	−6.2
1.5	∞	278	246	259	+5.4	−5.7
1.0	∞	280	247	259	+4.6	−5.3
0.0	∞	283	247	262	+3.1	−6.1
2.0	4.0	281	249	260	+5.0	−6.9
2.0	3.5	282	251	266	+4.3	−6.0
2.0	3.0	290	253	273	+3.6	−5.3
2.0	2.5	293	255	277	+2.8	−7.0
1.5	3.0	289	253	272	+2.9	−5.4
1.5	∞ [g]	278	250	266	+2.3	−5.6
1.7	3.0 [h]	277	250	263	+2.9	−5.5

[a] pNa ≡ $-\log[Na^+]$; pM ≡ $-\log[CH_3HgOH]_{added}$.
[b] Maximum of the positive dichroic band. $\Delta\varepsilon \equiv \varepsilon_L - \varepsilon_R$.
[c] Minimum of the negative dichroic band. $\Delta\varepsilon \equiv \varepsilon_L - \varepsilon_R$.
[d] Location of the cross-over point.
[e] Rotational strength R of the positive dichroic band.
[f] Rotational strength R of the negative dichroic band. The data in this column are less reliable than the data pertaining to the positive band because of the increased noise at wavelengths below 260 nm.
[g] Alkali-denatured DNA at pH 12.5. For further details see text.
[h] Solution contains also 0.01 M NaCN. The solution pH of about 10 (hydrolysis) is insufficient to denature the DNA by itself. For further details see text.

pNa 1.7). For comparison, the CD spectrum of calf thymus DNA at pH 12.5 (pNa 1.5) is also shown (dashed curve); it is practically identical with the CD spectrum of the cyanide-treated sample. Thus, while the red shift can be reversed by removing the methylmercury bound to DNA, the organomercurial-induced decrease of the area under the positive dichroic band cannot be reversed under the experimental conditions given. The reversibility of the UV red shift has been demonstrated in detail elsewhere and need not be repeated here [1].

Discussion

We have shown on several occasions [1, 2, 15, 16] that exposing native DNAs to appropriate concentrations of CH_3HgOH results in their denaturation. The reaction is brought about by methylmercury interacting with the nitrogen binding sites essential for Watson-Crick base pairing, and it is the disruption of this base pairing and the concomitant unstacking of the bases that makes itself noticeable by the hyperchromicity observed with UV. Thus, the intent of this study has not been to prove denaturation again but, rather, to see how CD measurements permit one to monitor this reaction and, hopefully, to obtain further information on the nature of the denatured (and methylmercurated) state of DNA.

Not many studies seem to have been performed that would have used CD as a means to follow DNA helix-to-coil transitions, but the few studies undertaken [7, 10–12] agree in one respect: namely that exposing DNAs (or double-stranded RNA [11]) to conditions, say of temperature [7, 11, 12] or solvent composition [10], that are known to disrupt the double helix results in the decrease of the area under the long-wavelength positive absorption band. We see this happening also in the case of CH_3HgOH at ambient temperature or with NaOH at pH 12.5 (*cf.*, Figs 1 and 4).

DNA helix-to-coil transitions are cooperative, also those induced by methylmercury [1, 2, 16], and one would therefore like to have a parameter of dichroism that permits the demonstration of cooperativity in the observed spectral phenomena. Samejima *et al.* [11] showed that both the intensity of the positive dichroic band at λ_{max} (260 nm in the case of double-stranded rice dwarf virus RNA) and the red shift of the cross-over point varied with temperature in a highly cooperative fashion when going through the 70 – 90 °C temperature zone while at temperatures above and below the transition region the two parameters were independent of temperature. Usatyi and Shlyakhtenko [12], looking at the temperature dependence of the CD spectra of a variety of natural DNAs, among them also calf thymus DNA, found that the maximum of the positive dichroic band of calf thymus DNA at 275 nm varied with temperature in a monotonous fashion but that the variation of the minimum at 245 nm with temperature, *i. e.*, the negative band, displayed cooperativity when going through the 50 – 60 °C melting region. Melting was performed in 0.1 × SSC.

With respect to the methylmercury-induced DNA denaturation, we found neither the maximum of the long-wavelength positive dichroic band at λ_{max} nor the extremum of the negative band at λ_{min} to display cooperativity when plotted against pM, and also the red shift of the cross-over point at 259 nm, although increasing cooperatively with pM at low and intermediate concentrations of CH_3HgOH, failed to "level off", *i. e.*, become independent of the methylmercury concentration, at the highest organomercurial concentrations employed. This was noticeable particularly in the more concentrated salt solutions. A reliable measure of cooperativity, however, was found in the rotational strength R of the long-wavelength positive band. Its normalized decrease Ω (see Experimental) has been defined in such a way as to achieve formal agreement with the variation of the hyperchromicity H^λ as a function of pM. Both H^λ and Ω are displayed in Fig. 5; they were

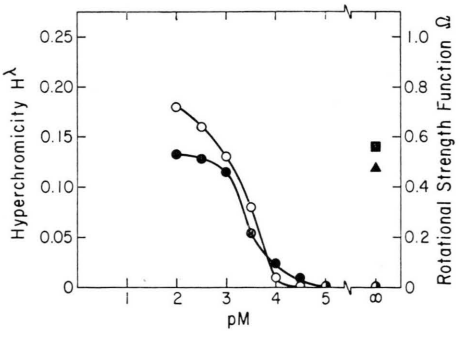

Fig. 5. Plot of hyperchromicity H^λ and normalized decrease in rotational strength of the long-wavelength positive dichroic absorption band, Ω, as a function of CH_3HgOH concentration and at pNa 1.5. pNa ≡ −log[Na^+]. pM ≡ −log·[CH_3HgOH]$_{added}$. (○) H^λ data; (●) Ω data; (■) Ω at pH 12.5 and in absence of CH_3HgOH; (▲) Ω after the addition of 0.01 M NaCN to DNA in pNa 2.0/pM 3 (pNa 1.7) [(◑) represents overlap of open- and closed-circled data points]. For further details see text and Table I.

evaluated from data gathered at pNa 1.5. As can be seen, and as already described above under Results, at CH_3HgOH concentrations up to about pM 4, there is no or very little interaction occurring between native DNA and the organomercurial. Reaction starts at pM < 4 with the product being denatured DNA complexed by methylmercuy. The denaturation half-point is obtained from either plot as $pM_{1/2} = 3.4_5$. Previous measurements, on H^λ, had yielded the value $pM_{1/2} = 3.5_3$ for the same salt concentration [1]. Increasing ionic strength will shift both the Ω-pM and H^λ-pM curves to lower pM values (not shown) as has been demonstrated for the H^λ-pM relation in detail elsewhere [1]. That denatured DNA is indeed the final product can further be seen by the Ω data pertaining to alkali-denatured DNA or pM 3-treated DNA that had been stripped of the metal by the addition of NaCN (*cf.*, Fig. 4). Both values have been incorporated along the pM ∞ axis in Fig. 5. Their magnitude corresponds to the one displayed by DNA at pM 3-2, a range in which DNA is known to be denatured.

There is very little information available from the literature regarding the magnitude of the rotational strength R of the dichroic absorption bands of nucleic acids. In fact, we believe to be the first to have determined experimentally R of a DNA both as a function of polymer conformation and solvent composition (*cf.*, Table I). Yet the limited information available appears to be in complete harmony with what has been found by us. Brahms and Mommaerts [7] were the first to present R data on nucleic acids. For native calf thymus DNA (measured in 0.01 M NaCl, 0.01 M Tris, 1 mM EDTA, pH 7), they report the values $+5 \times 10^{-40}$ and -5×10^{-40} (in c.g.s. units) for the positive and negative CD absorption bands, respectively, which has to be compared with our values of $+5.6 \times 10^{-40}$ and -6.2×10^{-40} (in c.g.s.) at pNa 2.0 or with the slightly smaller values at pNa 1.5. Not surprisingly, also in dilute Na_2SO_4, native DNA displays a conservative CD spectrum. In this context, it is of interest to compare our data on rotational strength with those presented by Johnson and Tinoco [17]. Their theoretical calculations predict native DNA in the B-form to exhibit R values of $+5.8 \times 10^{-40}$ and -3.5×10^{-40} c.g.s. units, with the absorption maximum and minimum located at 280 and 238 nm, respectively, while single-stranded DNA in the B-form is to have R values of $+1.8 \times 10^{-40}$ and -4.8×10^{-40} c.g.s., with extrema located at 285 and 244 nm. With due consideration for the precision of our measurements and the approximations inherent in a simplified theoretical approach the data presented in Table I are in pleasantly good agreement with predicted values, this is particularly true as far as the rotational strengths of the long-wavelength positive bands are concerned.

Mercury is known to cause extensive red shifts in the UV absorption spectra of DNAs [1, 18, 19] or nucleosides [20] due to its interacting preferentially with the nitrogen binding sites of the bases. Similar red shifts, much larger than those resulting from temperature changes [11], are generated therefore also in the CD spectra. However, apart from the magnitude of the red shift, which is undoubtedly associated with the amount of organomercurial bound by single-stranded DNA, the similarity of the R values displayed by DNA, for instance, at pNa 1.5/pH 12.5 in the absence of CH_3HgOH, in a pNa 1.5/pM 3 mixture, or at pNa 1.7/pM 3 in presence of 0.01 M cyanide, strongly suggests that at a given ionic strength, here near pNa 1.5, denatured DNA is composed of only one group of molecules in near-identical conformation irrespective of the degree of methylmercury binding. This conclusion had not been obvious from previous results obtained from buoyant density measurements in a density gradient generated by ultracentrifugation [2] or from the rates of sedimentation displayed by methylmercurated DNA in a centrifugal field [21], but it is in complete agreement with recent findings that show the frictional coefficient of methylmercury-denatured DNA to be quite unaffected by the degree of mercury binding while affected dramatically by changes in the concentration of the supporting electrolyte [16].

Lastly, from Table I it can be seen that the rotational strength of the long-wavelength CD band of native DNA decreases by a factor of about 1.8 when going from pNa 2.0 to 0.0 and that a very similar change takes place with denatured DNA at pM 2.5 (*cf.*, Fig. 3, inset; the exact value is 1.6). Current interpretations of this salt-induced decrease, restricted, however, to native DNA in solution or in films [8, 22, 23], envision this change to arise from conformational alterations equivalent to B $\rightarrow$ C geometry transitions as they are known to occur in DNA fibers at appropriate relative humidities, salt strengths, and with the proper salt of the acid [24].

Since information concerning the geometry of denatured DNA is unavailable at present, we cannot offer any suggestions as to the meaning of the dichroic change at pM 2.5 in terms of DNA geometry although, as can be gathered from Fig. 3, the changes are quite similar despite the fundamental difference existing between native and denatured DNA. This is clearly a point that warrants further attention in future investigations. There is no doubt, however, that CD measurements permit the monitoring of methylmercury-induced DNA helix-to-coil transitions, particularly if the data are expressed in terms of *R*.

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