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Effect of Hg(II) on the spectroscopic properties of poly[d(A–T) · d(A–T)] and poly[d(A) · d(T)] and their constituent subunits (deoxyadenosine and thymidine monomers and dimers)

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Abstract

Adding, respectively, increasing amounts of $Hg(ClO_4)_2$ (≡ Hg(II)) to poly[d(A–T) · d(A–T)] (**I**), poly[d(A) · d(T)] (**II**) as well as to their constituent subunits 2′-deoxyadenosine (≡ dA), thymidine (≡ dT), 2′-deoxyadenosine-5′-monophosphate (≡ dAp), thymidine-5′-monophosphate (≡ dTp), 2′-deoxyadenylyl-(3′ → 5′)-2′-deoxyadenosine (≡ d(ApA)), 2′-deoxyadenylyl-(3′ → 5′)-thymidine (≡ d(ApT)), thymidylyl-(3′ → 5′)-2′-deoxyadenosine (≡ d(TpA)), and thymidylyl-(3′ → 5′)-thymidine (≡ dTpT)) – all dissolved in 0.1 M $NaClO_4$, 5 mM cacodylic acid, pH 7 – generates changes in their UV spectra that (a) progress in (**I**) in a pattern that is distinctly different from the one occurring in (**II**) and that (b) reveal a strong sequence dependence in the case of the dinucleoside phosphates. The spectroscopic parameters D (dipole strength), f (oscillator strength), and h (hypochromicity) were determined as a function of Hg(II) concentration for both polymers as well as for all dimers. Also determined were D and f of the monomers. D and f of dA, dAp, and d(ApA) display a different dependence on Hg(II) concentration than do D and f of dT, dTp, and d(TpT). The corresponding parameters of the mixed-sequence dimers d(ApT) and d(TpA) vary with Hg(II) in a 'mirror'-like fashion. Increase in base stacking subsequent to mercury binding is noted with d(TpT) and d(TpA). The opposite occurs in d(ApT). Hg(II) exerts only marginal effects on the base stacking in d(ApA). Both D and f of polymers (**I**) and (**II**) increase with increasing levels of Hg(II), i.e. Hg(II) binding decreases base stacking (loss of hypochromicity). The effect is very much stronger in (**II**) than in (**I**). In contrast to dimers, the spectra of the mercurated polymers do not reveal any sequence dependence, meaning that none of the mercurated dimer spectra persists in a polymer spectrum.

Keywords: Mercuric ion effect; Spectral property; Synthetic polynucleotide; Constituent monomer; Constituent dimer; Dipole strength, oscillator strength, hypochromism

1. Introduction

Mercuric ions (≡ Hg(II)) are known to interact strongly and yet reversibly with the nitrogen binding sites of purines and pyrimidines [1–5]. It is believed that with native DNA the metal is chelated between the Watson–Crick base pairs, forming strong bonds to the lone-pair electrons of the nitrogen atoms in a linear N–Hg–N configuration (sp hybridization) [3,5,6]. The phosphate backbone is thought not to participate in Hg(II) binding. The interaction is most conveniently fol-

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lowed spectrophotometrically because of the strong absorption of electromagnetic radiation in the ultraviolet (≡ UV) by the DNA bases. Employment of circularly polarized electromagnetic radiation reveals that Hg(II) binding is accompanied by changes in DNA chirality: the conformation of duplex DNA is altered (reversibly) from one with right-handed helicity (B-form DNA) to one with left-handed (Z-form like) screwness [7–14].

When studying the effect of Hg(II) on the circular dichroic (≡ CD) absorption spectra of the synthetic nucleic acids poly[d(A–T) · d(A–T)] (**I**) and poly[d(A) · d(T)] (**II**) [14] we were struck by the fact that the changes generated by Hg(II) in the CD of the polymers revealed a distinct base sequence dependence: the CD signals, in both sign and magnitude, progressed differently when varying the concentration of Hg(II) in the case of (**I**) than they did with (**II**). While it is well known that the base arrangement in (**II**) is quite different from that in (**I**) – being characterized by (i) a high propeller twist in the base pairs, which results in maximal base stacking, (ii) a novel system of bifurcated hydrogen bonds, and (iii) a narrow minor groove width of about 9 Å [15,16] (for a review, see Ref. [17]) – and, hence, could account for much of the CD variations noted, it was nevertheless of interest to see the sequence dependence of the CD signals also expressed in the spectra of the mercury complexes of their constituent subunits d(ApA), d(ApT), d(TpA), and d(TpT). It was found that while features of the CD spectra of mercurated d(ApA) and d(TpT) persist in the CD spectra of mercurated poly[d(A) · d(T)] there was little obvious agreement of the CD spectra of mercurated d(ApT) and d(TpA) with the CD of mercurated poly[d(A–T) · d(A–T)] [14].

The present contribution is an extension of the CD study [14]. Since the CD of nucleic acids originates almost exclusively with base stacking (this holds also for their dimeric subunits [18]) and since the magnitude of the Hg(II)-induced CD changes seen in (**I**) and (**II**) as well as in the constituent dinucleoside phosphates signal major perturbations in base stacking, we thought it worthwhile also to explore the effect of Hg(II) on the hypochromism of both AT-dimers and AT-polymers. Hypochromism, brought about by the interaction of a particular electronic excited state of a given base with different electronic states of closely spaced neighboring bases, can be studied in ordinary UV and can be evaluated quantitatively by using spectroscopic parameters such as the dipole strength D, or the oscillator strength f, of the underlying electronic transition [18]. Hypochromism can thus be a quantitative indicator of Hg(II)-induced perturbations in DNA base stacking. To this end, the UV spectra of the polymers, dimers, and monomers were recorded from 360 to 200 nm in the presence of varied amounts of Hg(II). Both the dipole strength D and oscillator strength f of the absorption bands were evaluated as a function of Hg(II) concentration. f was used to compute the hypochromism h of the compounds of interest as a function of Hg(II) concentration.

2. Materials and methods

The deoxynucleosides as well as mono- and di-nucleoside phosphates were purchased from Sigma as were the synthetic nucleic acids poly-[d(A–T) · d(A–T)] and poly[d(A) · d(T)]. Monomers, dimers, and polymers were dissolved in 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 7, at concentrations of $(1–2) \times 10^{-4}$ M(P). This very same salt solvent has also been used in all previous studies dealing with the binding of Hg(II) by nucleic acids (e.g., Refs. [7–14]), for perchlorate does not complex Hg^{2+}. Hg(II) was added in the form of $Hg(ClO_4)_2$. Hg(II) concentrations are expressed in terms of r values, whereby $r \equiv [Hg(II)]_{added}/[\text{monomer unit}]$. The brackets denote molar concentrations. Hg(II)-titration, as done previously, covered the r range 0; 0.01; 0.03; 0.05; 0.07; 0.09; 0.12; 0.15; 0.2; 0.3; 0.4; 0.5; 0.6; 0.75; 1; 2; and, on occasion, 3 and 5. Base dilution due to the titration was taken into consideration when computing concentrations.

Molar absorptivities (ϵ) of the monomers and dimers were taken from the literature [19–22]. Values reported for wavelengths other than 260 nm were converted to the 260 nm value, the

wavelength used in concentration evaluations. Corrections for pH were not necessary, for the literature values had either been determined at pHs very close to 7 or the absorption spectra did not vary much with pH at the wavelength of interest. The particular ϵ^{260} values employed in the study were: $\epsilon_T = 8833$; $\epsilon_A = 14900$; $\epsilon_{Tp} = 8500$; $\epsilon_{Ap} = 15300$; $\epsilon_{AA} = 13700$; $\epsilon_{AT} = 11400$; $\epsilon_{TA} = 11700$, and $\epsilon_{TT} = 8400$ l/mol/cm. The ϵ^{260} values for poly[d(A–T) · d(A–T)] and poly[d(A) · d(T)] were 6000 and 6650 l/mol/cm, respectively [11].

Spectra were recorded with the help of the Shimadzu-UV 160 spectrophotometer from 360 to 200 nm and at 25°C. Absorption bands were evaluated quantitatively by using the Jandel Scientific non-linear curve-fitting software PeakFit, version 3. The program employs the Marquardt–Levenberg fitting algorithm. Gaussian functions were used in the fitting process; r^2 was always better than 0.999.

The dipole strength D and oscillator strength f of an absorption band are given by

$$D = \left[3hc(2303)/8\pi^3 N_0\right] \int (\epsilon/\lambda)\, d\lambda, \qquad (1)$$

$$f = \left[2303mc^2/\pi e^2 N_0\right] \int (\epsilon/\lambda^2)\, d\lambda, \qquad (2)$$

where h is Planck's constant, c the speed of light, N_0 Avogadro's number, m and e are the mass and charge of an electron, and ϵ is the molar absorptivity of the compound in question. If the wavelength λ is given in cm, evaluation of the fundamental constants leads to $D = 9.180 \times 10^{-3} \int(\epsilon/\lambda)\mathrm{d}\lambda$ (debye)^2 and $f = 4.318 \times 10^{-9} \int(\epsilon/\lambda^2)\mathrm{d}\lambda$ (unitless). Computation of D or f requires thus the determination of the area under a given band. The Romberg integration procedure was used to integrate the area numerically. Cut-off points for band integration were the wavelength position at which the band has its minimum or merges with the zero absorbance line.

The hypochromism h of stacked dinucleoside phosphates may be computed from f by using the relation

$$h(\%) = (1 - f_D/f_M)100, \qquad (3)$$

where f_D is the oscillator strength of the dinucleoside phosphate in question and where f_M is the oscillator strength of an equimolar mixture of the components of the dinucleoside phosphate [23]. f_M was computed by employing f_A and f_{Ap} for d(ApA), f_T and f_{Tp} for d(TpT), f_A and f_{Tp} for d(ApT), and f_T and f_{Ap} for d(TpA). The hypochromicity of poly[d(A–T) · d(A–T)] and poly[d(A) · d(T)] was computed by replacing f_D with f_P (the polymer oscillator strength) and f_M with f_{D^*}. f_{D^*} is the oscillator strength of an equimolar mixture of the dinucleoside phosphates that make up a polymer strand. Thus, with poly[d(A–T) · d(A–T)], f_{ApT} and f_{TpA} were used; in the case of poly[d(A) · d(T)], f_{ApA} and f_{TpT} were employed.

Data were plotted by using the Jandel Scientific Sigma Plot, version 4.02. The Desktop Molecular Modeller, version 1.2 (Oxford University Press) was used to undertake a few exploratory energy minimization computations.

3. Results

3.1. Monomer spectra

The effect of Hg(II) on the absorption spectra of dA and dT and their 5′-monophosphates is shown in Figs. 1 and 2. The numbers with the curves are normalized Hg(II) concentrations (r values). While the spectrum of dA is minimally affected by Hg(II), with the exception of the small increase in absorbance between 280 and 300 nm, major changes are seen with dAp. Nevertheless, that Hg(II) does interact with dA is noted by the appearance of two isosbestic points (Fig. 3, panel (1)). Fig. 3, incidentally, contains the difference spectra of the various monomers, dimers, and polymers, displayed as a function of Hg(II) concentration. In the case of dAp, three isosbestic points come into being (Fig. 3, panel (2)).

Although major spectral variations take place when Hg(II) is added to dT or dTp (Fig. 2) they progress in nearly identical fashion, i.e. the presence of the 5′-phosphate group in dTp is not felt at all. Consequently, their difference spectra are

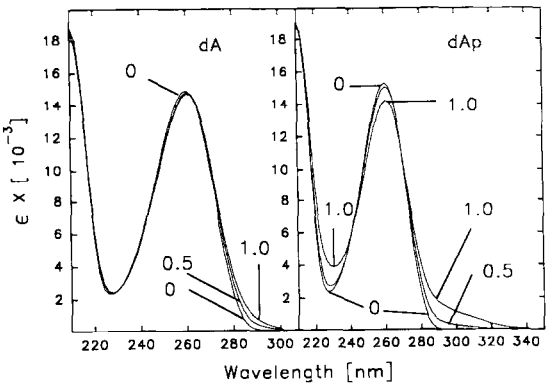

Fig. 1. Absorption spectra of 2′-deoxyadenosine (dA) and 2′-deoxyadenosine-5′-monophosphate (dAp), collected at different concentrations of $Hg(ClO_4)_2$ (≡ Hg(II)). The numbers with the curves refer to the normalized Hg(II) concentration r, whereby $r \equiv [Hg(II)]_{added}$ /[base monomer unit]. The brackets denote molar concentrations. Molar absorptivity ϵ is given in l/mol/cm units. Wavelength range: 210–360 nm. As solvent served 0.1 M $NaClO_4$, 5 mM cacodylic acid buffer, pH 7. Cells of 1 cm pathlength were used in the measurements. Further details are given in the text.

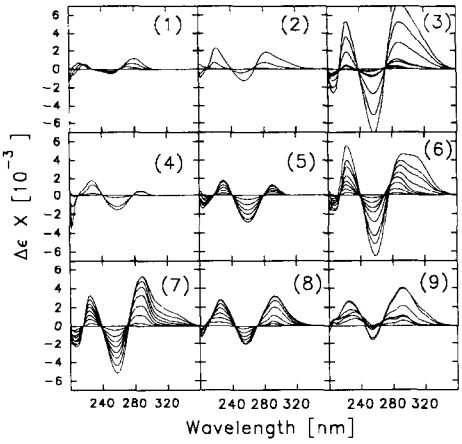

Fig. 3. Difference absorption spectra of dA (panel 1), dAp (panel 2), 2′-deoxyadenylyl-(3′ → 5′)-2′-deoxyadenosine (d(ApA)) (panel 3), dTp (panel 4), thymidylyl-(3′ → 5′)-thymidine (d(TpT)) (panel 5), thymidylyl-(3′ → 5′)-2′-deoxyadenosine (d(TpA)) (panel 6), 2-deoxyadenylyl-(3′ → 5′)-thymidine (d(ApT)) (panel 7), poly[d(A–T)·d(A–T)] (panel 8), and poly[d(A)·d(T)] (panel 9), collected in presence of varied amounts of $Hg(ClO_4)_2$. The ($r = 0$) spectra were subtracted from the ($r > 0$) spectra: $\Delta\epsilon \equiv \epsilon_r - \epsilon_{r=0}$ liter/mol/cm. Wavelength range: 200–360 nm. Spectral progression should be obvious, it follows sequentially the r values given in Figs. 1, 2, 4–6. dA, dAp, dT, and dTp are defined in the legends of Figs. 1 and 3. For further details, consult the legends to Figs. 1 and 2 as well as text.

superposable and, thus, only one spectrum (that of dTp) is shown in Fig. 3 (panel (4)).

3.2. Dimer spectra

Figs. 4 and 5 contain the absorption spectra of d(ApA), d(TpT), d(ApT), and d(TpA). There can be little doubt that the addition of Hg(II) to the dinucleoside phosphates causes spectral variations that are strongly sequence dependent. This

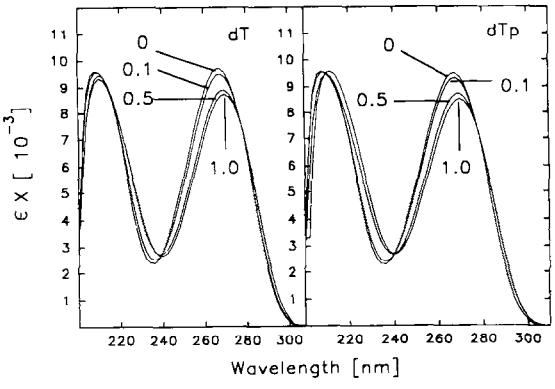

Fig. 2. Absorption spectra of thymidine (dT) and thymidine-5′-monophosphate (dTp), collected at different concentrations of $Hg(ClO_4)_2$. The numbers with the curves are r values. Their definition is given in the legend to Fig. 1. The molar absorptivity ϵ has the units l/mol/cm. Wavelength range: 200–360 nm.

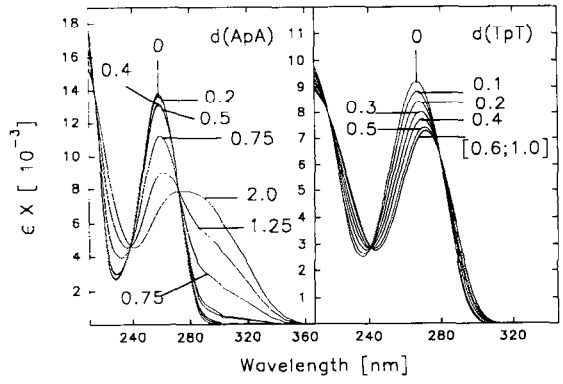

Fig. 4. Absorption spectra of d(ApA) and d(TpT), collected in absence and presence of $Hg(ClO_4)_2$. The numbers with the curves are r values; ϵ has the units l/mol/cm. Wavelength range: 200–360 nm. The bracketed numbers indicate that the corresponding spectra overlap with each other. For further details, consult the legends to Figs 1–3 as well as text.

is particularly evident when consulting the difference spectra displayed in Fig. 3 (panels (3), (5)–(7)). Long-wavelength red-shifts, giving rise to the positive $\Delta\epsilon$ bands located between 280 and 360 nm, are very pronounced in the case of d(ApA) (panel(3)), d(TpA) (panel (6)), and d(ApT) (panel (7)) but miniscule with d(TpT) (panel (5)). It is readily seen that the magnitude of the $\approx$ 280–360 nm $\Delta\epsilon$ band stems from the presence of the A residue. The same holds for the negative $\Delta\epsilon$ bands near 260 nm and the short-wavelength positive $\Delta\epsilon$ bands at 220 nm: their magnitude, too, reflects the presence of the A residue (compare, for instance, panels (3), (6), and (7) with panel (5)).

3.3. Polymer spectra

Fig. 6 shows the absorption spectra of mercurated poly[d(A–T) · d(A–T)] (left panel) and poly[d(A) · d(T)] (right panel). The corresponding difference spectra are contained in panels (8) and (9) of Fig. 3, respectively. In contrast to poly[d(A–T) · d(A–T)], whose spectrum varies smoothly with increasing levels of Hg(II), that of poly[d(A) · d(T)] does not. Most obvious is the change of ϵ_{max}: it decreases at first with increasing r (up to about $r \approx 0.25$) but increases thereafter with further increasing r. Curiously enough, neither panel (8) nor panel (9) (Fig. 3) show obvious evidence of base sequences playing a role: all $\Delta\epsilon$ bands are of similar magnitude, and the isosbestic points are located near identical wavelength positions. There is, however, one important difference between the two polymers as far as isosbestic points are concerned. Whereas the isosbestic points of poly[d(A–T) · d(A–T)] at 243.5 and 270.5 nm are invariant with Hg(II) concentration in the range $0 \leqslant r \leqslant 1$ (the experimental range covered in this case) – signaling that complexation involves the transition of the polymer between two well-defined states – the corresponding isosbestic points of poly[d(A) · d(T)] not only are are ill-defined to begin with (there is one near 247 nm and one near 260 nm) but they are in existence only in a limited concentration range of Hg(II). These two points exist only in the range $0 \leqslant r \leqslant 0.25$; there is, perhaps, another one formed near 243 nm at $r > 0.3$. This indicates that complexation of poly[d(A) · d(T)] by Hg(II) is governed by a minimum of two equilibria.

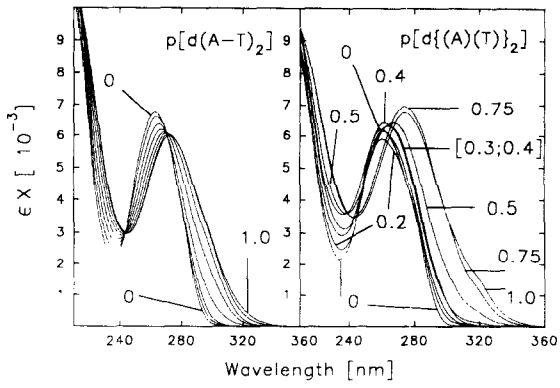

Fig. 6. Absorption spectra of poly[dA–T)·d(A–T)] (left panel) and poly[d(A)·d(T)] (right panel), collected in absence and presence of $Hg(ClO_4)_2$. The numbers with the curves are r values; they progress in the sequence 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0 (left panel); they are listed individually in the right panel. The bracketed numbers indicate that the corresponding spectra overlap with each other. ϵ has the units l/mol/cm. Wavelength range: 210–360 nm. For further details, consult the legends to Figs. 1–3 as well as text.

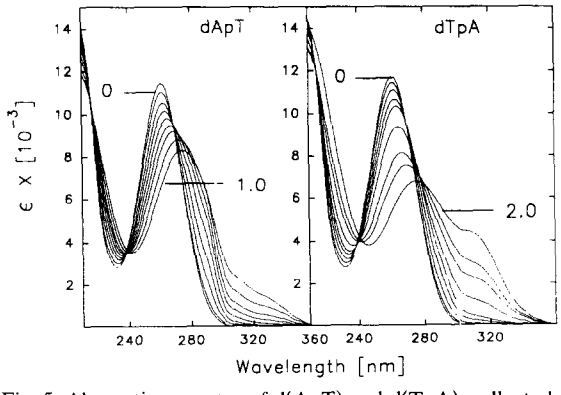

Fig. 5. Absorption spectra of d(ApT) and d(TpA), collected in absence and presence of $Hg(ClO_4)_2$. r values progress in the sequence 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 1.0 in the case of d(ApT) and in the sequence 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 2.0 in the case of d(TpA). ϵ has the units l/mol/cm. Wavelength range: 210–360 nm. For further details, consult the legends to Figs. 1–3 as well as text.

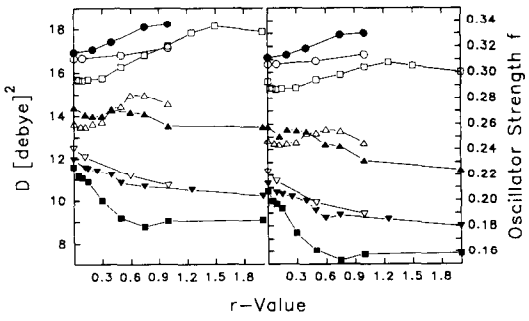

Fig. 7. Dependence of dipole strength D (left panel) and oscillator strength f (right panel) on $Hg(ClO_4)_2$ concentration: (●) dAp; (○) dA; (□) d(ApA); (△) d(ApT); (▲) d(TpA); (▽) dT; (▼) dTp; (■), d(TpT). D is given in units of $(10^{-18}$ esu cm cgs)^2 (≡ debye)^2; f is unitless. For a definition of monomer and dimers, consult legends to Figs. 1–3; for a definition of r, see legend to Fig. 1.

3.4. Effect of Hg(II) on the dipole strength D, oscillator strength f, and hypochromism h of the UV absorption bands

Fig. 7 shows the variation of D and f with r. Three 'families' are discernible: (1) the monomers and dimers of adenosine (dA, dAp, d(ApA)) see their D and f parameters increase in magnitude with increasing levels of Hg(II) (upper part of the left and right panels); (2) the monomers and dimers of thymidine (dT, dTp, d(TpT)) have D and f parameters that decrease in magnitude with increasing r values (lower part of the left and right panels); (3) in-between (center part of the left and right panels) are the mixed-sequence dimers d(ApT) and d(TpA). Their D and f parameters cross each other's path: both D and f of d(ApT) are initially numerically smaller than the corresponding parameters of d(TpA) ($r = 0$) but become larger at, say, $r = 1.0$.

Computing h with the help of Eq. (3) yields the following results (the numbers in parentheses are r values):

(1) d(ApA), $h = 5.1\%$ (0); $h = 7.1\%$ (0.10); $h = 7.1\%$ (0.40); $h = 7.3\%$ (1.00);
(2) d(TpT), $h = 5.0\%$ (0); $h = 6.4\%$ (0.10); $h = 8.1\%$ (0.15); $h = 12.1\%$ (0.40); $h = 17.4\%$ (0.60); $h = 19.0\%$ (1.00);
(3) d(ApT), $h = 4.8\%$ (0); $h = 4.8\%$ (0.10); $h = 3.8\%$ (0.20); $h = 3.3\%$ (0.40); $h = 0.4\%$ (0.50); $h = -1.1\%$ (0.60); $h = -3.5\%$ (0.75);
(4) d(TpA), $h = 3.5\%$ (0); $h = 4.9\%$ (0.10); $h = 5.6\%$ (0.75); $h = 6.6\%$ (1.00); $h = 10.8\%$ (1.25); $h = 13.7\%$ (3.00).

The conclusion is that complexation by mercury increases base stacking in d(ApA) very slightly, but it increases it in d(TpT) quite strongly. A similarly strong increase is noted with d(TpA), but the opposite occurs in the case of d(ApT): base stacking decreases here upon mercury binding (h becomes even negative at elevated levels of Hg(II)).

The strong increase in hypochromicity of d(TpT) subsequent to Hg(II) binding appears reasonable: Hg(II) is known to displace the hydrogen (as H^+) from the H–N(3) site of T [3,6] and, hence, addition of Hg^{2+} to N^- should not generate at all (or not by much) repulsive (+) charges. Accumulation of (+) charges can perhaps be invoked as explanation for the decrease of h in d(ApT) upon mercuration. However, charge repulsion fails to explain the situation in the case of d(ApA) and d(TpA), for their h increases, rather than decreases, with increasing r. It is clear that other structural features must additionally be operative.

Lastly, using the f_{D^*} data contained in Fig. 7 and the f_P parameters derived from the spectra of poly[d(A–T) · d(A–T)] and poly[d(A) · d(T)], one obtains the following h values for the polymers (the numbers in parentheses again represent r values):

(1) poly[d(A–T) · d(A–T)]: $f_P = 0.151$, $h = 40.2\%$ (0); $f_P = 0.150$, $h = 40.6\%$ (0.10); $f_P = 0.144$, $h = 42.5\%$ (0.20); $f_P = 0.149$, $h = 40.5\%$ (0.30); $f_P = 0.154$, $h = 38.4\%$ (0.40); $f_P = 0.158$, $h = 36.5\%$ (0.50); $f_P = 0.158$, $h = 36.5\%$ (0.75); $f_P = 0.160$, $h = 32.9\%$ (1.00);
(2) poly[d(A) · d(T)]: $f_P = 0.154$, $h = 38.4\%$ (0); $f_P = 0.146$, $h = 39.7\%$ (0.10); $f_P = 0.160$, $h = 33.5\%$ (0.20); $f_P = 0.166$, $h = 28.3\%$ (0.30); $f_P = 0.171$, $h = 26.1\%$ (0.40); $f_P = 0.184$, $h = 19.2\%$ (0.50); $f_P = 0.201$, $h = 10.9\%$ (0.75); $f_P = 0.195$, $h = 14.7\%$ (1.00).

The h value computed for poly[d(A–T) · d(A–T)] (at $r = 0$) agrees extremely well with the value obtained from melting experiments done in the

author's laboratory, and it is assumed that the value computed for poly[d(A) · d(T)] at $r = 0$ will be in agreement with whatever may be available from the literature.

The data enumerated above reveal that h of poly[d(A–T) · d(A–T)], after an initial increase from 40 to 42% in the range $0 \leqslant r \leqslant 0.25$, decreases with increasing levels of Hg(II) and reaches about 33% at $r = 1.00$. Still larger variations occur in poly[d(A) · d(T)]: h decreases here to 10–15% in the range $0.75 \leqslant r \leqslant 1.00$.

4. Discussion

The electronic transitions observed with the nucleosides dA and dT in the 360–200 nm wavelength range of this study correspond, respectively, to transitions I (275 nm), II (270 nm), III (213 nm), and IV (204 nm) of 9-methyladenine [24] and transitions I (275 nm) and II (207 nm) of 1-methylthymine [25]. They all are in-plane $\pi \rightarrow \pi^*$ transitions (the quoted wavelength positions apply to the crystal environment). However, due to the random motion of the base chromophores in solution, adenine transitions I and II are not seen individually but are hidden in the first (long-wavelength) band envelope of dA. Similarly, transitions III and IV of dA are burried in the second (short-wavelength) band envelope. In the case of dT, each band envelope (first or second) represents one transition only [26]. However, the possibility of the first band to be composite in nature has been discussed [27]. Thus, the D and f data collected for dA or dAp represent averaged values while those for dT or dTp are most likely not. Similar considerations apply to the dinucleoside phosphates: while the first band envelope for d(ApA) contains four electronic transitions, those for d(ApT) and d(TpA) are made up of three, and the one for d(TpT) represents two transitions.

In computing D, f, and h, only the first band envelopes were considered, for the short-wavelength bands are too close to the 200 nm cutoff point of the instrument in order to be reliable with respect to band shape and intensity. The observed solution (i.e. randomized) oscillator strength f for 9-methyladenine (first band envelope) is 0.29 [28]; this compares well with the values $f = 0.306$ (dA) and $f = 0.312$ (dAp) found in this study (cf., Fig. 7). Similarly, f for 1-methylthymine is 0.19 (crystal, first band) [29]; the corresponding (randomized) transitions are: dT, $f = 0.223$; dTp, $f = 0.213$ (Fig. 7). It is gratifying to note the close agreement, for there is a certain degree of arbitrariness in deciding which wavelength position to select as integration cutoff at the point at which the first and second band envelopes start overlapping with each other.

Since Hg(II) binding increases D and f in the purine derivatives, but decreases them in the pyrimidine derivatives, the consequence is that base stacking is strongly increased in d(TpT) subsequent to complexation by mercury while in d(ApA) the effect is more or less negligible. Most interesting is the effect of Hg(II) on f and D of the mixed-sequence dimers d(ApT) and d(TpA). Their 'mirror'-like progression as a function of Hg(II) concentration (Fig. 7) is real, for a differential effect is also noted in the circular dichroism of the dimers: a positive Cotton band appears in d(ApT) at elevated levels of Hg(II), with maximum near 340 nm, but in d(TpA), this band turns strongly negative [14]. Based on the variation of h with Hg(II) concentration, one sees that base stacking increases in d(TpA) subsequent to mercury binding but decreases in d(ApT). There can be little doubt that this behavior is related to their conformation. Energy minimization computations yield models that show the A in d(ApT) to be tilted away from the central phosphate group (distance of one of the hydrogens of the NH_2 group at C6 of A to the phosphorous atom is 11.18 Å) while in d(TpA), the A is tilted toward the group (distance of (NH)–H to P is 6.92 Å). It is therefore reasonable that mercury binding should produce differential effects in their spectra.

Since the present work employed nonpolarized electromagnetic radiation and measured the spectra of the monomers, dimers, or polymers in solution, nothing is known about how Hg(II) may affect the orientation of their transition dipole moments μ ($|\mu| = \sqrt{D}$). Thus,

whether the noted red-shifts, particularly of the adenine-containing spectra, reflect changes in the orientation of the monomer dipoles or indicate simply perturbations in the arrangement of the π electrons of the aromatic rings cannot be answered at present. Simpson [4] studied Hg(II) binding to (ribo)nucleosides spectrophotometrically and found that around neutral pH the affinity of mercury for the electron donor sites in the purines and pyrimidines decreases in the order N3(U) > N1(G) > N7(G) ≈ N3(C) > NH_2(A) > NH_2(G) > N1(A). Hug and Tinoco employed the CNDO-CI method to compute the spectra of purines and pyrimidines [26]. They found that the purine spectra could be divided into two groups, namely the adenine (A-type) group and the hypoxanthine (H-type) group. The H-type spectrum possesses a pronounced shoulder on the long-wavelength side of the first band envelope while the A-type spectrum does not. Since protonation of adenine at N1 or N3 was found to shift its spectrum from that of the A-type to the H-type [26], one could argue that mercury binding at N1 or N3 of adenine (in dA, dAp, d(ApA), d(ApT), d(TpA)) produces also an H-type shoulder (cf., Figs. 1, 3–5). On the other hand, the B → Z transition suspected to occur in the polymers, particularly in (**I**) [8,11], presupposes the binding of Hg(II) to N7 of the adenine residue in order to bring about the requisite anti → syn change of the purine base. Protonation of N7, however, is thought to preserve the A-type character of adenine [26] and, hence, mercuration of N7 should not be the cause of the marked spectral red-shift noted particularly in (**II**) (cf., Fig. 6). This demonstrates that some challenging interpretative problems remain.

Although, as anticipated, the non-polarized absorption spectra of the mercurated dimers show a strong sequence dependence (Figs. 3–5), which, incidentally, does not exist in the spectra of the untreated dimers, this dependency is lost when looking at the spectra of mercurated (**I**) or (**II**) (Fig. 6). There is, perhaps, a tendency that the variations noted with the alternating copolymer are more akin to the changes ocurring in d(TpT) while those taking place in the homopolymer resemble more the ones observed with d(ApA). Yet none of the features of the mercurated dimer spectra persists in the polymer spectra. Since, as pointed out above, the base pair arrangement in (**II**) is very much different from that in (**I**), the differential progression with Hg(II) concentration of the absorption spectra reflects thus their inherent differences in helix structure. It is therefore not surprising to see the polymers respond differentially to mercuration and, in particular, to see poly[d(A) · d(T)] (**II**) undergo a more severe base unstacking (decrease of h) than poly[d(A–T) · d(A–T)] (**I**) subsequent to mercuration. It should be noted that Hg(II) binding preserves the alignment of the bases in duplex DNA, i.e. does not cause denaturation. Consequently, removal of Hg(II) with the aid of a strong complexing reagent, such as cyanide, returns all spectra to the form they had prior to adding Hg(II). Removal of Hg(II) also reestablishes the biological activity of DNA [30].

Although the presence (as well as persistence) of the various isosbestic points during mercuration shows that all compounds, including the polymers, form Hg(II) complexes of defined composition, no attempts were made to determine their stoichiometry or gather information on their thermodynamic properties. Thus, whether complexation produces 'base paired' (i.e. crosslinked) monomers or dimers (as they would exist in the polymers) remains unclear at present. Neither Simpson [4] nor Eichhorn and Clark [31] cited evidence for the formation of L–Hg–L complexes (with L standing for a base) when they titrated nucleosides with Hg(II); hence, complexes of the form L–Hg–X (with X standing for any suitable anion, including OH^-) will certainly exist. Since "base pairing" is not a precondition of base stacking, it will presumably matter little whether the compounds are of the type L–Hg–L or L–Hg–X.

One final point. It is puzzling to see Hg(II) produce changes in the spectrum of dAp that are distinctly different from those found with dA (Fig. 1) as the mode of Hg(II) interaction with the purine ring (binding to N only) should be the same, irrespective of the presence of the 5′-phosphate group. This is certainly the case with dT and dTp: the spectral changes are practically the

same (Fig. 2). Incidentally, the very same situation is noted in the CD of the compounds: that of dT and dTp is unaffected by Hg(II) but the one of mercurated dAp differs in both magnitude and sign from the CD of mercurated dA [14]. This suggests that, at least in monomers, the 5′-phosphate group participates in Hg(II) binding. Energy minimization computations on dAp produce a molecule in which the base is *anti* about the $C_{1'}$–N9 link and in which one of the oxygens of 5′-PO_4 is 5.14 Å from N7 of the purine ring and, hence, bridgeable by Hg(II) (assuming sp hybridization). N1 and N3 are 7.64 and 6.70 Å apart from the $-O-PO_3^-$ group. Although, in dTp, the distance from the phosphate group to the ring nitrogen (N3 of pyrimidine) is about the same (5.41 Å), the model shows that the CH_3-group of T, protruding from the C5 of pyrimidine, interferes sterically with bond formation. No such interference is of course possible in dAp. While computer modeling certainly does not prove the correctness of the proposed mode of binding, it offers nevertheless a reasonable explanation. In this context it is of interest to note that in mercurated Gp Hg(II) is thought to connect the 5′-PO_4 group to the $-NH_2$ group at C6 [32].

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