A Mercuric Complex of Guanosine 5′-Monophosphate*

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ABSTRACT: Upon addition of mercuric ion to guanosine 5′-phosphate at pH 5.7 and a ratio of 1 or 2 moles of Hg per mole of nucleotide, a strong absorption band appears at a wavelength of 220 mμ.

The addition greatly shifts both the 252.5-mμ maximum and the 223-mμ minimum of the absorption spectrum of guanosine monophosphate to longer wavelengths, and also suppresses the maximum. Evidence is presented that the 220-mμ absorption band is exhibited by one molecular complex of guanosine monophosphate and mercury at a unimolecular ratio of the two components. Formation of the complex requires the amino and phosphate groups of the guanosine monophosphate molecule with the amino group uncharged and the secondary phosphate group in the acid form. Mercury must be the divalent cation. The formation also shows specificity with respect to the locations of both amino and phosphate groups. A probable molecular model for the complex is proposed.

Since Katz's discovery (1952) of reversible profound structural change of DNA produced by $HgCl_2$, there has been considerable work on the structures of complexes of Hg^{2+} ion with nucleic acids and nucleosides. Inorganic mercuric ion has been reported to bind to guanosine and, hence, to alter the absorption bands of the nucleoside near 260 mμ (Yamane and Davidson, 1961; Eichorn and Clark, 1963). It has been proposed that one Hg^{2+} ion complexes with two guanosine molecules by forming two colinear bonds with either the N_1 or the O_6 site on the base or by assuming a tetrahedral configuration with bonds to both O_6 and N_7 (Eichorn and Clark, 1963). This paper reports a strong absorption band at 220 mμ which is displayed rather specifically by a mercuric complex of GMP at a unimolecular ratio of the two components. Formation of the complex requires the amino group of the guanine moiety and the phosphate group of GMP with considerable degrees of specificity. A structure is proposed for the complex.

Materials and Methods

Guanine, guanosine, nucleotides, GDP, and GTP were obtained from the P-L Biochemicals; according to the manufacturer they were chromatographically pure. Their absorption characteristics were found to conform with the data in the manual of the company (Pabst Laboratory, 1967). Ribose 5′-phosphate was manufactured by California Biochemical Corp. Mercuric acetate, methylmercuric chloride, and propylmercuric chloride were manufactured by Merck & Co.

Unless specified otherwise, a reaction between GMP (or a GMP-related compound) and Hg^{2+} was carried out at pH 5.7, around 25° with the concentrations of the nucleotide and mercurial equal to 1×10^{-4} and 2×10^{-4} M, respectively. The medium was buffered with 0.01 M sodium acetate, or in many cases with 0.01 M sodium acetate–0.1 M sodium perchlorate. Sodium acetate at this concentration was chosen as buffer because it produces no spectral shift of the $HgCl_2$ spectrum (Yamane and Davidson, 1961). The molar concentration of GMP was determined from the optical density at the maximum near 260 mμ using the extinction coefficients given in the manual of P-L Biochemicals (Pabst Laboratory, 1967). For convenience, the inorganic mercuric ion was added in the form of chloride in most cases, although the same alterations in the ultraviolet absorption of GMP were observed when it was added as either acetate or nitrate. All measurements of the ultraviolet absorption spectra reported were made with a Cary 15 dual-beam recording spectrometer with nitrogen flushing to remove oxygen from the light path. Samples of GMP and its medium, with and without Hg^{2+}, were placed in a pair of matched cells of 5-mm path length. Cells of shorter and longer path lengths were employed for samples with higher and lower GMP concentrations, respectively.

Results

Figure 1 shows two alterations in the ultraviolet absorption spectrum of GMP produced by $Hg(Ac)_2$: first, the suppression of the 252.5-mμ maximum and the wavelength shifts of both the maximum and 223-mμ minimum to longer wavelengths, and second, the appearance of a new outstanding peak at 220 mμ. Both alterations appear to be instantaneous, since indistinguishable spectra were obtained at 5 min and at 30 min after the addition of $Hg(Ac)_2$. Qualitatively similar alterations in the maximum and minimum produced by mercuric ion were reported previously with guanosine under different experimental conditions (Yamane and Davidson, 1961; Eichorn and Clark, 1963). The 220-mμ absorption band has not been reported previously for any mercuric complex of nucleotide or nucleoside under any circumstance. Our attention has been focused on the molecular basis for the absorption band.

As shown below, mercuric ions were bound to GMP to a great extent. Thus, it appeared possible that the 220-mμ peak reflected the presence of a certain GMP-free mercuric complex with a strong minimum at that wavelength, whose

* From the Bio-Medical Division, Lawrence Radiation Laboratory, University of California, Livermore, California 94550. *Received April 26, 1969.* This work was performed under the auspices of the U. S. Atomic Energy Commission.

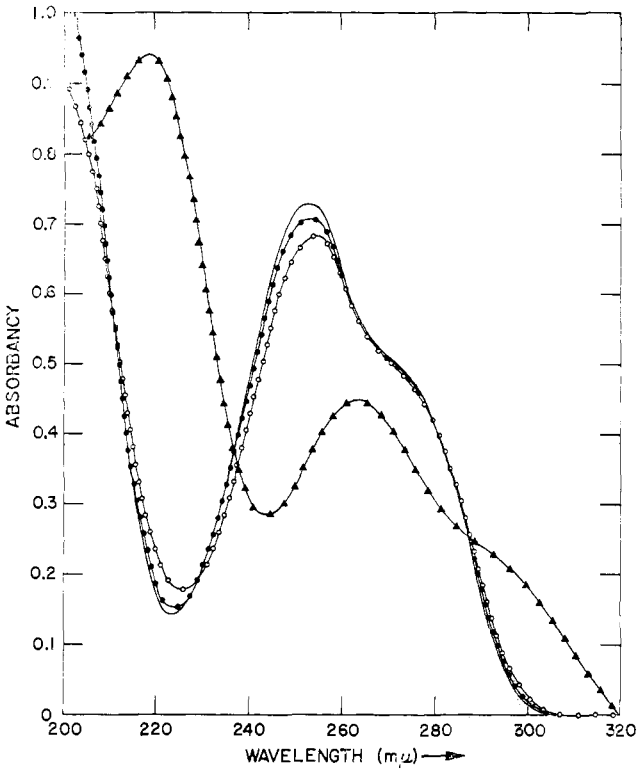

FIGURE 1: Effects of $Hg(Ac)_2$ on ultraviolet absorption spectra of GMP, guanosine, and guanine at 25°. (—) GMP alone, (▲—▲) GMP + $Hg(Ac)_2$, (O—O) guanosine + $Hg(Ac)_2$, and (●—●) guanine + $Hg(Ac)_2$. Concentrations: GMP, guanosine, and guanine, 1.0×10^{-4} M; Hg^{2+}, 2.0×10^{-4} M in all cases except for "GMP alone." The medium was 0.01 M sodium acetate (pH 5.7).

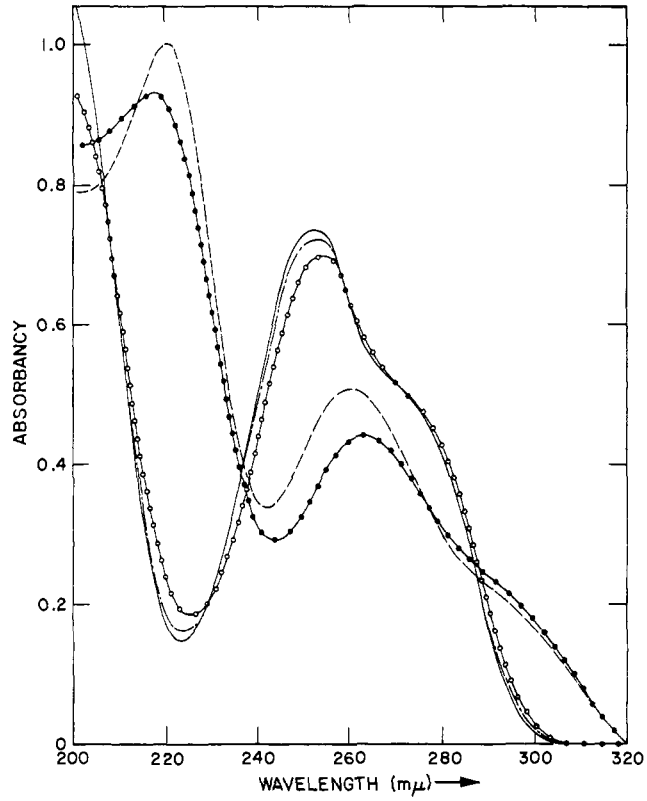

FIGURE 2: Effects of various mercurials on ultraviolet absorption spectrum of GMP. (—) GMP alone, (- — -) GMP + CH_3HgAc, (O—O) GMP + C_3H_7HgCl, (---) GMP + $Hg(Ac)_2$, and (●—●) GMP + $HgCl_2$. The mercurials were added to a final concentration of $2 \times Hg^{2+}$ of 10^{-4} M. The other conditions were the same as for Figure 1.

concentration was decreased by the binding which supposedly suppressed the minimum. However, the possibility was ruled out by several observations. For example, the optical density of a pH 5.7 solution of 1×10^{-4} M Hg^{2+} and 0.01 M sodium acetate decreases steadily from 0.14 at 218 mμ to 0.074 at 222 mμ. These absorbancy values are only a small fraction of the optical density (1.99) observed with such a solution in the presence of 1×10^{-4} M GMP at 220 mμ. Besides, essentially the same peak (except that the position was red shifted by 1 mμ) was observed, when the medium contained 0.1 M sodium perchlorate in addition to 0.01 M sodium acetate. Hg^{2+} would now exist mainly as complexes of perchlorate, were it inert to GMP. That is, the mercuric complex would be different from any mercuric ion species possibly present in the acetate buffer without perchlorate. Hence, observation does not support the possibility.

Anion Dependence. To determine the active form of mercuric ion for producing the observed effect, the influence of anions was further studied at 1×10^{-4} M GMP and 1×10^{-4} M Hg^{2+} with the following results. (1) A peak at 220 mμ of about 1.84 optical density units was observed for all the solutions containing only acetate at 4×10^{-4}–1×10^{-2} M, but not for solutions containing only chloride or perchlorate. (2) A peak at the same position with a similar magnitude was also observed when Hg^{2+} was added as $Hg(NO_3)_2$ to a solution of GMP in water and the resulting solution was then brought to pH 5.7 with NaOH. Thus, the active form of Hg^{2+} is a simple divalent inorganic ion and not a complex of acetate, chloride, or perchlorate.

Since both chloride and perchlorate ions form strong mercuric complexes, at sufficiently high concentrations they should inhibit the mercuric effects. This was found indeed to be the case. For a solution of 1×10^{-4} M GMP and 2×10^{-4} M Hg^{2+} in 0.01 M sodium acetate, chloride ion at concentrations of 8×10^{-4} and 1×10^{-3} M reduced the mercuric effects to levels approximately equivalent to those produced by Hg^{2+} concentrations of 1×10^{-4} and 0.5×10^{-4} M, respectively. The mercuric effects were completely abolished at chloride concentrations above 2×10^{-3} M. Moreover, at all these three levels of chloride concentration, the reduction in the peak height at 220 mμ was parallel to the inhibition of the mercuric ion induced alteration of the absorption maximum and minimum of GMP. The inhibition by perchlorate ion was found to be of a similar nature, with effective concentrations considerably higher than those of chloride. These results are consistent with the interpretation that chloride or perchlorate exerts its inhibition by reducing Hg^{2+} concentrations.

Methylmercuric Acetate and Propylmercuric Chloride. Figure 2 shows that unlike Hg^{2+}, these two monovalent organic mercurials at concentrations of two moles of mercurial per mole of GMP had rather small effects on the ultraviolet absorption of GMP. Propylmercuric chloride shifted the maximum and minimum of GMP to longer wavelengths

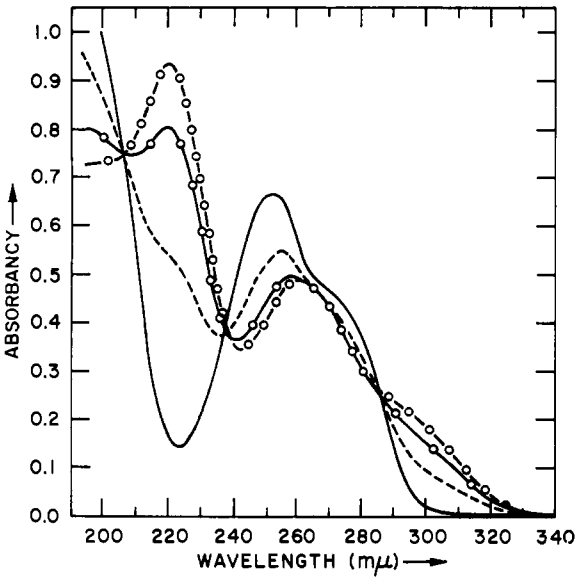

FIGURE 3: Effect of $Hg(Ac)_2$ on ultraviolet absorption spectrum of GMP at various molar concentration ratios of Hg^{2+} to GMP. (—) 0, (- - -) ratio 0.5, (O—O) ratio 1.0, and (O--O) ratio 2.0. The concentrations of GMP were 1.0×10^{-4} M. The temperature and the medium were the same as for Figure 1.

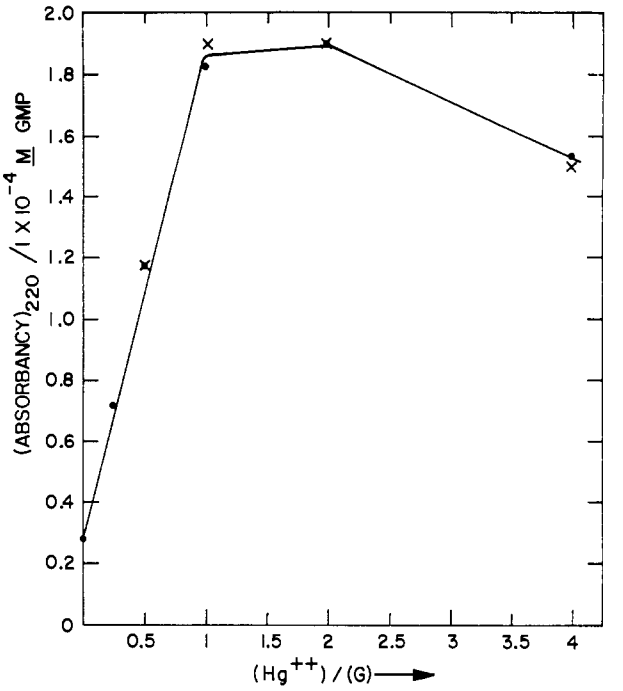

FIGURE 4: Dependence of absorbancy at 220 mμ upon the molar concentration ratio of Hg^{2+} to GMP. (●) GMP concentration maintained at 1×10^{-4} M and (×) Hg^{2+} concentration maintained at 2×10^{-4} M. The other conditions were the same as for Figure 1.

by 3 and 2 mμ, respectively. It also slightly suppressed the maximum. Methylmercuric acetate produced similar but even smaller effects. No peak was discernible between 210 and 250 mμ in either case.

Concentration of Mercuric Ion. For brevity, we shall follow the usual practice of denoting the number of moles of Hg^{2+} added per mole of GMP as r. Decreasing r from 2 to 0.5 diminished the positional shifts of both the maximum and minimum to longer wavelengths accordingly (Figure 3). However, the decrease reduced the height of the 220-mμ peak without a positional shift. These results indicate that the peak was exhibited by a single GMP–Hg complex. The observation that the absorption spectra for all r values in this range exhibit isosbestic points at 207 mμ gives the same indication.[1]

In considering the molecular basis for the 220-mμ peak, it is sufficient to regard a population of GMP molecules with Hg^{2+} as consisting of only two species: one, designated as G*, is the GMP–Hg complex exhibiting the 220-mμ peak. The other species exhibits no peak at 220 mμ; this will include unbound GMP, G, as well as the other GMP–Hg complex, if it exists. For $0 < r < 1$, the absorbancy at 220 mμ/mole of GMP added, A, varied linearly with r

$$A = (3.3 \times 10^3) + (1.50 \times 10^4)r \qquad (1)$$

The same linear dependence was observed when the concentration of either GMP or Hg^{2+} was kept constant (Figure 4).

[1] At $r = 4$, the spectrum did not pass the isosbestic point and showed no peak between 210 and 240 mμ. However, both the maximum and minimum were altered in the manners described above and to a degree about twice that observed at $r = 2$. These data indicate that the GMP–Hg complex at $r = 4$ differed from that at $r \leq 2$ and will not concern us here.

For a given r value, the concentrations added of GMP, and also Hg^{2+}, in these two sets of experimental data differed in general, and by a factor as high as 4 in the extreme case. Hence the identity in linear dependence indicates that the reaction forming G* from GMP and Hg^{2+} was strongly in favor of G*. It follows that

$$A = (3.3 \times 10^3) + [A^* - (3.3 \times 10^3)]r \qquad (2)$$

where A^*_{220} is the molar absorbancy at 220 mμ of G*. Upon comparing eq 1 and 2, one obtains 1.83×10^4 for A^*. This value is consistent with the finding that A had an elevated plateau at 1.85×10^4 for r values from 1 to 4. That the plateau was reached at $r = 1$ indicates that one Hg^{2+} atom is bound to one GMP molecule in the complex.

The following results on the functional groups of the complex displaying the 220-mμ peak were obtained with the molar concentration ratio of mercury to GMP or GMP-related compound equal to 2.

Guanine Moiety. Since the peak was not observed when GMP was replaced by ribose 5′-phosphate, guanine moiety is evidently involved in the complex formation. The replacement of GMP with another nucleoside 5′-phosphate such as UMP, AMP, CMP, IMP, and XMP also failed to display the peak (Figure 5), indicating base specificity.

It may be noted that the GMP molecule differs from IMP and XMP only in the group attached to the C_2 of the purine base: NH_2 for GMP, H for IMP, and OH for XMP. Thus, the amino group is required and cannot be replaced by either the H or the OH group. That neither adenine nor cytosine, which contain an amino group, can replace guanine indicates

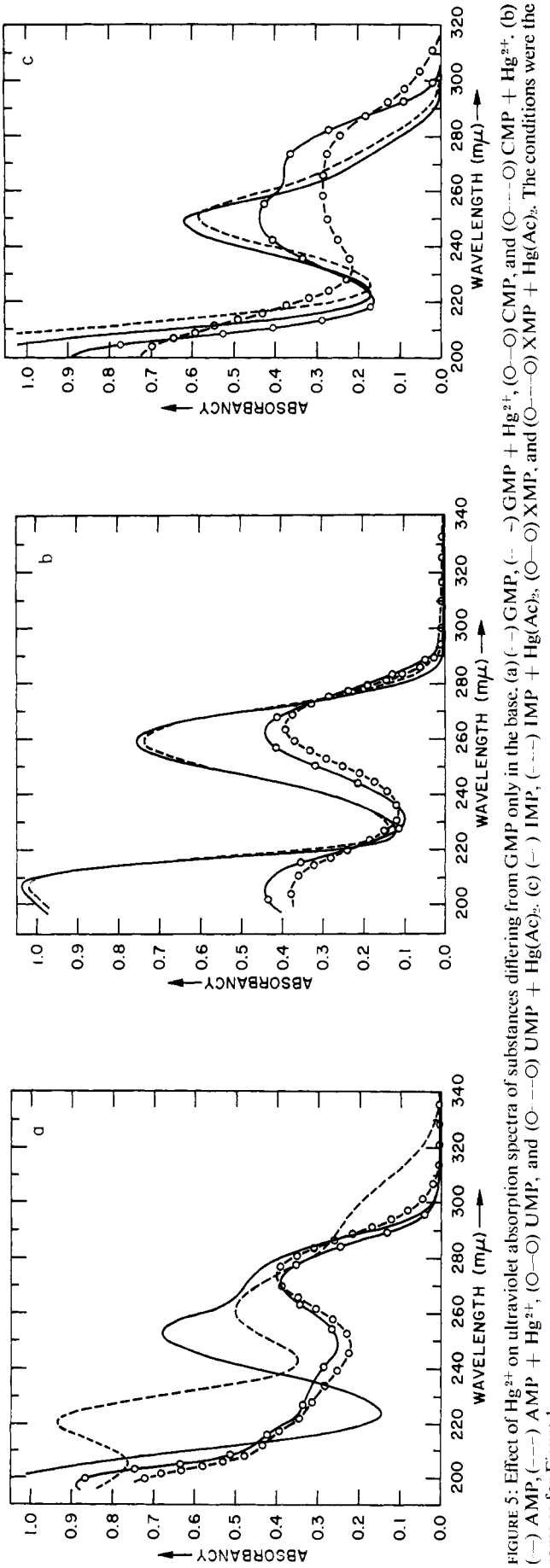

FIGURE 5: Effect of Hg^{2+} on ultraviolet absorption spectra of substances differing from GMP only in the base. (a) (– –) GMP, (– –) GMP + Hg^{2+}, (O—O) CMP, and (O– –O) CMP + Hg^{2+}. (b) (– –) AMP, (– –) AMP + Hg^{2+}, (O—O) UMP, and (O– –O) UMP + $Hg(Ac)_2$. (c) (– –) IMP, (– –) IMP + $Hg(Ac)_2$, (O—O) XMP, and (O– –O) XMP + $Hg(Ac)_2$. The conditions were the same as for Figure 1.

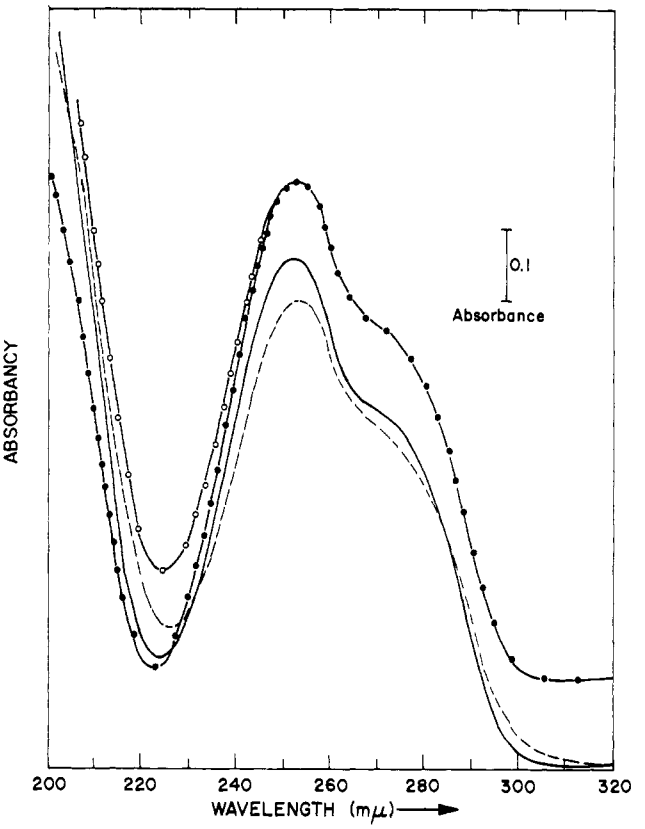

FIGURE 6: Effect of Hg^{2+} on ultraviolet absorption spectra of (a) guanosine + P_i and (b) guanine + ribose 5′-phosphate. (—) a, (– – –) a + Hg^{2+}, (●) b, and (O) b + Hg^{2+}. The concentrations of guanosine, P_i, guanine, and ribose phosphate were 1.0×10^{-4} M. The concentrations of Hg^{2+} were either 0 or 2.0×10^{-4} M. The medium was the same as for Figure 2.

some specificity of the ring to which the amino group is attached.

To further test the requirement for amino group, the amino group of GMP was masked by incubating the nucleotide at 1×10^{-2} M with 1% formaldehyde in 0.001 M potassium phosphate, pH 6.8, for 22 hr at 37° (Fraenkel-Conrat, 1954). The reaction mixture was then diluted 100-fold into the perchlorate–acetate buffer containing 2×10^{-4} M $HgCl_2$. The ultraviolet absorption spectrum of such a system gave no peak around 220 mμ. On the other hand, a peak similar to that observed with GMP without formaldehyde was exhibited by the control, which differed from the above system only in that the formaldehyde was not mixed with GMP until $HgCl_2$ was added. These results further demonstrate the requirement for the amino group. They are in contrast to the reported mercuration of guanosine under different experimental conditions, which was not blocked by the formaldehyde treatment (Eichorn and Clark, 1963).

Phosphate Group. Figures 1 and 6 show that the role of GMP in the complex was irreplaceable by either guanine, guanosine, guanine with ribose 5′-phosphate, or guanosine with P_i. Thus, the formation of complex requires the presence of phosphate as well as the guanine moiety. Moreover, the base and phosphate must be on one and the same molecule.

There is also specificity with respect to the position of the phosphate group on the GMP molecule. GMP with the

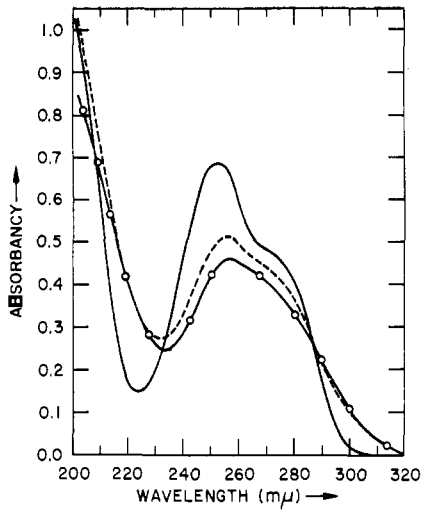

FIGURE 7: Effect of Hg^{2+} on ultraviolet absorption spectrum of 2′(3′)-GMP. (—) 2′(3′)-GMP, 1.0×10^{-4} M; (– – –) 2′(3′)-GMP, $1.0 \times 10^{-4} + 2.0 \times 10^{-4}$ M Hg^{2+}; (○) 2′(3′)-GMP, $1.0 \times 10^{-4} + 6.0 \times 10^{-4}$ M Hg^{2+}. The medium and the temperature were the same as for Figure 2.

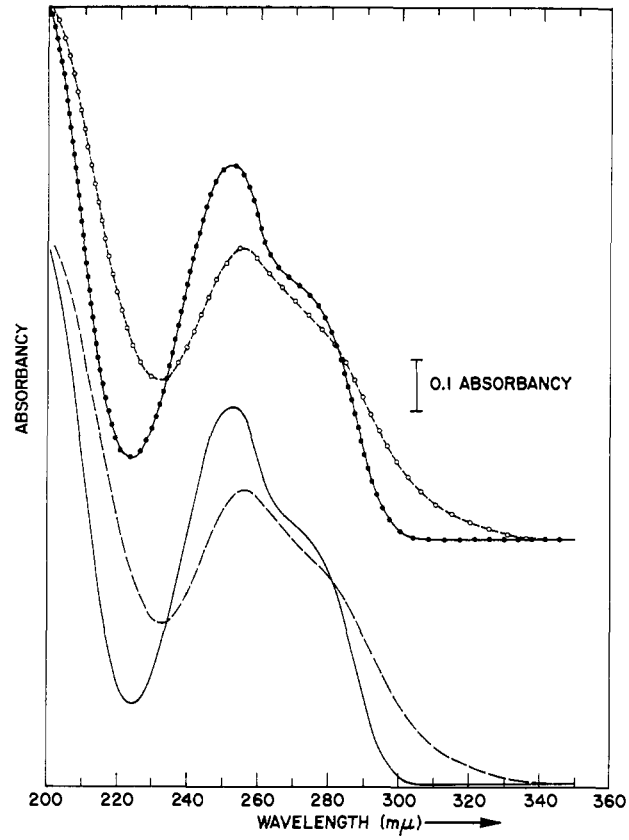

FIGURE 8: Effect of Hg^{2+} on ultraviolet absorption spectra of GDP and GTP. (●) GDP, (○) GDP + Hg^{2+}, (—) GTP, and (– – – –) GTP + Hg^{2+}. The concentrations of GDP and GTP were 1.0×10^{-4} M. Concentrations of Hg^{2+} were either 0 or 2.0×10^{-4} M. The medium and the temperature were the same as for Figure 2.

phosphate group at C_2' or C_3', instead of C_5' of the ribose exhibited no peak at all from 210 to 230 mμ with $Hg(Ac)_2$ (Figure 7). Neither did GDP nor GTP (Figure 8). As evident from the binding with manganous ion, the terminal phosphate of GDP or GTP has a greater affinity for certain metallic ions than does the phosphate group at C_5'. It should be noted that mercury did modify the maximum of ultraviolet absorption of 2′(3′)-GMP, GDP, or GTP, although in a manner different from that with GMP. At $r = 2$, mercury shifted the maximum and minimum of 2′(3′)-GMP, GDP, or GTP to longer wavelengths by 3–3.5 and 9–11 Å, respectively. It also suppressed the maximum by 24.8 to 26.6%. However, a similar positional shift of the maximum of GMP was observed at $r = 0.75$, with a 16-Å shift of the minimum and 21% suppression of the maximum. In addition, mercuration of GMP at $r = 0.75$ gave a distinct peak at 220 mμ with an absorbance of 1.45. These results indicate that 2′(3′)-GMP, GDP, and GTP were mercurated at sites different from that with GMP.

Replacement of D_2O for water in the reacting system of GMP with mercuric ion produced no significant alteration in the entire ultraviolet absorption spectrum from 210 to 320 mμ. Thus, deuteration of the amino or phosphate group of GMP does not affect the 220-mμ peak.

Ribose Moiety. It has been proposed that the C_2'-hydroxyl group forms a hydrogen bond with a phosphate–oxygen (Spencer *et al.*, 1962; Langridge and Gamatos, 1963) or N_3 of purine (T'so *et al.*, 1966). However, the involvement of the hydrogen atom in the GMP–Hg complex has been ruled out by the finding that Hg^{2+} with deoxyguanosine 5′-phosphate displayed the same ultraviolet absorption spectrum as with GMP (Figure 9). The observation that Hg^{2+} caused practically no change in the ultraviolet spectrum of either guanosine or ribose 5′-phosphate under the same experimental conditions is consistent with the interpretation that an Hg^{2+} bridge between the ribose moiety and guanine or phosphate is not responsible for any of the observed alterations of the absorption spectrum of GMP produced by Hg^{2+}.

pH Dependence. Since the amino and phosphate groups of GMP are required for the formation of the mercuric complex, the pH dependence of the reaction was studied at $r = 1$ to determine the active forms of these two groups. It was found that the differential optical density at 220 mμ of GMP with mercuric nitrate or mercuric chloride over that of GMP without mercuric ion decreases by 50% or more when the pH was changed from 5.7 to either 6.6 or 5.2. Moreover, the wavelength of the maximum of the differential optical density curve increases with the pH.

Since the amino group of GMP has a pK' value of 2.4, the uncharged state is undoubtedly the active form for the GMP–Hg complex at pH 5.7. This finding renders unacceptable those molecular structures of the complex that require the amino group to be in a positively charged state, including the one involving electrostatic interaction between the amino and phosphate groups.

The pK' values of the secondary phosphate of GMP are estimated to be about 6.1 and 6.3 at ionic strengths around 0.1 and ≤ 0.01 μ, respectively.[2] Thus, at pH 5.7, 72 and 80%

[2] The estimation was made as follows: Pabst Laboratory (1967) gives the pK' in 0.1 M NaCl as 6.1. However, the pK' values in tetra-*n*-propylammonium ion were reported to be 6.51 and 6.49 at 0.01 and

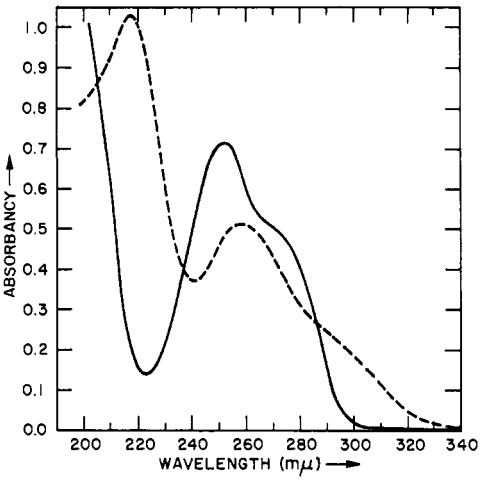

FIGURE 9: Effect of Hg^{2+} on ultraviolet absorption spectrum of dGMP. (—) dGMP and (– – –) dGMP + Hg^{2+}. Concentrations of dGMP and Hg^{2+} were 1.0×10^{-4} and 2.0×10^{-4} M, respectively.

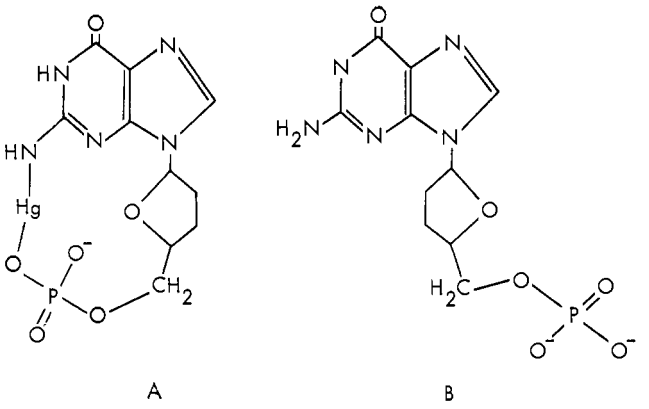

FIGURE 10: Structures of GMP and mercuric complex of GMP's at pH 5.7 and $r < 2$. (A) The mercury complex, in *syn* conformation and (B) GMP in *anti* conformation.

of the secondary phosphate group exists in acid form in these two corresponding regions of ionic strength. It was found that the concentration of GMP–Hg complex at $r = 1$, as measured by the optical density at 220 mμ, decreased by 15% on increasing the ionic strength from <0.01 to 0.1 μ. These data are compatible with participation of the acid form of the secondary phosphate group in the mercury complex at pH 5.7.

Molecular Structure. The present experimental results indicate that the molecule exhibiting the 220-mμ peak has the following structural properties: (a) The ratio of GMP to mercuric ion is unity. (b) The amino group on the base and the secondary phosphate group of GMP are required, with the former uncharged and the latter in the acid form. (c) The mercury must be divalent inorganic ion. These properties are consistent with a structure having a mercuric bond between the amino and the phosphate groups of GMP (Figure 10A). From the molecular model study with Coutauld atomic models, it was found that such a structure is indeed sterically feasible. Besides, the two linkages from Hg can be perfectly colinear, in agreement with the fact that the atom often forms two colinear bonds.

From the molecular models, it was also found that no mercury bond is feasible between the amino and phosphate groups of either CMP or AMP. This conclusion is in complete agreement with the aforementioned absence of the 220-mμ peak in the absorption spectra of the Hg^{2+} complexes of these nucleotides. This agreement further strengthens the view that Figure 10A is a reasonable model for the GMP–Hg complex. Such a molecular structure differs markedly from that in Figure 10B, the conformation for 5′-deoxyguanylyl residue in a double-stranded DNA, in the spatial relationships among the moieties of base, ribose, and phosphate. For example, although the mercuric complex allows the ribose and sugar planes to be perpendicular to each other, it does not require this as does the conformation in the double-stranded structure (Donohue and Trueblood, 1960). Second, Figure 10A is in the *syn* conformation, whereas Figure 10B is in the *anti* conformation. Although the proton magnetic resonance data have been interpreted to indicate the presence in solution of 5′-nucleotides (Danyluk and Hruska, 1968; Schweizer *et al.*, 1968) and 3′→5′-dinucleotides (Ts'o *et al.*, 1969) in *anti* conformation, 5′-deoxyguanosine is *syn* in the crystal of 5′-bromodeoxycytidine–deoxyguanosine complex—the only guanosine-containing crystal has been studied (Haschemyer and Rich, 1967). A perfect colinear mercury bridge between the amino-nitrogen and the phosphate-oxygen can also be formed in guanosine 3′-phosphate, but not in guanosine 2′-phosphate. However, the complexes with guanosine 2′-phosphate and with guanosine 3′-phosphate are in *anti* conformation. It remains to be seen how this conformational difference contributes to the observed difference between guanosine 3′-phosphate and guanosine 5′-phosphate in the 220-mμ absorption band.

Discussion

The basic site for guanosine has been identified as N_7 (Sobell and Tomita, 1964; Miles *et al.*, 1963; Jardetzky and Jardetzky, 1960), and the site of guanosine for mercury under different conditions was proposed to be either N_1 or O_6 (Eichorn and Clark, 1963). In contrast to these reports, the present structure involves mercuration of GMP solely at the site of the amino group. The difference in binding site is undoubtedly due to some differences in experimental conditions. Data in the literature have indicated that the binding site could vary with the kind of cation. As suggested by the results presented above, the binding site for Hg could vary from guanosine to GMP, and with pH or r, etc.

Any reasonable model for the mercuric complex of GMP should provide a satisfactory structural basis for the observed alterations of ultraviolet absorption spectrum, *i.e.*, the suppression of the maximum and the shifts to longer wavelengths of the maximum and minimum. The alterations result from a disturbance of the conjugated π-electron system of the

0.11 ionic strength, respectively (Phillips *et al.*, 1965). Since the presence of 0.2 M Na^+ lowers the pK' value of the terminal phosphate groups of AMP, ADP, and ATP from those obtained with tetra-*n*-propylammonium ion by 0.16, 0.32, and 0.54, respectively (Smith and Alberty, 1956), the pK' values of GMP in 0.1 and ≤0.01 M Na^+ are estimated to be around 6.3 and 6.5, respectively. Using the same correction for Na^+ concentration the pK' in ≤0.01 M NaCl is estimated to be around 6.3 in accordance with the data of P-L Biochemicals.

guanine moiety. Since the amino nitrogen is not a part of the purine ring, one may wonder whether such mercuration can cause the observed alterations at the maximum and the minimum.

The ultraviolet absorption study of the methylation of guanosine reveals that complete methylation of the amino group shifts the absorption maximum by 9 mμ at pH 1 and 4 mμ at pH 13, respectively, whereas methylation at N-1 guanosine shifts the maximum by 2 mμ at both pH values (Smith and Dunn, 1959). These results definitely indicate that a modification at the amino group could disturb the conjugated π-electron systems. Smith did not report the effects of methylation on the minimum and the bands around 190 mμ (Voet *et al.*, 1963). However, such effects are expected to exist, in general, in view of the suggestions of Mason (1954): the principal transition of the bands at the absorption maximum is being polarized in the direction of the molecular axis, while the main band near 190 mμ may be presumed to be polarized essentially at right angles to the above direction. Accordingly, since the amino group at C-2 is disposed at an angle of about 30° to the molecular axis, one would expect that any shift of the maximum to a longer wavelength by modification of the amino group will, in general, be accompanied by a similar shift of the bands near 190 mμ, and hence also of the minimum. In the present Hg–GMP complex the positive charge on the Hg atom should also contribute to the disturbance of the conjugated double-bond systems. Hence, qualitative considerations indicate that the proposed structure can account for the ultraviolet absorption data.

Mercuration of the amino group could alter the π-electron system on the guanine base by another and indirect mechanism–through the induction of mercuration at some ring site such as N_1 or even a distant site such as N_7. Such an induction may appear strange, but there is supporting experimental evidence. For example, the presence of mercury at the N_1 of adenosine or N_3 of cytosine has been reported to promote the mercuration at the amino group. The mercuration at N_1 and at N_7 of inosine mutually enhance each other (Simpson, 1964). However, it is sterically not feasible to have the mercury atom that forms a bridge between the amino and phosphate groups of GMP also bond any atom on the ring, considering that the ligands to the mercury have a tetrahedral configuration. Hence, such a mechanism is not favored.

It may be interesting to note that several lines of the foregoing experimental evidence can also be well interpreted by another structure with two mercuric atoms between two GMP molecules. Such a structure would still have the unimolar ratio between mercury and GMP. Each mercury atom forms a bridge between the amino group of one GMP molecule and the N_1 of another GMP. In this case, we can consider that the induction effect discussed in the preceding section is operative, and it is immaterial whether the mercuration at N_1 is enhanced by the prior mercuration at NH_2 or *vice versa*. In addition, the coordination bonds of mercury would still be colinear, a known stable configuration. However, a dimer structure of this type does not provide an obvious explanation for the observed positional specificity with respect to the amino and phosphate groups.

Acknowledgment

We thank Mr. Howard Alford of our Graphic Arts Division for his assistance in the study with the molecular models.

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