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Binding of Hg(II) to poly(dA) : poly(dT) and its component single strands

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Mercuric binding studies at pH 10 revealed that poly(dA) : poly(dT) exhibits a more dramatic absorption spectral alteration than the alternating polymer poly(dA-dT) : poly(dA-dT) and induces a unique intense positive CD band at 296 nm during the spectral titrations. Comparative studies with its component single strands suggest that the spectral alterations exhibited by poly(dA) : poly(dT) are consistent with a binding model in which the mercuric ions initially bind to thymines and cause the eventual strand separation of the duplex, with subsequent high cooperative binding to the poly(dA) strands. This interpretation is supported by the binding isotherms indicating much stronger mercuric binding to poly(dT) than to poly(dA), with saturation binding densities of 1 Hg(II) per 2 bases and 1 Hg(II) per base, respectively, and very high binding cooperativity for poly(dA). Striking spectral alterations are exhibited by the mercuric binding to poly(dA), likely the consequence of binding to the amino group of dA in an alkaline solution. The mononucleoside dA exhibits minor spectral alterations upon similar mercuric chloride additions whereas the dinucleoside monophosphate d(AA) exhibits significant spectral changes, albeit less pronounced than those of poly(dA). Some sequence effects on the mercuric binding are observed in the dinucleotide studies. Our CD results on the mercuric binding to polynucleotides do not support the contention of (ψ)-type condensed complex formation.

1. Introduction

Metal ions are known to exert a wide variety of structural effects on the biologically relevant macromolecules. Among those capable of interacting with nucleic acids, mercury(II), Cu(II), and silver(I) are known to bind at the bases and result in dramatic conformational alterations in DNA [1,2]. The reversible binding of Hg(II) to DNA has received considerable attention since it was first investigated by Katz [3]. It is by now well established that Hg(II) binds to the purine and pyrimidine bases [4–6] rather than to the phosphate group [7] and favors the AT-rich regions of DNA [3,8,9]. A chain slippage mechanism for the binding of Hg(II) to poly(dA-dT) : poly(dA-dT) was proposed by Katz [10] whereby each Hg(II) ion is attached to two thymine moieties on opposite strands of the polymer. The two protons released per Hg(II) bound have been attributed to the imino N-H bonds of thymines. Other investigators have also found the N3 of thymine or uridine to be the site of highest affinity for mercury binding [6,11–13]. Kosturko et al. [14] reported the X-ray crystal structure of 1-methylthymine-mercury complex to consist of an N(3)-Hg-N(3′) linkage between the two thymine moieties. Their results are, thus, consistent with the proposed chain slippage mechanism of Katz [10]. The Hg(II) cross-linking via thymine N3 atoms for poly(dA-dT) : poly(dA-dT) was also supported by a proton NMR study. Based on the observed chemical shifts of the bases, Young et al. [15] further refined the model to suggest that the cross-linked thymines

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alternate with coplanar stacks of adenine residues which are themselves hydrogen-bonded.

No such detailed understanding on the binding of mercury to its sequence isomer poly(dA):poly (dT) has been forthcoming, although some studies have been made on the related ribonucleic acid. Kawade [16] noted that in contrast to other nucleic acids, the absorbance of poly(A):poly(U) increases initially with increasing r, [Hg(II) added]/[DNA]. Parallel sedimentation velocity studies on poly(A):poly(U) and its components led to the suggestion that the initial hyperchromic effects resulted from an extensive breakdown of the double helix. Williams and Crothers [17] investigated the binding kinetics of Hg(II) on some polynucleotides and showed that the reaction of Hg(II) with poly(A):poly(U) occurs in two phases which differ in time scale by a factor of about 100. The fast phase is second order whereas the slow phase is first order and exhibits cooperative behavior. The measured second-order rate constant is nearly three orders of magnitude smaller than that found for poly(U) alone. This rate difference suggests that the reactive sites are blocked by double-helical formation, and become available for reaction with Hg(II) only through a structural fluctuation. A mechanism consisting of an initial Hg(II) binding to the transiently opened base-pairs and a subsequent structural rearrangement with mercuric binding to primarily poly(U) was proposed to account for these observations.

To gain further insights into the nature of DNA binding by mercuric ion, parallel spectroscopic titrations are hereby carried out with poly(dA):poly(dT) and the alternating polymer poly(dA-dT):poly(dA-dT). Studies have also been made with its single-stranded components poly(dT) and poly(dA) to investigate the role of strand separation on the mercuric binding to poly(dA):poly(dT). Measurements with dinucleoside monophosphates and mononucleosides were also made to elucidate the roles played by base sequence and base stacking.

2. Materials and methods

Synthetic polynucleotides were purchased from Pharmacia. Extinction coefficients at 260 nm of 8400, 13 700, 6600, and 6000 cm^{-1} M^{-1} for poly(dT), poly(dA), poly(dA-dT):poly(dA-dT), and poly(dA):poly(dT), respectively, have been used for their concentration (per phosphate) determinations. Absorbance value at high temperature (90 °C) was used for poly(dA) to minimize the hypochromic effect due to base stacking. Dinucleoside monophosphates and mononucleosides were obtained from Sigma and the extinction coefficients from the Handbook of Biochemistry and Molecular Biology have been used for their concentration determinations. Mercuric chloride was purchased from Fisher Scientific and used without further purification. As mercuric chloride causes precipitation in a Tris buffer, all experiments were carried out in chelexed water (water in equilibrium with the Chelex-100 resin purchased from Bio-Rad) containing 0.01 M NaCl and exhibiting a pH of about 10. Freshly prepared mercuric chloride solutions were used for each study, as the effectiveness of Hg(II) was observed to decrease considerably in a matter of days. Concentrations of the stock mercuric chloride solutions were determined by weight.

Absorption spectra were measured with a Cary 210 spectrophotometric system and the stirrer accessory was used during the absorption spectral titrations. CD measurements were made with a Jasco J-500 recording spectropolarimeter at appropriate temperatures, using a water-jacketed cylindrical cell of 2 cm path length. Temperatures were maintained by a Neslab RTE-8 refrigerated circulating bath. Both absorbance and CD titrations were carried out by adding aliquots of $HgCl_2$ to a solution of DNA or polynucleotide of 20 μM concentration. Thermal denaturation experiments were carried out with 1-cm semimicro cells by monitoring absorbance at an appropriate wavelength and collecting data at 15-s intervals with an Apple II microcomputer. A heating rate of 0.5 °C/min was maintained by a Neslab RTE-8 refrigerated circulating bath and an EPT-4RC temperature programmer. Numerical differentiations were performed to obtain differential melting profiles from which melting temperatures were determined from the maxima.

Spectral titrations were carried out by adding aliquots of $HgCl_2$ stock to the DNA solution.

Absorbance or ellipticity was monitored at appropriate wavelength to ensure equilibration prior to each measurement. The measured spectra were then normalized to the same DNA concentration via dilution corrections. The conformations of poly(dA-dT):poly(dA-dT) and poly(dA):poly(dT) do not appear to be altered at pH 10, as their CD spectra are nearly identical to those at neutral pH.

3. Results

3.1. Absorption spectral titrations of poly(dA):poly (dT) and poly(dA-dT):poly(dA-dT) with $HgCl_2$

Both poly(dA-dT):poly(dA-dT) and poly(dA):poly(dT) exhibit a spectral maximum around 256 nm in the absence of Hg(II). Binding of Hg(II), however, results in dramatically different spectral alterations for these two polynucleotides, as can be seen in fig. 1. For poly(dA-dT):poly(dA-dT), additions of $HgCl_2$ result in initial moderate hypochromic effects and further slight bathochromic shifts at higher r (= [$HgCl_2$ added]/[nucleotide]) values (fig. 1A). Absorbance at longer wavelengths also becomes progressively more prominent but without the presence of apparent isosbestic points. Absorbance changes at selective wavelengths are shown in fig. 1B. As is apparent, the intensity decrease at 255 nm is accompanied by a concomitant absorbance increase at 290 nm and both level off at an r value around 1 whereupon the 320 nm absorbance becomes progressively more prominent. A change in slope around $r = 0.25$–0.3 is evident for the 290 nm plot, suggesting the saturation of a stronger binding mode. This complex most likely corresponds to the interstrand

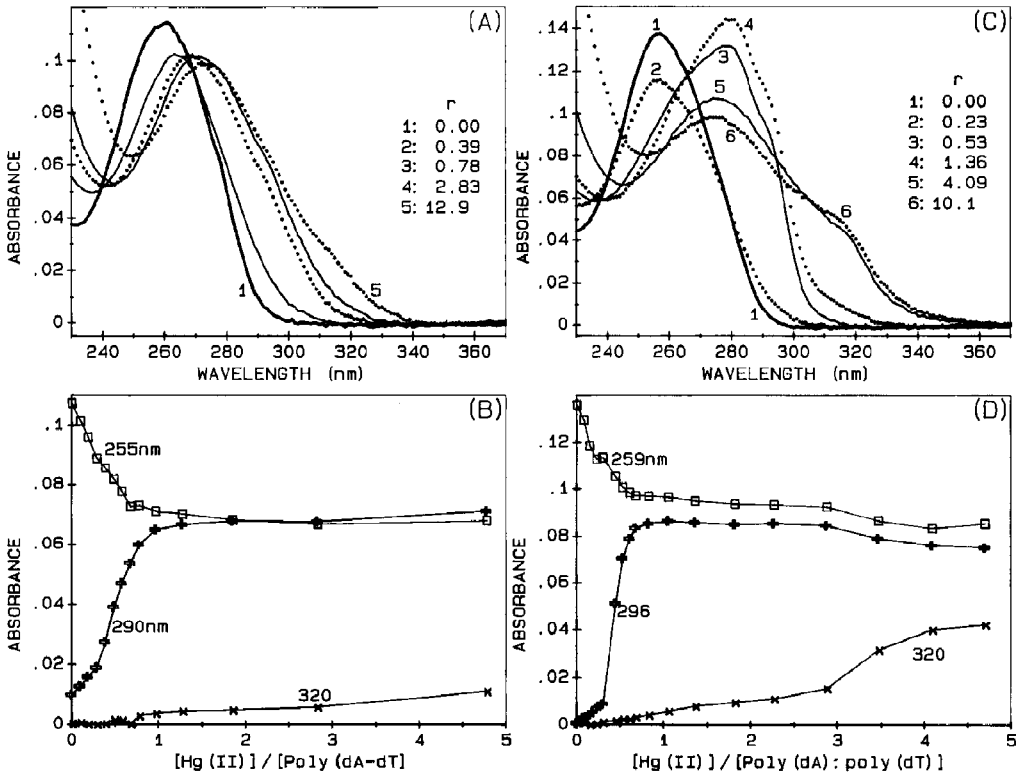

Fig. 1. Representative absorption spectra during the mercuric titrations with 17 μM poly(dA-dT):poly(dA-dT) (A) and 22 μM poly(dA):poly(dT) (C). The corresponding absorbance vs r (= [$HgCl_2$ added]/[DNA nucleotide]) plots at selected wavelengths are shown in panels B and D, respectively. Spectra were measured at 25 °C in chelexed water of pH 10 containing 0.01 M NaCl.

cross-link of (dT)N3-Hg-N3(dT) as proposed in the chain slippage model of Katz [10].

The spectral alterations of poly(dA):poly(dT) are, however, more dramatic, as can be seen in fig. 1C. Initial additions of Hg(II) result in hypochromic and hyperchromic effects at 256 and 296 nm, respectively, with an isosbestic point maintained at 280 nm for r values below 0.25. An abrupt spectral shift is seen to occur at an r value slightly above 0.25 with the appearance of a spectral maximum at 282 nm which reaches the highest intensity at an r value near 1. Further increase in the mercuric concentration results in the appearance of a significant absorbance at 320 nm with a concomitant hypochromic effect at 282 nm. These features can be seen more clearly with the absorbance vs r plots at selected wavelengths, as shown in fig. 1D. The absorbance decrease at 259 nm is seen to be accompanied by a strong absorbance enhancement at 296 nm (note particularly the abrupt change of slope around $r = 0.25$–0.30) and both appear to level around 0.75. Further increase in mercuric concentrations only results in a slight intensity depression at both wavelengths and a progressive enhancement of the 320 nm intensity. Around $r = 3$, a prominent intensity increase of the 320 nm shoulder is accompanied by moderate absorbance reductions at the two shorter wavelengths.

3.2. CD spectral features are strikingly different for the two A:T polynucleotides

The CD spectral features for the corresponding mercuric titrations are shown in fig. 2. As can be

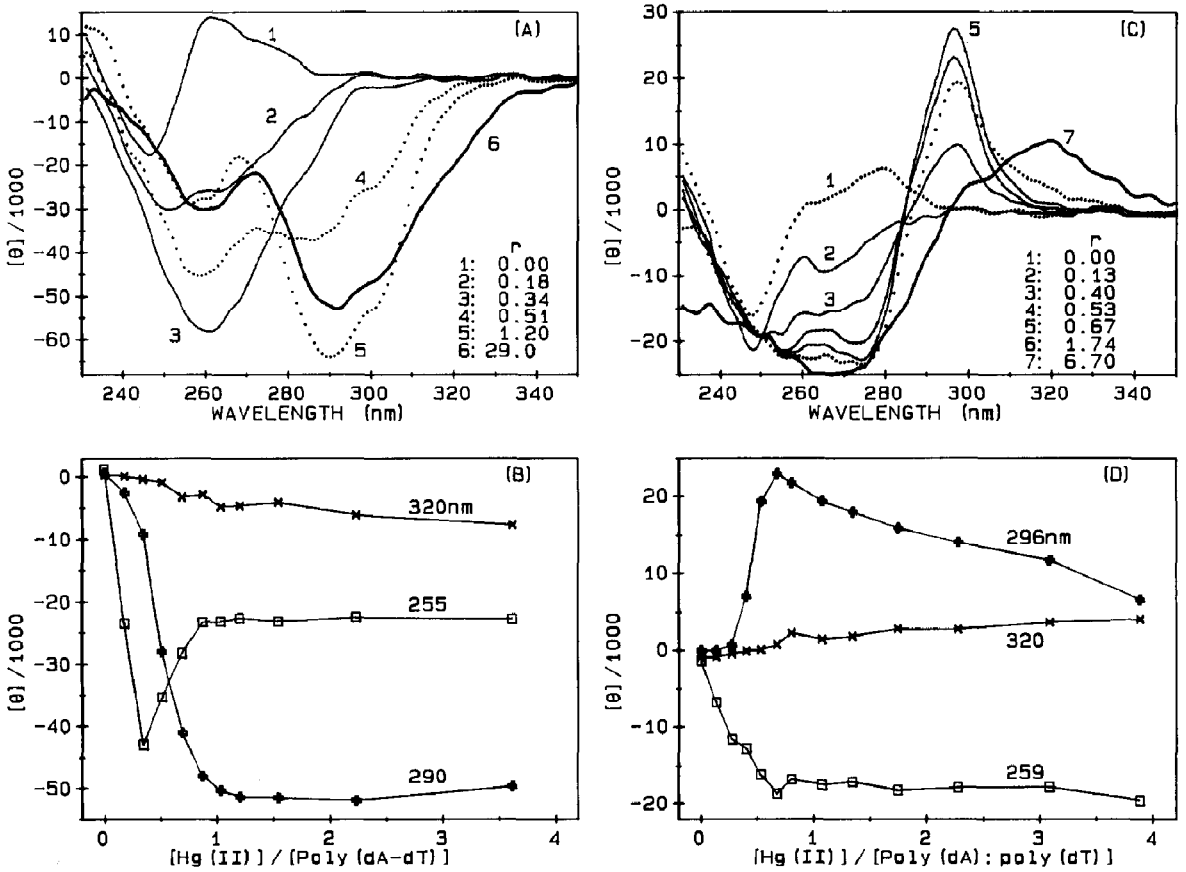

Fig. 2. Representative CD spectra during the $HgCl_2$ titrations with poly(dA-dT):poly(dA-dT) (A) and poly(dA):poly(dT) (C) with the corresponding ellipticity vs r plots at selected wavelengths appearing in B and D, respectively.

seen, the spectral alterations are dramatically different for these two A:T polynucleotides. In the absence of Hg(II), poly(dA-dT):poly(dA-dT) exhibits moderately strong positive and negative ellipticities at 256 and 247 nm, respectively (fig. 2A). Additions of Hg(II) result in the appearance of a strong negative CD maximum around 260 nm which reaches the largest amplitude around 0.25–0.3. Further increase in the mercuric concentration results in a progressive decrease of this band and a concomitant appearance of a negative shoulder around 290 nm which blossoms into a negative maximum and levels around $r = 1$. Further additions of Hg(II) cause the depression of the 290 nm band and the appearance of a shoulder around 320 nm. Molar ellipticities as a function of r at selective wavelengths are shown in fig. 2B. The 255 nm plot clearly exhibits a peak at 0.25–0.3 and leveling around 1. The saturation near $r = 1$ is also apparent from the 290 nm plot. Only a progressive enhancement of the negative ellipticity at 320 nm is seen. These CD spectral features agree well with those reported earlier [18] and are consistent with the results of absorbance titrations presented in the previous section.

The CD features for the poly(dA):poly(dT) titrations, however, are distinctly different from those of poly(dA-dT):poly(dA-dT). As can be seen in fig. 2C, poly(dA):poly(dT) initially exhibits positive and negative CD maxima at 275 and 247 nm, respectively. Additions of mercuric chloride result in the appearance of a broad nega-

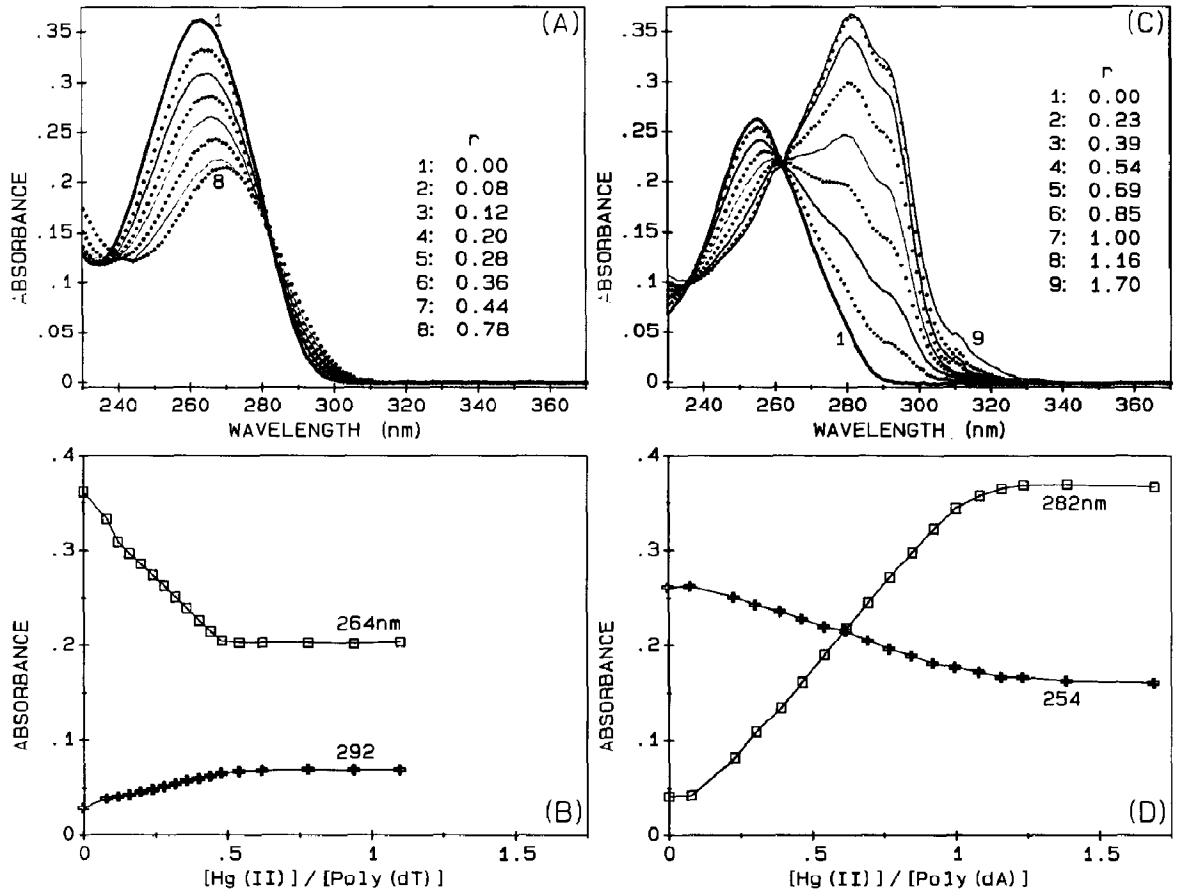

Fig. 3. Absorption spectral titrations of mercuric chloride with poly(dT) (A) and poly(dA) (C). The corresponding absorbance vs r plots at two selected wavelengths are shown in B and D, respectively.

tive CD band around 264 nm initially and continued additions of Hg(II) result in further intensity increase of this band but is now accompanied by the appearance of a strong positive CD band around 296 nm which reaches optimal amplitude around $r = 0.75$. A clean isoelliptic point is seen to be maintained at 282 nm. Additional Hg(II) leads to the depression of this band with a concomitant appearance of a 320 nm shoulder and an eventual appearance of a maximum. These features are illustrated in fig. 2D for selected wavelengths. The abrupt intensity increase around $r = 0.25-0.3$ and a peak at around $r - 0.75$ of the 296 nm plot are particularly noted. The 259 nm plot suffers breaks and saturations at roughly the same r values. It should be noted in passing that the appearance of a strong positive CD band at 296 nm is a unique consequence of basic conditions (pH 10) employed in our measurements, as mercuric titration of poly(dA):poly(dT) at pH 6 was devoid of such a feature [18].

3.3. Mercuric binding characteristics of poly(dT) and poly(dA)

To investigate the possibility of mercury-induced strand separation and subsequent binding to single strands, spectral titrations were also made with poly(dT) and poly(dA) separately. For poly(dT) (fig. 3A), progressive suppressions of the absorbance at 264 nm and slight bathochromic shifts are seen as more $HgCl_2$ is added. A concomitant slight hyperchromic effect around 292 nm is also observed and a clean isosbestic point is

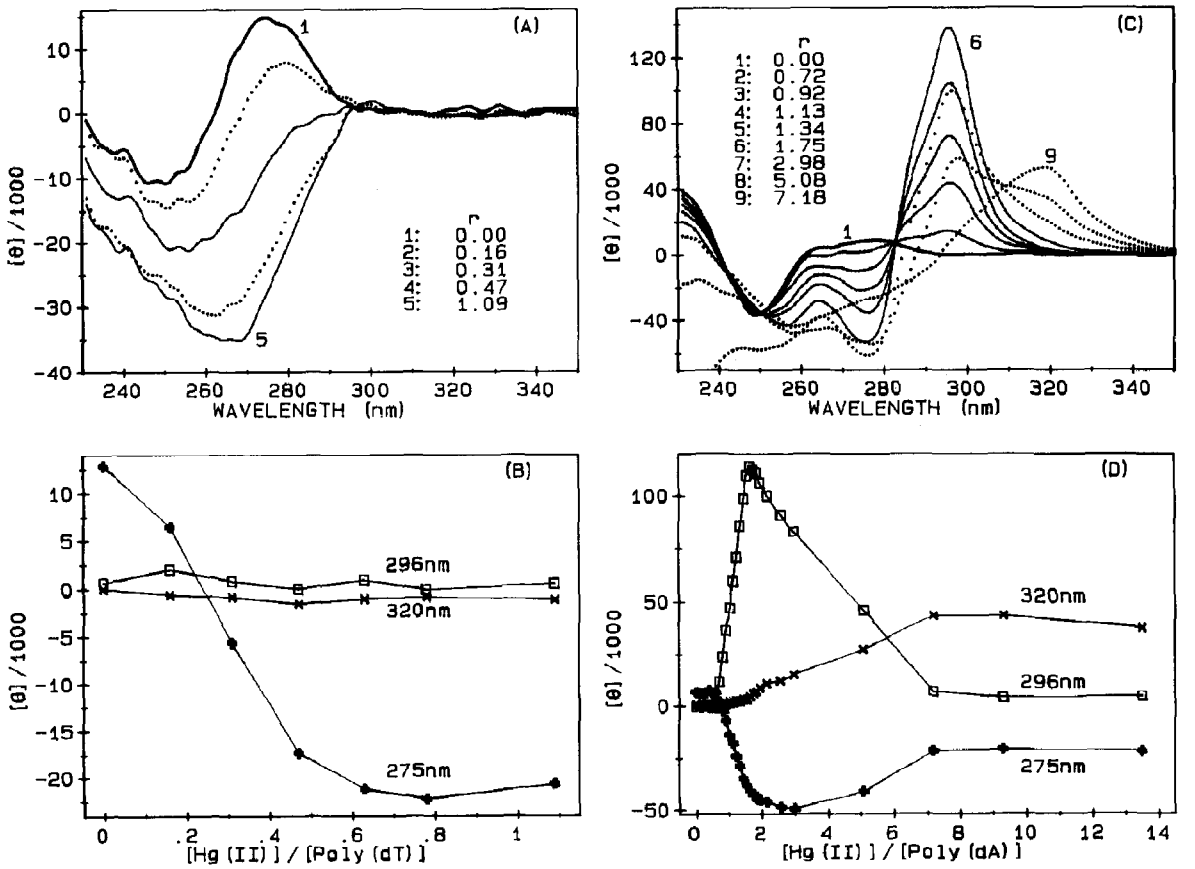

Fig. 4. CD titrations of mercuric chloride with poly(dT) (A) and poly(dA) (C) and the corresponding ellipticity vs r plots at selective wavelengths are shown in B and D, respectively.

maintained at 282 nm, suggestive of a two-state equilibrium. A titration break is clearly seen at $r = 0.5$ for both 264 and 292 nm monitorings (fig. 3B). In contrast, binding of Hg(II) to poly(dA) induces a much more striking absorption spectral change, as is apparent from fig. 3C. Absorbance around 282 nm is dramatically enhanced and is accompanied by a smaller hypochromic effect at 256 nm. Distinct isosbestic points are maintained around 262 and 237 nm for r values less than 2. In contrast to the saturation at $r = 0.5$ for poly(dT), poly(dA) appears to level roughly at $r = 1$ with both 254 and 282 nm monitorings (fig. 3D).

The corresponding CD titrations are shown in fig. 4. The appearance of a broad negative band around 264 nm is apparent in the titration of poly(dT) (fig. 4A). Consistent with the absorbance results, a plot of molar ellipticity vs r at 264 or 275 nm does indicate a saturation around $r = 0.5$ (fig. 4B). No appreciable ellipticity is induced at 296 or 320 nm. In contrast, CD spectral alterations for poly(dA) due to mercuric binding are distinctly different (fig. 4C). An intense positive Cotton effect around 296 nm with a concomitant negative CD at 278 nm of lesser intensity is observed and an isoelliptic point is clearly maintained at 282 nm for r less than 2. Further ad-

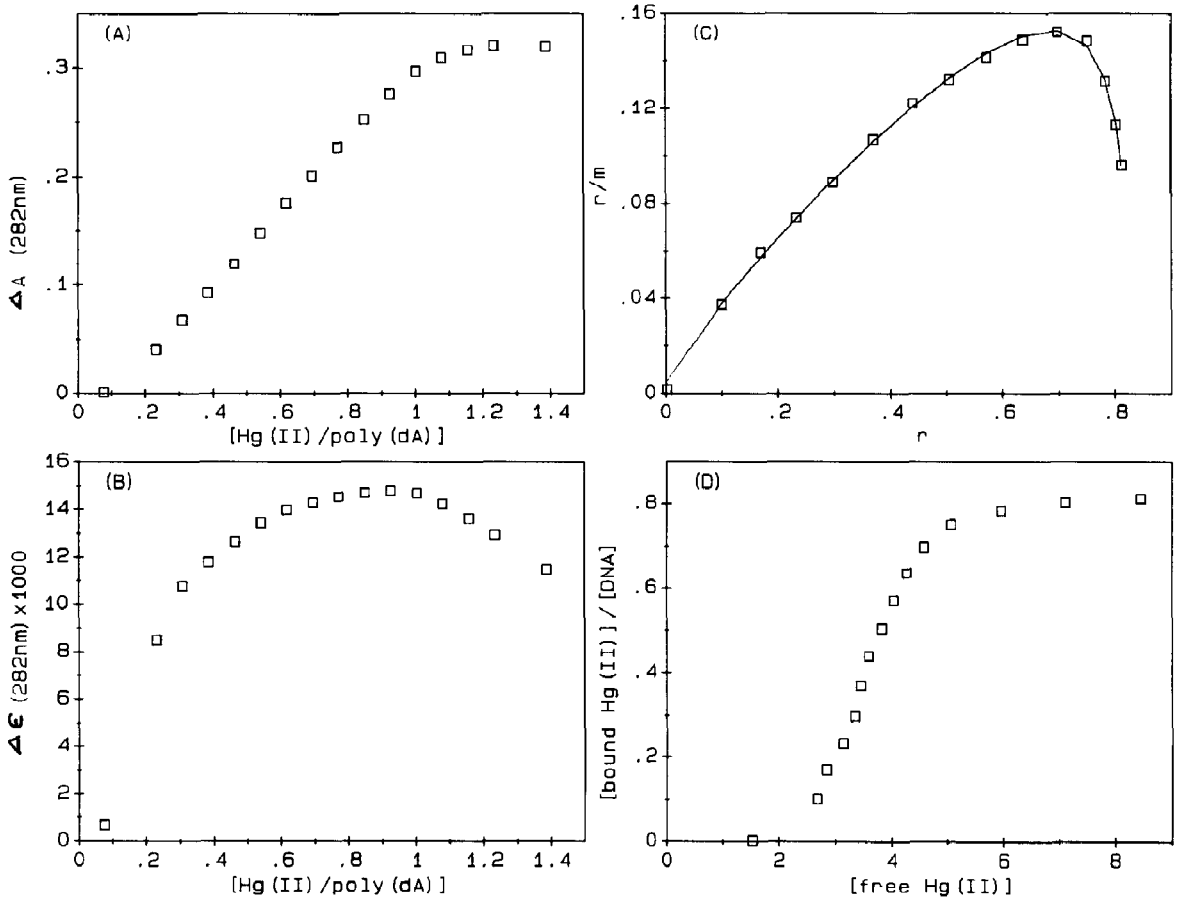

Fig. 5. Binding isotherms for mercuric titrations of poly(dA). (A) Plot of net absorbance changes at 282 nm vs r. (B) Dependence of apparent extinction coefficients on r. (C) Scatchard plot using the bound extinction coefficient obtained from intersection of lines formed from the first three and the last three points in B. The solid curve corresponds to a nonlinear least-squares fit with the McGhee-Von Hippel equation, using $K = 0.004\ \mu M^{-1}$, $n = 1.1$, and $w = 70$. (D) Plot of r vs m. In C and D, r and m represent [bound Hg(II)]/[DNA] and [free Hg(II)] in μM, respectively.

ditions of Hg(II) result in a reduction of this positive band and a concomitant appearance of the 320 nm shoulder which leads to the eventual appearance of a maximum at this wavelength for r greater than 7. These features are more clearly seen in the ellipticity vs r plots for selected wavelengths as shown in fig. 4D. Negligible ellipticity alterations are induced for r less than 0.5. Abrupt ellipticity increase and decrease at 296 and 275 nm, respectively, occur around $r = 0.7$ which result in the appearance of a peak for the former and a leveling for the latter at r less than 2, and further increase in mercuric concentrations results in intensity reductions for both wavelengths until $r = 7$. Significant ellipticity increase at 320 nm is seen to commence around $r = 1.5$ and reaches a plateau also around $r = 7$. It is noted that the CD spectral alterations are reminiscent of the poly(dA):poly(dT) results, particularly the appearance of an intense positive 296 nm ellipticity and its eventual demise at higher r values.

3.4. Mercuric binding to poly(dA) is highly cooperative

Scatchard plot with CD data of poly(dT) yields a binding constant of 10 μM^{-1} and a saturation binding density of 1 Hg(II) to 2 bases (not shown). Poly(dA), on the other hand, exhibits a concave downward Scatchard plot, suggestive of a cooperative binding. The characteristic features are illustrated with absorbance titrations in fig. 5. The absorbance change at 282 nm due to the Hg(II) binding has been replotted as a net change vs r in fig. 5A. The corresponding apparent difference extinction coefficients are plotted in fig. 5B which is seen to exhibit a maximum. When the bound extinction coefficient obtained from the intersection of straight lines formed by the first three points and the last three points is used, a striking concave downward Scatchard plot is obtained as shown in fig. 5C. Such a binding isotherm is characteristic of a highly cooperative binding and is analyzed with the formula of McGhee and Von Hippel [19]:

$$r/m = K(1-nr)\{[(2w-1)(1-nr)+r-R]/[2(w-1)(1-nr)]\}^{n-1} \times \{[1-(n+1)r+R]/[2(1-nr)]\}^{2},$$

where $R = \{[1-(n+1)r]^2 + 4wr(1-nr)\}^{1/2}$, r denotes the ratio of the number of bound ligands to the total number of lattice sites (base), m is the concentration of free ligands in the solution, n the binding site size, and w the cooperative parameter. An excellent fit is found, as indicated by the solid curve, and a binding constant of 4 mM^{-1}, cooperativity factor of 70, and a binding site size of roughly 1 base per Hg(II) are deduced. The high binding cooperativity can also be seen from a [bound Hg(II)]/[DNA] vs [free Hg(II)] plot as shown in fig. 5D.

3.5. Binding studies with di- and mononucleosides

To investigate the effects due to sequence and base stacking, spectral titrations of mercuric chloride with dinucleoside monophosphates d(AA), d(AT), d(TA), and d(TT) have also been made. As can be seen in fig. 6, Hg(II) binding induces much grosser spectral alterations in d(AA) (fig. 6B) than in d(TT) (fig. 6A) and some sequence dependence is demonstrated by a larger spectral change in d(AT) (fig. 6D) than in d(TA) (fig. 6C). The mercury-induced spectral alterations in dinucleosides are less dramatic than the related polynucleotides, as to be expected if stacking interactions play important roles. The corresponding CD features are shown in fig. 7. It is apparent that, in contrast to the absorbance titrations, the CD spectral features exhibited by the dinucleoside monophosphates do not correspond to those of the related polynucleotides. For example, in contrast to poly(dT), mercuric titrations with d(TT) yield a positive CD band around 285 nm, in addition to a strong negative CD around 263 nm (fig. 7A). The prominent presence of the intense positive 296 nm band in poly(dA) is conspicuously absent in the d(AA) titrations (fig. 7B). Consistent with the greater absorption spectral change, the CD spectral alterations for d(AT) (fig. 7D) are more pronounced than those of d(TA) (fig. 7C). It is noted, however, that in contrast to d(AT), no positive Cotton effects above 285 nm are observed for

poly(dA-dT):poly(dA-dT) during the mercuric titrations. Finally, the importance of base stacking appears to be supported by the lack of significant changes in either absorbance or circular dichroism during the mercuric titrations with mononucleosides dA and dT under similar conditions (not shown).

3.6. Thermal stability of some complexes

As the two A:T polynucleotides appeared to form unique complexes at D/P ratio around 0.25, thermal stabilities of $r = 0.35$ solutions were investigated. The binding of mercuric ion at this ratio dramatically stabilized the poly(dA-dT):poly(dA-dT) complex to result in a melting temperature near 100 °C, a more than 55 °C increase from the 38 °C melting temperature without Hg(II). A less pronounced melting transition is also observed at 50 °C which has been attributed by Young et al. [15] to the A:A hydrogen bond breaking in the dT-Hg(II)-dT cross-linked complex. Thermal stabilization of the mercuric complex is also observed for poly(dA):poly(dT) in which a broad melting transition is seen but without signs of completion even at a temperature as high as 98 °C.

To elucidate further the nature of thermal stabilities of poly(dA):poly(dT) complexes, melting experiments were also carried out for Hg(II) complexes of poly(dT) and poly(dA). Although these single strand polynucleotides by themselves do not

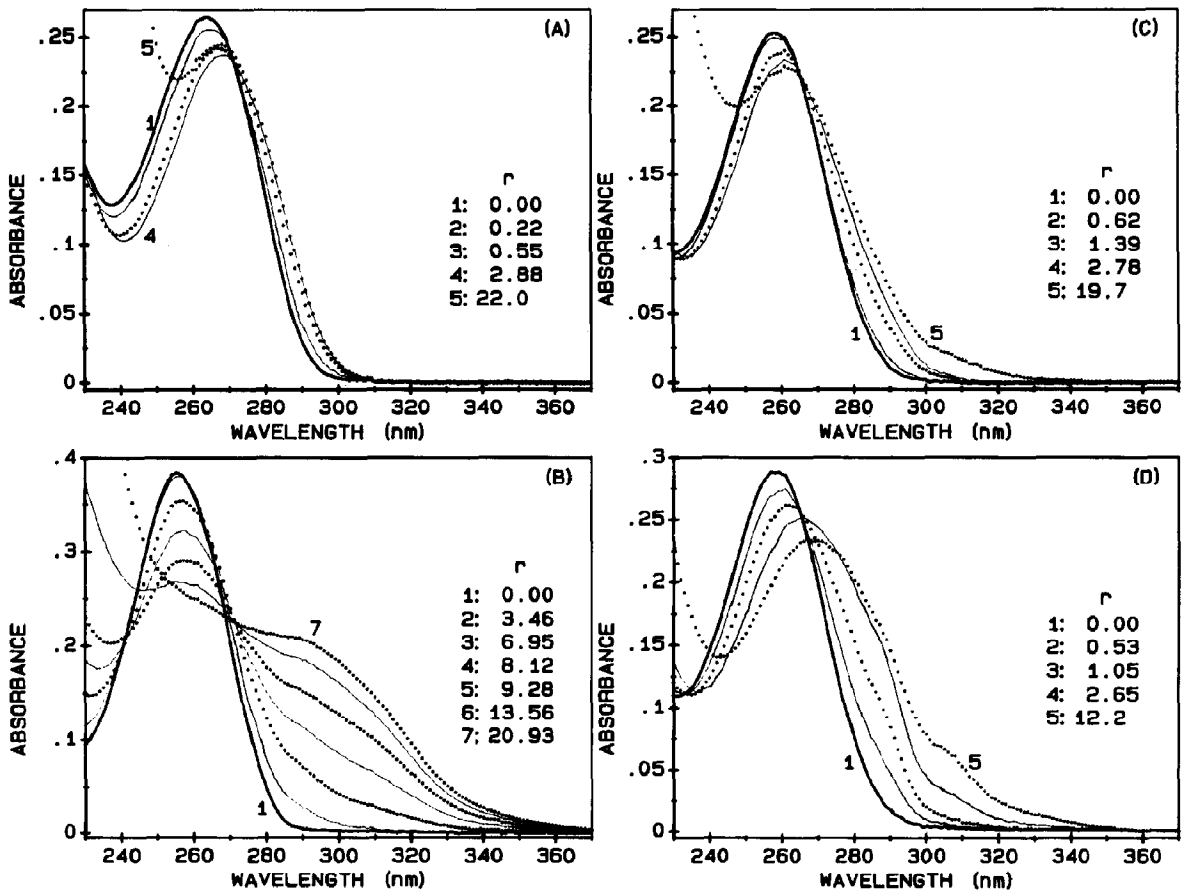

Fig. 6. Absorption spectral titrations of mercuric chloride with dinucleoside monophosphates. (A) d(TT), (B) d(AA), (C) d(TA), and (D) d(AT).

exhibit structured conformations (except for the possible stacking of dA), their mercuric complexes are highly ordered (as evidenced by the large induced CD intensities). The cross-linked complex of mercury with poly(dT) in the $r = 1$ solution appears to be quite stable and melts around 100 °C. Indeed, at 96 °C the CD amplitude at 264 nm still retains about 75% of the 2 °C value. The melting behaviors of Hg(II)-poly(dA) complexes were investigated at r values of 1 and 12 and their temperature-dependent CD spectra are shown in fig. 8. The intense positive 296 nm band is clearly evident for the $r = 1$ solution (fig. 8A). This prominent band, however, is strongly temperature dependent and exhibits dramatic intensity reduction near 35 °C with a concomitant ellipticity increase around 315 nm. This longer wavelength ellipticity enhancement reaches a maximum around 40 °C and a further temperature increase results in the intensity depression of this band. An isoelliptic point is maintained at 282 nm and the longer wavelength ellipticities disappear around 96 °C. The biphasic nature of this melting is made more apparent by the temperature dependent ellipticity plots at 296 and 320 nm, as shown in fig. 8B. The melting transition at 35 °C is strongly cooperative as indicated by the temperature dependent ellipticity change of 296 nm. These results suggest that around 35 °C the 1:1 mercuric-poly(dA) complex which induces the intense 296 nm CD band is being cooperatively transformed into conformations which exhibit a CD maximum at 315 nm. The altered conformations eventually release the mercuric ions from the polynucleotide at higher

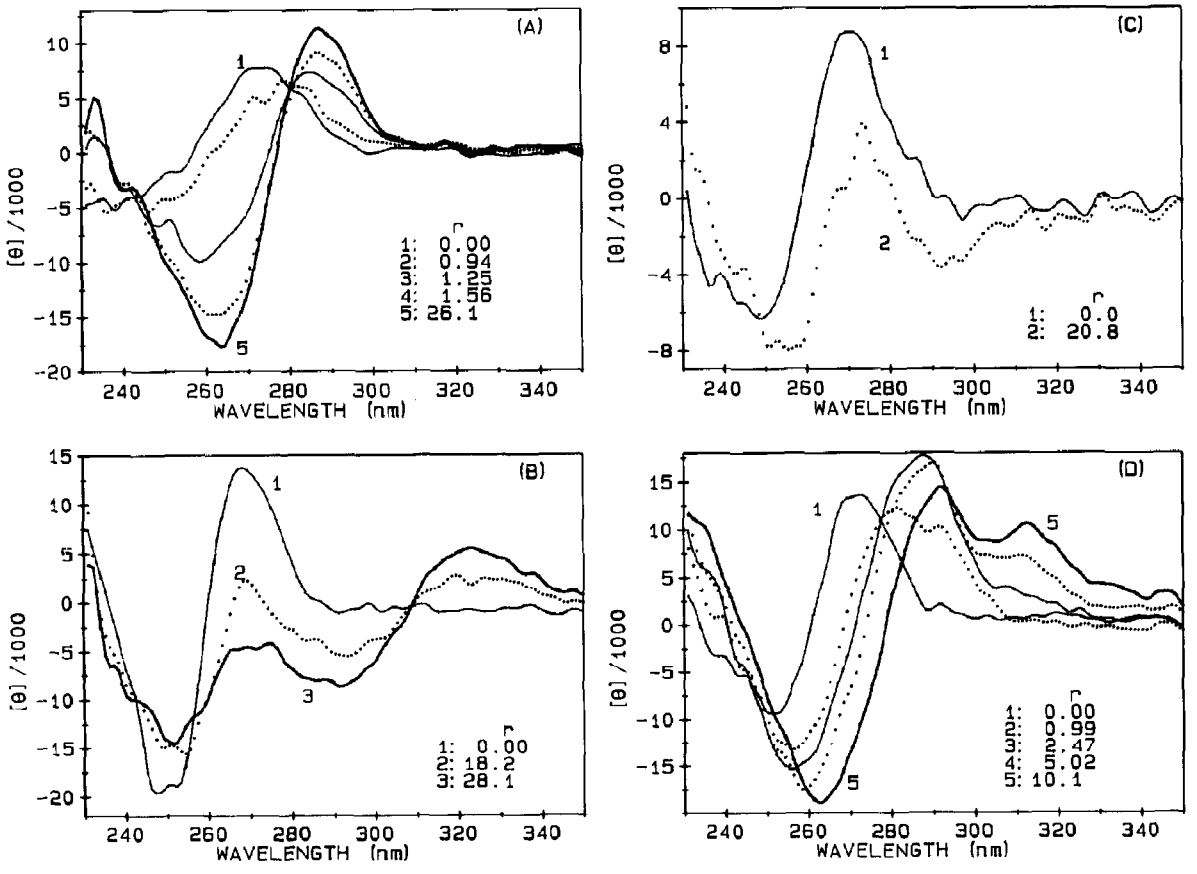

Fig. 7. Representative CD spectra during the mercuric chloride titrations with (A) d(TT), (B) d(AA), (C) d(TA), and (D) d(AT).

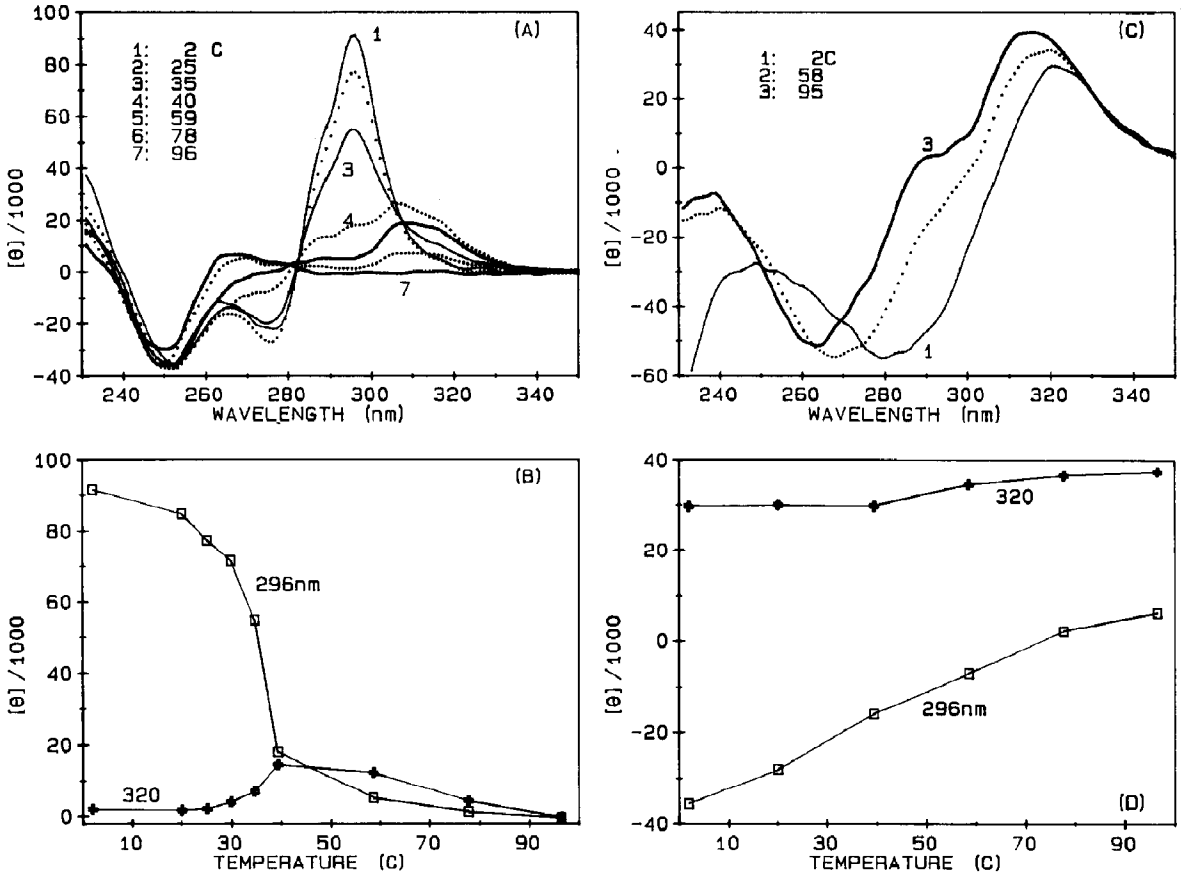

Fig. 8. Temperature-dependent CD spectra for $r = 1$ (A) and $r = 12$ (C) solutions. Ellipticity vs temperature plots at selected wavelengths are shown in B and D.

temperatures. The CD spectra of the $r = 12$ solution is characterized by a pair of positive and negative CD bands at 320 and 280 nm, respectively. Temperature increase, however, does not reduce their intensities but results in a spectral blue shift with an intensity enhancement of the long wavelength band. The spectral features, CD as well as absorbance, suggest that the mercury is still tightly bound to poly(dA) even at 95 °C with some alterations in complex conformation.

4. Discussion

The binding of Hg(II) to poly(dT) in a basic solution is characterized by a hypochromic effect at 256 nm and the presence of an isosbestic point at 280 nm in absorbance titrations, and an induced negative ellipticity at 264 nm in CD spectral titrations. The stoichiometry of the complex formed is 1 Hg(II) : 2 nucleotides (or $r = 0.5$). Mercuric binding to poly(dA), on the other hand, induces a dramatic absorbance enhancement at 282 nm along with an isosbestic point at 264 nm and the appearance of an intense positive CD band at 296 nm with an isoelliptic point at 282 nm. A complex is formed with approx. 1 : 1 ratio (or $r = 1$). Scatchard plots suggest a much stronger mercuric binding to poly(dT) than to poly(dA), with saturation binding densities of 1 Hg(II) per 2 bases and 1 Hg(II) per base, respectively. Very high cooperativity, however, is exhibited by

poly(dA). The spectral alteration of poly(dA):poly(dT) during the mercuric titration is chartacterized by an initial hypochromic effect at 256 nm with an isosbestic point maintained at 280 nm, characteristic of poly(dT) binding, until around $r = 0.25$. This is followed immediately by an abrupt appearance of the 282 nm absorbance maximum and a positive CD band at 296 nm and a subsequent dramatic intensity enhancement of these bands at higher mercuric concentrations, characteristics of the poly(dA) binding, until around $r = 0.75$ (i.e., $0.25 + 0.50$). Formation of other complexes is also apparent at even higher mercuric concentrations.

These results suggest that the spectral alterations exhibited by poly(dA):poly(dT) are consistent with a binding model in which the mercuric ions cause the strand separation of the duplex and the initial preferential intramolecular cross-linking of one Hg(II) to N3 of two thymines in poly(dT) ($r_1 = 0.5/2$) and subsequent metal ion binding to the poly(dA) strands at higher mercuric concentrations ($r_2 = 1.0/2$). This model agrees with that proposed for the binding of Hg(II) to poly(A):poly(U) via binding [16] and kinetic [17] studies.

Our investigations have been greatly aided by the striking spectral alterations exhibited by the mercuric binding to poly(dA). These dramatic spectral effects are likely the consequence of binding to the amino group of dA [6,11], as our titrations were carried out in a basic condition of pH 10. Indeed, our pH titrations with Hg(II)-poly(dA) solutions revealed that the gross spectral alterations occur at pH values above 8 (results not shown). Our observations of initial hypochromic, rather than hyperchromic, effects at 256 nm and the overall gross absorbance spectral alterations in the poly(dA):poly(dT) titrations appear to be at variance with the results of Young et al. [15]. Such discrepancies, however, most likely arise from the difference in pH (7 vs 10) and the smaller r range used in their measurements. Such pH difference and/or structural factors may also account for the fact that an $r = 1$ complex is found in our Hg(II)-poly(dA) titrations whereas an $r = 0.5$ complex formation of Hg(II)-poly(A) was observed by Ding and Allen [20]. Our 1:1 stoichiometry, however, is consistent with the results of pd(AAAA) titrations by Walter and Luck [18]. The dramatic absorbance enhancement at 282 nm and the intense positive CD at 296 nm may be useful in characterizing the presence of poly(dA) stretches in DNA under basic conditions. The presence of the intense positive CD band at 296 nm is unique for poly(dA) in strong basic condition, as our mercuric titrations with other natural DNA as well as synthetic polynucleotides all exhibit negative CD bands between 250 and 320 nm.

Stacking interaction of the bases must have played an important role in the observed striking spectral effects. This is based on the finding that titrations with d(AA) exhibit significant spectral changes, albeit less pronounced than poly(dA), whereas mononucleoside dA exhibits little spectral alterations upon similar mercuric chloride additions. Although the detailed structure of a 1:1 complex in poly(dA) is not clear, it may be that Hg(II) cross-links the adjacent amino groups of the stacked adenine bases in the same strand and each amino group is attached to two mercuric ions on each side.

The thermal denaturation measurements suggest that the cross-linked complex of Hg(II) with poly(dT) is thermally quite stable and appears to melt near 100°C. Several complexes are formed with poly(dA) depending on the mercuric concentration and temperature. The complex which induces the intense positive CD band at 296 nm (1:1 complex) melts cooperatively around 35°C to other conformations prior to the metal release while the higher r complexes are thermally much more stable. For example, the complexes in the $r = 12$ solution do not exhibit signs of melting even at 100°C.

The results of our binding titrations with poly(dA-dT):poly(dA-dT) appear to be consistent with the modified slippage model of Young et al. [15] with interstrand cross-linking of dT and self base-pairings of dA. CD studies with polynucleotides of known sequence have led Ding and Allen [20] to suggest that a cross-strand binding of Hg(II) to the two thymines of the base-paired first-neighbor unit TpA to be more important than ApT. Our mercuric binding studies with dinucleoside monophosphates indicate that d(AT) exhibits greater

spectral changes than d(TA), at variance with the suggestion of Ding and Allen. It should be cautioned, however, that the results from dinucleotide systems may not be strictly applicable to the double helical polynucleotides due to differences in factors such as base pairing and base stacking. For example, consistent with the results of poly(dA) and poly(dT) the absorption spectral changes are more prominent for d(AA) than for d(TT) during mercuric titrations, the CD spectral alterations of these dinucleosides, however, do not readily correspond to those of polynucleotides.

Finally, CD measurements of DNA in the presence of Hg(II) led Walter and Luck [21] to suggest a structural transition to a more condensed state resembling the (ψ)-like character. Electric dichroism and sedimentation velocity experiments by Ding and Allen [22], however, led to the conclusion that there is neither an intermolecular aggregation nor a tertiary structural formation in the complexing process. The increase of the sedimentation coefficient of DNA-heavy-metal-ion complexes was attributed to an increase in molecular weight and a decrease in the partial specific volume upon binding. Our CD results tend to support the contention of Ding and Allen. This is based on the observation that even though very strong CD bands are induced upon Hg(II) binding to DNA, their intensities are still several-fold less than the psi-structure of DNA induced by other ligands [23]. Furthermore, in contrast to significant tailings exhibited by the ψ-type condensed complexes, no ellipticity is apparent above 350 nm for the mercuric complexes formed.

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