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Influence of Mercuric Ions on the Phosphorescence and Photochemistry of DNA*

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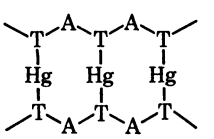
Abstract. Partial mercuration of DNA, such that roughly one mercuric ion is bound for every two thymine residues, results in an enhancement of the phosphorescence by a factor of ten and a small enhancement of the photosteady thymine dimer yield. Complete mercuration of DNA [one Hg(II) added per momoner unit] results in quenching of the phosphorescence intensity and an inhibition of thymine dimer production. The enhancement of the phosphorescence is interpreted in terms of a heavy-atom effect caused by the preferential binding of Hg(II) to the thymine residues. The quenching of both the thymine phosphorescence and the rate of thymine dimerization upon complete mercuration is probably due to energy transfer from thymine to another base, presumably adenine, which when mercurated acts as an energy trap.

We present in this paper some preliminary results on the influence of mercuric ions on the luminescence and photochemistry of DNA and related polynucleotides. As Thomas[1] first pointed out, mercuric ions bind directly to the purine and pyrimidine bases in DNA and not to the phosphoryl groups. This interaction causes large changes in the physical structure of the DNA, as evidenced by the drastic decrease in viscosity.[2] The binding of Hg(II) to DNA also causes profound changes in the optical absorbance[1,3] and circular dichroism.[4] These changes have usually been recorded as a function of r, the ratio of the number of moles of Hg(II) present per mole of monomer units. The binding of Hg(II) to DNA is reversed by an excess of an anion, such as CN^- or Cl^-, which strongly complexes with Hg(II).

Katz[5] has proposed a mechanism, based mainly on the experimental results of Yamane and Davidson,[3] for the binding of Hg(II) to poly(dA-dT) and DNA, in which the binding occurs preferentially to two thymine residues located on opposite strands: a proton from each thymine is displaced, presumably from the N_1 position, and a cross-link is formed. Such a binding mechanism supposes that an axial chain-shift of one base spacing occurs to give, for the case of poly (dA-dT) at $r = 0.25$:



Recent circular dichroism measurements by Aktipis[4] also support the hypothesis that the binding of Hg(II) to DNA takes place initially at the AT-rich regions of DNA. Davidson *et al.*[6] have made use of the preference of Hg(II) for A·T rather than G·C regions to separate poly(dA-dT) from DNA.

We have examined the influence of Hg(II) on the luminescence of DNA at 77°K. It has been shown previously[7] that the intrinsic phosphorescence of both poly(dA-dT) and DNA is nearly exclusively that of a triplet localized at a thymine residue. Since thymine has the lowest triplet state of the four bases in DNA,[8] energy transfer at the triplet level from the other bases to thymine is possible and may account, in part, for the absence in DNA of phosphorescence from the other bases. We show in this paper that the binding of small amounts of Hg(II) to DNA and poly(dA-dT) results in a large enhancement of the thymine phosphorescence. This phenomenon should be useful in studies of the excited states of nucleic acids.

We have, in addition, measured the effect of Hg(II) binding on the formation of thymine dimers in DNA. Photodimerization in DNA has been shown previously to be strongly dependent upon the presence of bound dyes such as proflavin,[9] which supposedly act as singlet energy traps.[10] We have found that the rate of photodimerization in DNA is greatly reduced by a high concentration of Hg(II) ($r = 1$, when bases other than thymine are mercurated). Hence, mercuric ion binding can serve as a useful tool for varying the photochemical yields in DNA and may have application in certain photobiological investigations.

Materials and Methods. The synthetic polynucleotides poly A, poly(dA-dT), poly C, poly I, and poly G were obtained from the Miles Laboratory; poly dT was a gift from F. J. Bollum, University of Kentucky. Calf thymus DNA was purchased from Sigma Chemical Co. *Escherichia coli* and *Clostridium perfringens* DNA were obtained from Worthington Biochemical Corp., and thymine-labeled *E. coli* DNA was given to us by W. L. Carrier. *Haemophilus influenzae* DNA was given to us by J. K. Setlow. Deaminated DNA (C→U, A→I, and G→X) was prepared from calf thymus DNA by treatment with nitrous acid as described by Zmudzka *et al.*[11] Deamination was also used to prepare poly X from poly G. All emission measurements were made on samples in ethylene glycol–water 1:1, which forms a glass at 77°K; the pH (6.8) was maintained with 0.01 M phosphate buffer. Mercuric ions were added in the form of $HgCl_2$, purchased from J. T. Baker Chemical Co. Absorbance measurements were made at 25°C with a Beckman recording spectrophotometer, model DK-1A.

The phosphorescence at 77°K from samples in quartz tubes of inner diameter 3 mm was observed by means of an instrument previously described.[12] Photodimerization was induced with 254 nm irradiation from a 15-W low-pressure mercury lamp (General Electric). Thymine dimers were isolated by paper chromatography in *n*-butanol–water–acetic acid 80:30:12. Once separated, the percentage of thymine present as the dimer was measured by liquid scintillation techniques.[13]

Results. Absorbance measurements: The absorption spectra of the polynucleotides, including DNA, undergo red-shifts when mercuric ions are bound.[3,14,15] The interaction of polynucleotides with Hg(II) can be followed spectrophotometrically by means of the increase in absorbance at 300 nm, a wavelength at which absorbance in the absence of Hg(II) is very small. The results for poly A, poly dT, poly(dA-dT), and DNA are compared in Fig. 1. The spectrum of poly dT is shifted only slightly to the red by Hg(II), hence

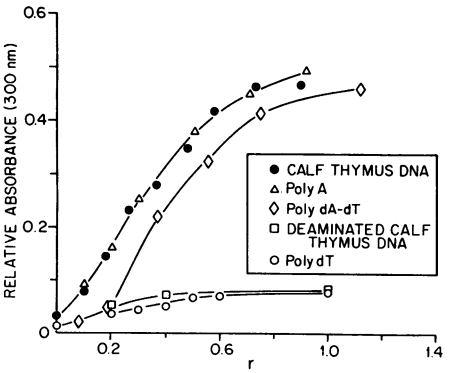


FIG. 1. Relative absorbance at 300 nm as a function of r. The ordinate represents the absorbance at 300 nm divided by the absorbance at 260 nm for $r = 0$. All samples were in 0.01 M phosphate buffer at pH 7.

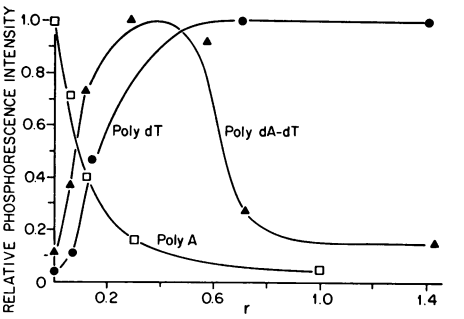


FIG. 2. The relative phosphorescence intensity measured at the spectral peak (~450 nm) and plotted as a function of r. Excitation wavelength was 260 nm.

the increase in relative absorbance at 300 nm is much smaller for poly dT than it is for poly A.[14] Poly A shows a much greater red-shift upon mercuration than any of the other polynucleotides studied, including poly G, poly X, and poly C.

The curve for poly(dA-dT) at low values of r ($r < 0.25$) coincides with that of poly dT, while for $r > 0.25$ the curve breaks sharply and parallels that of poly A. The slight increase in absorbance of mercurated poly(dA-dT) for $r < 0.25$ implies that the binding of Hg(II) to poly(dA-dT) occurs initially at the thymidyl residues, as suggested by Katz.[5] Then, after one mercuric ion is bound for every two thymidyl residues, binding to the adenyl residues occurs, along with a much greater increase in the 300 nm absorbance.

The binding of Hg(II) to DNA also produces a large red-shift;[1,3] however, it is difficult to tell from the initial part of the titration curve (Fig. 1) whether the binding is exclusively to thymine or not. In DNA, as well as with the other systems studied here, the interaction with Hg(II) appears to be complete by $r = 1$, although for poly dT the absorbance changes are complete by $r = 0.5$. We assume that all the Hg(II) present is bound at low r values. This assumption for the case of DNA is supported by the experimental results of Davidson and co-workers.[3,16]

Deaminated DNA was not as red-shifted by Hg(II) binding as DNA itself. This result is in part explained by our observation that mercuration of inosine (in poly I) results in a red-shift which is much smaller than that obtained upon mercuration of adenosine (in poly A).

Emission measurements: The phosphorescence intensity of poly A and poly dT was measured as a function of r. As shown in Fig. 2, these two molecules differ greatly in their response to Hg(II) binding. Poly A has its phosphorescence quenched more than 10-fold when sufficient Hg(II) is bound. Similar results (not shown) were obtained with poly G. In contrast, the phosphorescence of poly dT increases more than 10-fold upon mercuration. Poly C (not shown)

behaved similarly. For all four of these polynucleotides, the change in the phosphorescence intensity with r was complete by $r = 0.5$. The phosphorescence spectrum of poly(dT)·Hg ($r = 1$) was slightly more structured than that of poly dT and was red-shifted by 10–15 nm. The residual phosphorescence of fully mercurated poly A was less structured than that of poly A and showed no appreciable shift in the peak maximum. Neither poly I nor poly X showed any appreciable phosphorescence enhancement when fully mercurated.

For the case of poly(dA-dT), the phosphorescence intensity, as shown in Fig. 2, increases with r for $r < 0.25$. However, when r is increased above 0.25 the phosphorescence intensity decreases. These results are compatible with the preferential binding of Hg(II) to the thymine residues at $r < 0.25$ and the consequent enhancement of thymine phosphorescence as in poly dT. The decrease in intensity for $r > 0.25$ occurs when Hg(II) binds to the adenine residues. As discussed later, this quenching probably corresponds to some kind of energy transfer from mercurated thymine residues to mercurated adenine residues.

The phosphorescence intensity of three different DNAs, varying in their A+T content, was measured as a function of r. The results are given in Fig. 3, where it is clear that (*a*) Hg(II) enhances the phosphorescence of all three DNAs at low r values; (*b*) the relative amount of Hg(II) needed to achieve a phosphorescence maximum varies roughly in proportion to the amount of thymine present in the DNA (a ratio of one Hg(II) per 2–4 thymine residues needed for a maximum), and (*c*) at high concentrations of Hg(II) ($r \sim 1$), the phosphorescence decreases more than 10-fold for each DNA. Also shown in Fig. 3 is the result obtained upon deaminating calf thymus DNA. At low concentrations of Hg(II), the phosphorescence of deaminated DNA was enhanced, as with native DNA; but phosphorescence was not quenched at high r values.

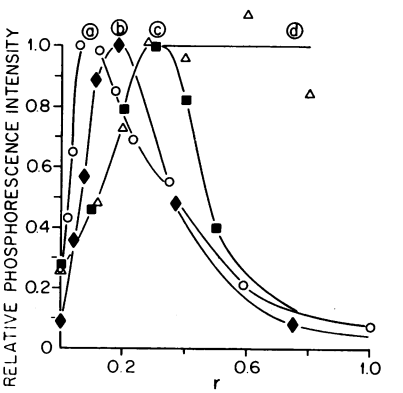


FIG. 3. Variation in the relative phosphorescence intensity (450 nm) as a function of r for (*a*) *E. coli* DNA (0.93); (*b*) calf thymus DNA (1.36); (*c*) *C. perfringens* DNA (2.7); (*d*) deaminated calf thymus DNA. The (A+T)/(G+C) ratio is given in parentheses after each DNA type. The r value for deaminated DNA was based on an estimated extinction coefficient of 7.94×10^3.

The phosphorescence spectra of mercurated poly dT ($r = 1$), poly(dA-dT) ($r = 0.3$), and calf thymus DNA ($r = 0.2$) were found to be identical. Presumably in all three systems, the same excited triplet state, namely that of thymine, is emitting. Furthermore, in every case, mercuration led to a 5-fold reduction in triplet lifetime, e.g., from 0.3 to 0.06 sec for DNA. In contrast, mercuration of poly C led to an approximately 60-fold reduction in lifetime, from 0.5 to 0.008 sec.

In all the polymers studied, mercuration ($r \cong 0.5$) led to a complete quenching of the fluorescence. The fluorescence and phosphorescence quenching curves of poly A and poly G were superimposable.

Photochemistry: The photosteady concentration of thymine dimers formed in both *H. influenzae* and *E. coli* DNA for 254-nm irradiation is shown in Fig. 4 as a function of r. (The higher yields of dimer formed in *H. influenzae* DNA reflect the higher A+T content of this DNA.) We observe that the yield of dimers increases slightly with r and is maximal when one Hg(II) is present for about every four thymine residues. Higher concentrations of Hg(II) reduce the dimer yield such that by $r = 1$ the photosteady yield is 10–15% of that when $r = 0$. Similar results were obtained with denatured DNA.

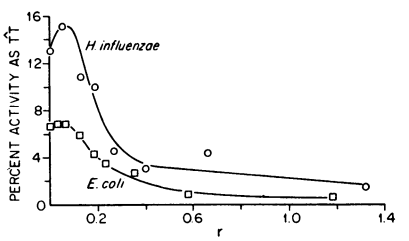


FIG. 4. Photosteady concentrations of thymine dimer in two different DNAs as a function of r. Each sample was given a dose of 1×10^5 ergs/mm^2 at 254 nm.

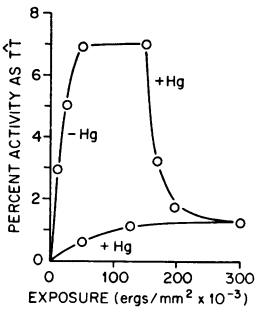


FIG. 5. Concentrations of thymine dimer in *E. coli* DNA as a function of dose (254 nm). Samples were irradiated in the presence ($r = 1$) and in the absence of Hg(II) ($r = 0$). Dimer formation was reversed by adding Hg(II) to a sample previously irradiated in the absence of Hg(II) and continuing the irradiation in the presence of Hg(II).

The rate of formation of thymine dimers as a function of dose (254 nm) is shown in Fig. 5 for *E. coli* DNA with ($r = 1$) and without bound mercuric ions. The initial rate of dimer formation with Hg(II) present is about one-tenth of that with Hg(II) absent. Of interest is the ability of Hg(II) to shift the photosteady equilibrium established between monomers and dimers. As indicated in Fig. 5, the addition of Hg(II) to a sample containing 7% dimer [obtained by previous irradiation in the absence of Hg(II)] followed by further irradiation, results in a decrease in the dimer yield from 7% to the new, photosteady concentration of 1%.

Discussion. It has been generally observed that the interaction of a heavy atom with an aromatic molecule results in profound changes in the emission properties of the molecule.[17] These changes include a decrease in the phosphorescence lifetime, a decrease in the fluorescence quantum yield, and sometimes an increase in the phosphorescence quantum yield. It has been established that these heavy-atom effects are brought about by an enhancement of the spin orbit coupling of the aromatic system. Spin orbit coupling is necessary for transitions to occur between singlet and triplet states.

The changes in the emission properties of poly dT and poly C upon mercuration are indicative of a heavy-atom effect. In both of these polymers there is a

shortening of the phosphorescence lifetime, in addition to fluorescence quenching and phosphorescence enhancement.

In poly A and poly G, mercuration quenches the fluorescence, but it also quenches the phosphorescence instead of enhancing it as in poly dT and poly C. Since both the radiative and the nonradiative transition from the triplet to the ground state can be enhanced by mercuration, the phosphorescence quenching in poly A and poly G may indicate that in these systems the nonradiative transition is preferentially enhanced, so that most of the triplets decay without emitting a photon. This explanation is valid provided one assumes that the concomitant fluorescence quenching is due to an increase in the intersystem crossing ($S^1 \rightarrow T$): that is, energy is lost from the singlet state by transfer to the triplet state. We cannot, however, rule out the possibility that in poly A and poly G the fluorescence quenching with Hg(II) may be due to an increase in the internal conversion process ($S^1 \rightarrow S^\circ$). If this were so, one would not expect to find any increase in phosphorescence intensity because of the decrease in the excited singlet-state population.

The changes in phosphorescence intensity of poly(dA-dT) and DNA upon mercuration are more complex than those of the homopolymers. In these systems there is both an increase in intensity, at low r values, and a decrease in intensity, at high r values, upon mercuration. The enhancement at low r values is consistent with the preferential binding of Hg(II) to the thymine residues, since (*a*) only poly dT and poly C show a phosphorescence enhancement upon mercuration; (*b*) the phosphorescence spectrum and the triplet lifetime of mercurated DNA are very close to those of mercurated poly dT but not to those of poly C; and (*c*) the r value needed to achieve the maximum phosphorescence intensity for each of the three different DNAs studied increased in proportion to the thymine content of the DNA.

The quenching of the phosphorescence at high r values in poly(dA-dT) is probably due to energy transfer from mercurated thymine to mercurated adenine, which does not emit. A similar process may also occur in DNA, with either mercurated adenine or guanine acting as traps. The quenching in DNA does not appear to depend upon any specific structural changes since denatured DNA behaves in the same way as native DNA. However, the phosphorescence of deaminated DNA, in which the A and G residues have been converted to I and X, is not quenched at high concentrations of Hg(II), which implies that mercurated A and G residues act as traps. If energy transfer is the mechanism by which quenching occurs, the question arises as to whether this transfer is at the singlet or triplet level or both.

From the position of the long-wavelength edge of the absorbance, one would conclude that adenine has the lowest-lying singlet state of any of the mercurated bases. Hence, this base is the most likely to be a singlet energy trap. This conclusion is supported by the absence of phosphorescence quenching by Hg(II) in deaminated DNA.

We cannot rule out transfer at the triplet level from thymine to some other base which, when mercurated, has a triplet lower than that of thymine. In the absence of Hg(II), thymine has been shown to have the lowest triplet in DNA.[8]

Mercuration lowers the triplet of thymine since the phosphoresence of mercurated poly dT is appreciably red-shifted. On the other hand, the short-wavelength edge of the weak phosphorescence of mercurated poly A is only slightly red-shifted relative to poly A itself. Hence, it is likely that in mercurated poly (dA-dT) the thymine triplet is still lowest, in which case one might expect to find that energy transferred at the singlet level from T to A transfers back at the triplet level from A to T. Since the thymine emission disappears in fully mercurated poly(dA-dT), we must assume that an insignificant amount of energy finds its way back into the thymine triplet state. Therefore, one would conclude that either the quenching of the adenine fluorescence proceeds via internal conversion ($S^1 \rightarrow S^\circ$), or else triplet transfer from A to T cannot compete with the mercury-enhanced triplet-to-ground state transition rate in the mercurated adenine residues. Further studies are needed to determine accurately the relative positions of the energy levels in the mercurated polynucleotides, especially those with weak emission, if we are to have a clearer picture of the quenching mechanism in mercurated DNA.

We note that quenching of both the phosphorescence and the rate of dimerization, as a function of r, follow parallel curves, i.e., begin to decrease at roughly the same value of r. Our studies on TpT and poly dT have shown that Hg(II), when bound to the thymidyl residues in these systems, does not decrease the quantum yield of dimerization at 25°C. Hence, it is reasonable that when Hg(II) is bound only to the thymine residues in DNA there should be no quenching of dimerization. Binding of Hg(II) to other residues at higher r values may, as already noted, create traps which quench the thymine-excited states, and cause a decrease in the rate of dimerization.

We have also observed that the spore photoproduct obtained by irradiating DNA at −80°C[18] is also formed at a much slower rate when Hg(II) is bound to the DNA. Thus the quenching of photoproduct formation in DNA is not confined to thymine dimerization and probably involves some excitated-state precursor which these products have in common, such as the excited singlet state.

We thank W. L. Carrier for the gift of labeled *E. coli* DNA, F. J. Bollum for the gift of poly dT, and J. K. Setlow for the gift of *H. influenzae* DNA.

Abbreviation: r, ratio of moles of Hg(II) present per mole of base monomer.

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