Interaction of Aquated *cis*-$(NH_3)_2Pt^{II}$ with Homopolynucleotides[1]

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Abstract: The binding of aquated *cis*-$(NH_3)_2Pt^{II}$ to polyriboadenylic acid, polyribocytidylic acid, and polyriboguanylic acid has been studied at pH 7, 37 °C, and an ionic strength of 0.1. The total number of binding sites per nucleotide in each polynucleotide is one; the magnitude of the intrinsic binding constant increases in the order poly(C) < poly(A) < poly(G). The CPD binding profiles for poly(A) and poly(C) are similar, displaying a noncooperative behavior, while the binding profile for poly(G) is more complex and may be described by a number of alternative binding models. The data are consistent with the model in which the CPD binds very strongly to the four-stranded poly(G) aggregate up to $\bar{r}$ values of 0.25, at which point the aggregate is disrupted and the subsequent binding affinity, perhaps to the same or a different site, is greatly reduced. This is the first binding data which supports the four-stranded model for poly(G) in solution. The binding affinity of CPD to homopolynucleotides is greater than to the corresponding nucleosides. The results of these studies are compared and discussed relative to similar investigations reported for other metal ions binding to nucleic acids.

Introduction

The interaction of ions and small molecules with biomacromolecules has a fundamental influence on their structure, conformation, and therefore biological activity. It is well known that the conformation of a nucleic acid is especially sensitive to protonation, metalation, salt concentration in the medium, and the binding of a variety of small molecules. Clearly in the case of many drug-nucleic acid interactions, the structure and/or local conformational change resulting from the binding perturbation may also alter the replication, transcription, and/or translation process.[2-6]

Although many studies have focused on a variety of aspects related to the binding of moieties to nucleic acids,[7-10] this investigation will be concerned predominantly with a quantitative expression of the binding process. Previous studies of electrostatic metal ion binding to nucleic acids are extensive;[11-13] however, the literature is considerably smaller on the reversible covalent binding of metal ions or metal complexes to the bases in the nucleotide units, with detailed investigations reported on only Ag(I), Hg(II), CH_3Hg^{II}, and Cu(II).[14-16] Silver ion is known to bind reversibly to homopolynucleotides, with proton liberation occurring above pH 5 in the case of poly(U) and poly(I), while no deprotonation occurs for poly(A) or poly(C).[17] Daune et al.[9] have also investigated the quantitative binding of Ag(I) to homopolynucleotides and a number of DNA samples with different % G + C content. The binding affinity to the homopolynucleotides is high ($K \approx 10^5-10^7$) and exhibits a relatively strong cooperative binding interaction. The binding affinity of Ag(I) to poly(A) is decreased with increasing concentration of phosphate buffer and binding to oligo(A) species is influenced by the chain length.

Two consecutive reactions occur with DNA which are classified according to the value of $\bar{v}$, the ratio of [Ag(I) bound]/[nucleotide]. Yamane and Davidson[18] suggest that the first complex ($0 < \bar{v} < 0.2$) involves binding of Ag(I) to N-7 or N-3 of the purine, while in the second complex ($0.2 < \bar{v} < 0.5$), Ag(I) adds to the remaining base pairs. The magnitude of the binding constant for the first complex (K_1) in the different DNA samples is ca. 3×10^6, while the value of the binding constant for the second interaction (K_2) ranges from ca. 10^4 to 10^5. In both interactions the binding exhibits a characteristically cooperative behavior. The Hg(II)-DNA binding studies yield similar results, again with large K values, cooperative binding, and the suggestion that the metal cross-links between the strands. The binding affinity of CH_3Hg^{II} to DNA is considerably weaker with the binding occurring predominantly to denatured DNA with no cross-linking of the chain.[19] Miller and Bach[20] have used polarographic techniques to determine the extent of Cu(II) and Cd(II) binding to both native and denatured DNA at zero potential. The value of this binding constant, K^0, for Cd(II)-DNA binding is ca. 200 and 80 M^{-1} for denatured and native DNA, respectively, while the K^0 values are ca. 1500 M^{-1} for the Cu(II) interaction to either DNA form. In addition, the binding interaction decreases with an increasing degree of binding, caused by the reduction in the electrostatic potential on the DNA molecule.

The binding of *cis*-$(NH_3)_2PtCl_2$ to nucleic acids is of interest on its own merits since it is now established that this inorganic coordination complex is an effective antineoplastic agent.[21] The binding is known to selectively inhibit DNA synthesis in mammalian cells, both in vitro and in vivo.[22-24] Studies have suggested that the *cis*-$(NH_3)_2PtCl_2$ acts as a pro-drug and that the active form may well be the aquated *cis*-$(NH_3)_2Pt^{II}$.[23,25] Both of these forms of *cis*-$(NH_3)_2Pt^{II}$ interact effectively with only guanosine, adenosine, and cytidine on the nucleoside level.[26,27] The extent of interaction observed in the equilibrium formation of the 1:1 complexes is indicated by the log K values of 3.7, 3.6, and 3.5, respectively.[27] Chu et al.[28] and Mansy et al.[29] have presented Raman data which indicate that at high (nucleotide (side))/*cis*-$(NH_3)_2$-$Pt(OH_2)_2$ 2+ ratios, the anionic 2:1 *cis*-$(NH_3)_2PtL_2{}^{2-}$ complex is the major species in solution, when L = 5′-GMP^{2-} or 5′-AMP^{2-} and the neutral 2:1 complex when L = Cyd. Reaction with uridine does occur, but is significantly slower, and if the reactions are carried out below 7 and in an oxygen atmosphere, a platinum-uracil "blue" is produced.[30] Although no quantitative binding profiles have been carried out for CPD interacting with the homopolynucleotides, Wherland et al.[31] report that the reaction of the *cis*- or the *trans*-$(NH_3)_2PtCl_2$ with poly(A) in unbuffered solution causes precipitation to occur at a 2/1 mole ratio of poly(A) to either platinum complex. Similarly for poly(dA), poly(C), poly(I), and poly(G), precipitation occurs at a mole ratio of 1.0. In addition, Chu et al.[28] report that the Raman spectrum of the mixture CPD with poly(G) at an r value of 0.60 reveals no significant changes from that of the spectrum for poly(G) itself. An unusually high frequency for ν(Pt-N) provides the basis for the authors to suggest, however, that the ammine groups are strongly hydrogen bonded to the polynucleotide.

Munchausen and Rahn[32] report that the saturation r value for *cis*-$(NH_3)_2PtCl_2$ binding to poly(C), poly(A), and poly(G) is 0.4-0.5 and point out that this is consistent with each platinum coordinating to two adjacent bases.

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Studies to date on the *cis*-$(NH_3)_2PtCl_2$ saturation binding to DNA samples from different sources yield conflicting results. Munchausen and Rahn[32] report that saturation in DNA is linearly related to the % (G + C) content, with r at saturation ranging from 0.1 to 0.2. Horacek and Drobnik,[33] however, obtained an r value of 0.4. The use of different solution conditions, in addition to perhaps obtaining measurements before equilibrium had been established, may explain, in part, these discordant results.

Macquet and Theophanides[34] indicate that the interaction of a number of platinum salts with DNA is complex and suggest that at least three types of reactions are occurring. The first interaction involves platinum binding to guanine at N-7 with no proton release. A second interaction involves a more complex binding with a proton liberated in the process. A maximum of six platinum atoms per (AT + GC) unit (i.e., one platinum atom/1.5 nucleotide phosphate) was determined.

Roos[35] has utilized equilibrium dialysis to study the binding of *cis*-$(NH_3)_2PtCl_2$ to *E. coli* DNA. Using both the Scatchard model and the more complex treatment of Schellman[36] or McGhee and von Hippel,[37] he arrived at the same apparent association constant of 25 500 mol^{-1} dm^3 and a value of 0.58 ± 0.01 for the saturation binding value. In this way, he obtains a value of 1.27 for the average number of lattice residues involved per bound platinum and interprets this to mean that approximately 30% of the bound platinum is bifunctionally bound to two bases. This is a very high estimate, especially when considered in comparison to others which are at least a factor of 10 or more lower.[38-40]

The present study presents binding data for CPD interacting with the single stranded homopolynucleotides, poly(A) and poly(C), and with poly(G) at 37 °C. The specific aims were, in each case, to determine (1) the total number of binding sites per nucleotide unit; (2) the magnitude of the intrinsic binding constant (or constants); and (3) the degree of cooperativity exhibited in the binding process.

Experimental Section

Materials. The potassium salt of each of the homopolynucleotides was purchased from Sigma Chemical Co., all having a molecular weight greater than 100 000. The *cis*-$(NH_3)_2PtCl_2$ was on loan from the Mathey Bishop Co., Malvern, Pa. The silver nitrate was Reagent ACS Code 2179 and all other chemicals were Baker analyzed reagent quality.

Solution Preparation. All glassware was scrupulously cleaned and then dried in an oven at 140 °C before use. Distilled-deionized water was used in all solutions. Unbuffered solutions of CPD and the polynucleotides were prepared as described earlier by Scovell and O'Connor.[27] The buffered solution of CPD was prepared by initially dissolving *cis*-$(NH_3)_2PtCl_2$ in water and then adding a stoichiometric amount of standard $AgNO_3$ solution. The AgCl was filtered from the solution using a 0.22-μ Millipore filter. This solution was added to a concentrated aliquot of $NaClO_4$ and KH_2PO_4 and this mixture was diluted to near the final volume. The pH was then adjusted to 7.0 and the solution diluted to final volume to obtain the stock solution, 0.1 in $NaClO_4$ and 0.01 M in phosphate buffer. This CPD solution, and the buffer solution which was used to prepare the polynucleotide solution, was filtered again using a 0.22-μ Millipore filter before the reaction mixtures were prepared.

The analysis for the free CPD concentration was done spectrophotometrically. In order for Beer's law to be followed and to obtain reproducible results, the procedure of Berman and Goodhue[41] was modified. Use of the unmodified procedure gave values of the extinction coefficient which were low and also time dependent. The modified procedure essentially required (1) that the acid $SnCl_2$ solution be prepared immediately before use, (2) repeated aqua regia treatment of the samples to ensure complete oxidation and complexation of the platinum, and (3) that the chloride ion concentration be increased to eliminate the time dependence in the extinction coefficient at 400 or 310 nm. The detailed study and modified procedure will be published elsewhere.

Experimental Procedures. A. Equilibrium Binding Study. The reaction mixtures were prepared with the polynucleotide concentration at 1.0×10^{-4} M in all cases and varying amounts of CPD, such that the initial (CPD)/poly(N) mole ratio (the poly(N) concentration is expressed in terms of nucleotide units) varied from ca. 7 to 0.01 in most cases. The reaction mixtures were sealed in vials and incubated for 6.5 days in a sealed container in a water bath at 37 ± 1 °C. The mixtures were removed from the bath and ultrafiltration of the samples was used to separate an aliquot of the solution containing only CPD. This sample was then analyzed spectrophotometrically for free platinum. Only a 10-mL aliquot of the 40 mL in the ultrafiltration cell was filtered. This procedure utilized the Millipore Pellicon type PT membrane filters which have a nominal molecular weight limit of 10 000, in conjunction with the Millipore stirred cell. Low nitrogen pressure facilitated more rapid filtering. Initially samples of only CPD and of only polynucleotide were separately filtered to ensure that CPD passed through the filter quantitatively (no binding to the membrane was detected) and that no free polynucleotide passed through the membrane. It was assumed that the CPD-polynucleotide complex would likewise be retained. The Donnan effect was minimized to an insignificant level by use of 0.1 M $NaClO_4$.

The filtered CPD aliquots were prepared for platinum analysis according to the modified spectrophotometric procedure. Cells of 1 and 5 cm were used and the analyses were carried out using a Beckman Acta MIV spectrophotometer. The platinum determination can be conveniently analyzed at either λ 400 or 310 nm. The data reported here were obtained utilizing the 400-nm band for which ϵ_{400} 7.8×10^3. Beer's law plots for CPD were obtained over the concentration range 1.0×10^{-4} to 2.0×10^{-6} M. The stock CPD concentration was also determined using this plot, while the polynucleotide concentration was determined using reported extinction coefficients.[15,42]

B. Kinetic Profiles. Before the equilibrium studies were undertaken, the general kinetic profile for the reaction was monitored by the change in the UV spectrum at λ_{max} for the CPD-polynucleotide complex. Reaction mixtures were incubated at 37 °C and the absorbance was checked periodically over a 7-day period. The initial absorbance increase observed in all three systems changed only slightly after 6-7 days. We therefore, considered the reaction carried out in buffered conditions very close to, if not at, equilibrium at 6.5 days.

Calculations

The initial binding of CPD to the homopolynucleotide may generally be expressed as in eq 1.

$$\mathrm{CPD} + \mathrm{poly(N)} \rightleftharpoons [\mathrm{CPD\text{-}poly(N)}] \quad (1)$$

Since each poly(N) has many potential binding sites, one can represent the CPD binding to poly(N) by n' such expressions, where n' is the maximum number of binding sites per *polynucleotide*. When the number of sites per molecule is large, it is not possible to determine the concentration of each of the many complex species in solution. The general approach to such a problem is to determine experimentally the number of moles of ligand, (A), *bound* per mol of polymer, expressed in terms of the repeating monomer concentration, which in this case is a nucleotide.[43] This mole ratio is defined as $\bar{\nu}$. In this representation, the binding interaction described involves n, the maximum number of binding sites per nucleotide unit.

Assuming that the magnitude of all the stepwise binding constants is the same, except for the statistical factor, i.e., n equivalent and independent binding sites, the resulting Adair equation can be simplified to eq 2, which relates $\bar{\nu}$ to n, the number of binding sites per nucleotide, K, the intrinsic binding constant, and [A], the concentration of unbound ligand.

$$\bar{\nu} = \frac{nK[A]}{1 + K[A]} \quad (2)$$

This equation also assumes that the ligand binds to only one repeating unit on the polymer lattice. Ligand binding which involves two or more adjacent units requires a more complex analysis which not only involves ligand binding as a function of the number of ligands already bound, but also considers the distribution of the ligands on the polymer lattice.[37] This last assumption is expected to be valid for these studies; although

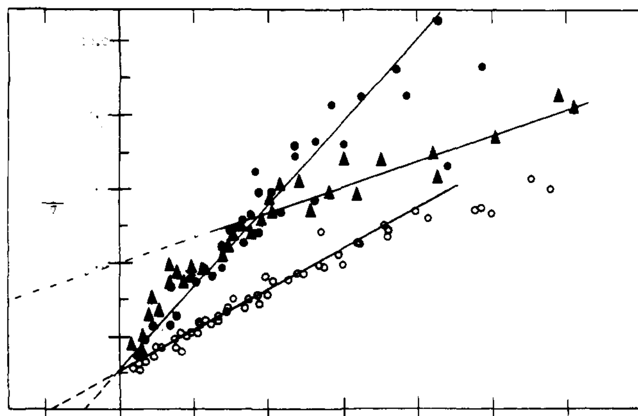

Figure 1. Double reciprocal plots for CPD interaction with poly(C) (●), poly(A) (○), and poly(G) (▲).

it has been shown that cross-linking occurs in DNA and it has been suggested in homopolynucleotides, there is no clear evidence to date that multisite binding (inter- or intrastrand cross-linking) contributes significantly in the overall binding in the systems of interest here.

Expression 2 may be rearranged and plotted in the form of the double reciprocal plot[44] or alternately the Scatchard plot.[45] Of the two methods, the Scatchard plot is often more sensitive to deviations from ideality and therefore is generally more revealing. Other methods of exhibiting the data are also available, but will not be used. The values of n and K can be derived directly from these plots. The n value could, in theory, also be obtained from a Bjerrum plot[46] ($\bar{v}$ vs. log [CPD]); however, the data are not sufficiently inclusive to draw conclusions from this nonlinear representation of the data.

One finds that the data for many, if not most, systems are not fit well with this binding model. In such cases, these plots are convenient and useful, however, for testing the validity of the inherent assumptions.

If the binding interactions are not ideal as defined above, a more complex binding model must be invoked. Such a model may consider either (1) interactions between the equivalent binding sites in the one set or (2) more than one set of equivalent and independent sites. Introduction of further complexities into the binding model at this point of our understanding of CPD-polynucleotide binding seems unwarranted.

In the first modified binding model, the initial interaction is considered to modulate the binding affinity of subsequent interactions. Generally this may be represented by the inclusion of an exponential term, $\exp(2w\bar{v})$, which accounts for the variance in K as a result of the changing electrostatic interactions as a function of $\bar{v}$.[45] Equation 3 does just this and will be used and referred to as the modified Scatchard expression.

$$\bar{v} = \frac{n[A]K\exp(2w\bar{v})]}{1 + [A]K[\exp(2w\bar{v})]} \qquad (3)$$

This equation was originally utilized by Scatchard to explain the interaction of small ions with proteins. It has also been utilized in studies concerned with the covalent binding of metal ions to synthetic single-stranded and double-stranded polynucleotides and to a variety of DNA samples.[9]

The parameter w is adjustable and can be correlated with the degree of cooperative interaction between the sites. Positive values of w indicate negative cooperativity or anticooperative behavior, while negative w values are indicative of a positive cooperativity or simply a cooperative character in the binding. A cooperative interaction implies that the initial binding enhances the binding affinity for subsequent binding, while an anticooperative interaction indicates that the initial binding decreases the subsequent binding affinity. The value of n can be obtained from either the Bjerrum, Klotz, or, in some cases, from the Scatchard plot.

When two (or more) different interactions occur, the binding is described in terms of two sets of equivalent and independent sites, where n_{total} is now the sum of n_1 and n_2, the number of binding sites associated with each set. Equation 4 expresses the general summation for m sets of such sites.

$$\bar{v} = \sum_{i=1}^{m} \frac{n_i K_i[A]}{1 + K_i[A]} \qquad (4)$$

Table I summarizes the equations utilized in each binding model and, for the first two models, lists these equations rearranged in linear form(s).

Table I

Model I: One Set of Equivalent and Independent Sites

$$\left(\frac{1}{v}\right) = \left(\frac{1}{n}\right) + \left(\frac{1}{nK}\right)\frac{1}{(A)} \qquad (2)$$

$$\left(\frac{\bar{v}}{(A)}\right) = k(n - \bar{v})$$

Model II: One Set of Equivalent but Interacting Sites

$$\log\left(\frac{\bar{v}}{A}\right)\left(\frac{1}{n - \bar{v}}\right) = \log K - 0.87w\bar{v} \qquad (3)$$

Model III: Two or More Sets (m) of Equivalent and Independent Sites

$$\bar{v} = \sum_{i=1}^{m} \frac{n_i K_i[A]}{1 + n_i K_i} \qquad (4)$$

Results

Kinetic Profiles. The rate of the CPD-poly(N) interaction was monitored by the UV absorbance change at λ_{max} for the complex. At 37 °C, $\mu = 0.1$, and pH 7 (0.01 M phosphate buffer), the absorbance change after 6 days was small. We therefore considered equilibrium to be attained in all three systems at 6.5 days under these conditions. In following the reaction profile by this means, it appeared that the three reactions had comparable rates. This is a qualitative observation and not in complete agreement with earlier reports in which the measurements may perhaps have been more quantitative.[47]

Monitoring of the CPD-poly(A) reaction under comparable conditions, but in unbuffered medium (pH 7 ± 1), indicates that the reaction rate was significantly faster, with equilibrium being attained in ca. 30 h. The phosphate buffer presumably competes kinetically with the polynucleotide for CPD and thereby inhibits the rate of reaction by about a factor of 5.

Binding Profiles. If the binding interaction were ideal (model I), the values of n and K can be determined directly from the double reciprocal plot. Figure 1 shows the data plotted in this manner for the CPD interaction with poly(C), poly(A), and poly(G). The plots for poly(C) and poly(A) appear linear, while the data for poly(G) are clearly nonlinear. Assuming that the constraints of model I are valid for the poly(C) and poly(A) reactions, the n and K values for the binding of CPD to poly(C) are 1 and ca. 5000, and 1 and ca. 9000 for poly(A). The data for the poly(G) reaction, however, indicate that the interaction is not simple and is not consistent with model I. The straight line representing the data points at low $\bar{v}$ values, if extrapolated to the $(1/v)$ axis, intersects at a value of 4.0. However, the data profile begins to change abruptly from this initial character at $(1/v)$ values of ca. 5, with the resulting data points fitting a straight line which crosses the $(1/v)$ axis at 1.0. The double

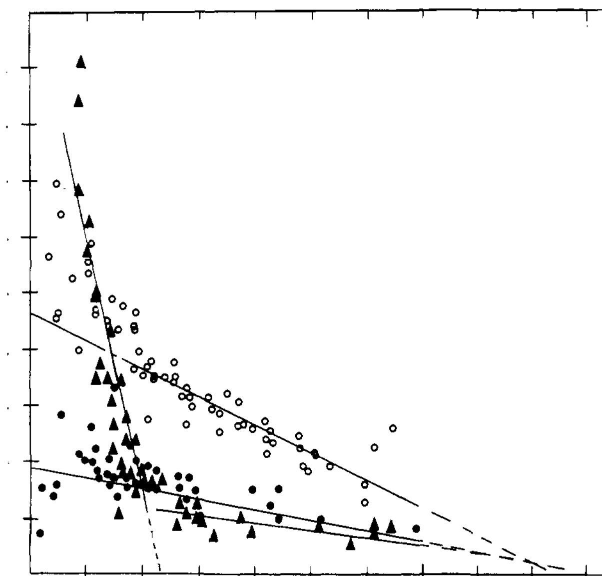

Figure 2. Scatchard plots for CPD interaction with poly(C) (●), poly(A) (○), and poly(G) (▲).

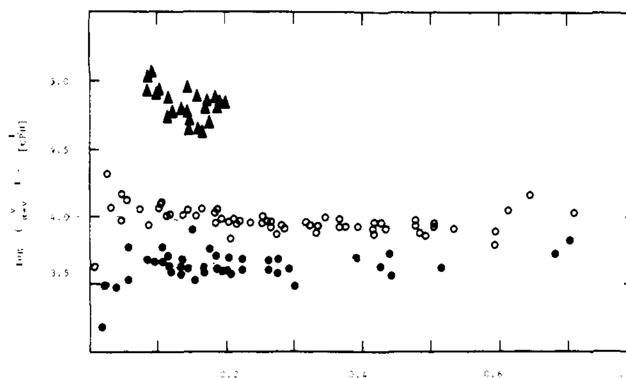

Figure 3. Modified Scatchard plots (eq 3) for the interaction of CPD with poly(C) (●), poly(A) (○), and poly(G) (▲).

reciprocal plot suffers from at least one disadvantage in that it inherently overemphasizes the data at high $\bar{v}$ values and therefore often is not sensitive to subtle deviations from ideality. For this reason, the data for the three systems were evaluated also by an alternative representation.

The Scatchard plot of the data is generally regarded as more sensitive to detection of nonideal binding behavior, although subtle features can also be masked here. Figure 2 exhibits the Scatchard plots of the data for the three systems. If CPD binding occurs at one set of n equivalent and independent sites, the plots should be linear. Clearly, the Scatchard plot for the poly(G) data exhibits pronounced curvature, a finding not unexpected and, in fact, in agreement with the double reciprocal plot results, while the poly(C) and poly(A) plots appear linear except for an apparent slight curvature at low $\bar{v}$ values. Curvature in the Scatchard plot is generally an indication of either (a) some cooperativity in the binding interaction or (b) more than one set of equivalent and independent sites.

If one assumes that there exists only one set of equivalent, but interacting, sites (vide infra), the data may be plotted according to model 2 assuming $n = 1$ in each case. Since there are only slight deviations in the Scatchard plots, the n value and the K value may, to a very good approximation, be obtained from this plot. In the case of the poly(C) and poly(A) systems, both the double reciprocal and the Scatchard plot indicate that the maximum number of CPD binding sites per nucleotide is one. Although the extrapolated line for the poly(G) system is less certain, the n value also appears to be ca. 1.

The modified Scatchard plot for the three systems is shown in Figure 3. The plots for the poly(A) and poly(C) systems, which extend from $\bar{v} = 0$ to 0.7, are linear and yield calculated w values of +0.3 and 0 and log K values of 4.0 and 3.7, respectively. Therefore, the CPD interaction with poly(C) is noncooperative, while the data suggest that CPD interaction with poly(A) may be slightly anticooperative. The value of $w = +0.3$ for the poly(A) reaction depends, to a large degree, on the data obtained at $\bar{v} < 0.1$, which are the data points of greatest uncertainty. Because of the difficulty of obtaining clearly reliable data in this $\bar{v}$ range, together with the small value calculated for w, our conclusion that this interaction is probably noncooperative appears justified. It should also be pointed out that the data for $\bar{v} < 0.05$ in the poly(C) system do deviate from the linear relationship observed for the data for $\bar{v} \geq 0.05$. Although the data in the low $\bar{v}$ region is very important to the analysis of the CPD interaction and to our understanding of this binding profile at biologically significant conditions, the uncertainty in this data below $\bar{v} = 0.05$ precludes us from strongly weighting it in our analysis. This data will certainly serve as a helpful guide in future studies which pursue this binding interaction in the low $\bar{v}$ region.

The poly(G) data are shown only for the first interaction which extends from $\bar{v} = 0.10$ to 0.25. The data exhibit a distinct negative slope for which $w = +1.9$ and log $K \simeq 5.0$. Therefore, this model for the binding of CPD to poly(G) at low $\bar{v}$ values indicates that the interaction is anticooperative in nature and clearly different from the two other polynucleotide systems investigated.

Discussion

A. Binding Sites. Although there remains considerable controversy concerning the nature of the binding of CPD to polynucleotides, there is no evidence strongly supporting any mode of binding which differs significantly than that suggested at the nucleoside level.[26,27] There appears to be general agreement that at low (CPD/nucleoside) mole ratios, the binding sites on the nucleoside involve the N-3 position in cytidine, the N-1 (or N-9) in adenosine, and the N-7 position in guanosine. The suggestion has been made that CPD chelates to guanosine,[48] although X-ray cyrstallographic studies on *cis*-$(NH_3)_2Pt[5'\text{-IMP}]_2^{2-}$ [49] and enPt$[5'\text{-GMP}]_2^{2-}$ [50] reveal monodentate binding at only the N-7 position. The structure for [enPt(5′-CMP)]$_2$[51] also agrees with the postulation of N-3 binding for cytidine, in addition to the phosphate being implicated in binding to the second enPt^{II} in the dimeric unit. Although the structures have other subtle features, the only other significant interaction in the complex is the strong hydrogen bonding present in all three complexes involving the hydrogens on the amine attached to the platinum and the phosphate group of the nucleotide. The significance of this latter interaction is speculative, but that it exists is now established. The magnitude of such an interaction is difficult to quantitate accurately, but it may well increase the binding stability of the complex by as much as 10–20 kcal/mol. Whether this secondary interaction is related to the antineoplastic activity is open to question; however, it is clear that many active complexes have such hydrogens available, while in many inactive complexes, the existing amine hydrogen(s) is (are) shielded by hydrophobic groups in the amine from participating strongly in a hydrogen-bonding interaction. Generally, with substituted amines in which the hydrogens are completely substituted by alkyl or aryl groups, little or no activity is observed.

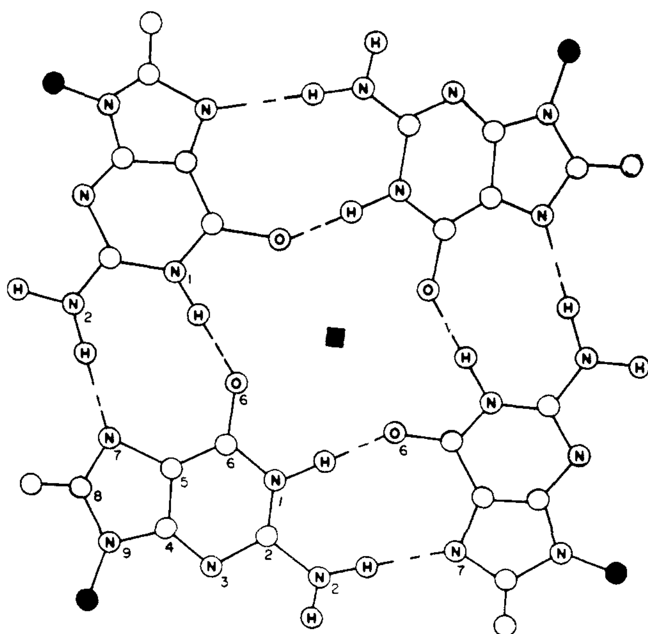

Figure 4. A layer of the proposed structural arrangement of guanine bases in the four-stranded poly(G) helix.

Studies on the number of heavy metal ions or complexes binding to nucleosides and the corresponding polynucleotides indicate that the primary site of interaction on the nucleoside remains the primary binding site on the polynucleotide level. Data can be cited from Hg(II), CH_3Hg^{II},[14,19,52-54] and OsO_4[7] binding studies at both levels of complexity which provide the basis for this hypothesis. Considering that this trend may be extended to the *cis*-$(NH_3)_2Pt^{II}$ studies, it is assumed that the primary binding sites in the homopolynucleotides are the same as those suggested in the nucleosides.

B. Reversibility. A number of studies on the interaction of *cis*-$(NH_3)_2PtCl_2$ with homopolynucleotides and with DNA have concluded that the reaction is irreversible.[55,34] In these studies, it has not been fully appreciated that the dissociation for the CPD-polynucleotide complex must be extremely slow. Although the detailed kinetics of this interaction is not known, it is clear that (1) the forward reaction (association reaction) is quite slow and (2) the binding constant is large. This implies generally, or at least in simple reactions, that the dissociation constant will be especially small and the dissociation rate will, therefore, be very slow. In support of our assumption of reversibility, Roos[35] has carried out the classic experiments for reversibility in the interaction of $Pt(en)Cl_2$ with DNA and concludes that the reaction is indeed reversible.

C. Stoichiometry and the Nature of the Binding Interaction. In the analyses of the data, the first binding model tested assumed that CPD binding occurs at sites which are equivalent and noninteracting. Therefore, any deviation observed from this ideal behavior may be attributed to a fault in either or both assumptions. The data for both the CPD binding to poly(C) and poly(A) support ideal behavior and the noncooperative binding is understood as a direct consequence of the law of mass action, i.e., linear double reciprocal and Scatchard plots.

The data for the CPD-poly(G) interaction indicate that model I is inappropriate for the analysis. The nonlinear Scatchard plot may result from (a) cooperativity effects between the sites if only one set of equivalent sites is assumed; (b) a considerable fraction of the bound CPD which forms *intrastrand* cross-links; (c) the occurrence of multiple (two in this case) sets of equivalent and independent sites; or (d) the CPD binding to poly(G) drastically altering the integrity of the poly(G) unit at a particular value of CPD/poly(G) mole ratio such that the subsequent binding affinity, whether to the same site or to a different site, is markedly decreased.

Only the inclusion of cooperativity has been considered thus far. However, the analysis assuming a strong anticooperative behavior for poly(G), after the method of Schwarz,[56] is not completely satisfactory, and, if no other complexities are introduced, the data are not fit well. It has been suggested that CPD binding to poly(G) may produce some degree of intrastrand cross-links,[57] although its quantitative significance remains to be resolved. The stereochemical constraint inherent in such an interaction is also apparent. These two uncertainties, coupled together, suggest that this mode of binding is presently just an unnecessary complication.

Studies using a variety of physical techniques support the conclusion that in neutral solution poly(A) and poly(C) are single stranded.[58] Poly(G) is, however, quite different in that, in salt solution, it exhibits a remarkably stable secondary structure, quite unlike poly(A), poly(C), and also poly(I). For example, little or no change in physical characteristics is observed in thermal denaturation studies.[59-61] The stability of poly(G) is such that its melting temperature is predicted to be greater than 100 °C, while under comparable solution conditions, poly(A), poly(C), and poly(I) melt well below 100 °C.[58] X-ray fiber diffraction studies of poly(G) and poly(I) indicate that both homopolynucleotides are complex units having four strands.[62,63] Figure 4 illustrates the suggested arrangement of the four guanine bases in a layer of the four-stranded helix, with the hydrogen bonding interactions as shown. There are two hydrogen bonds per base in poly(G), while the similar poly(I) structure would have only one hydrogen bond per base. This crystallographic description is also consistent with the solution characteristics.[59,60,64]

Considering that the four-stranded complex exists in solution, it is of interest to note that the Scatchard plot may be resolved into two lines indicative of two interactions of different CPD binding affinity. The initial slope in the Scatchard plot, extrapolated to the $\bar{v}$ axis, results in an intercept at $\bar{v} \simeq 0.25$, while the second and weaker interaction intersects the $\bar{v}$ axis at ca. 1.0. These results are consistent with CPD initially interacting with the four-stranded helix, presumably binding at the N-7 position of guanine. Since the N-7 position is involved in hydrogen bonding in the poly(G) aggregate (as shown in Figure 4), CPD binding progressively disrupts the structure until, on an average, one CPD is bound per four nucleotides ($\bar{v} = 0.25$), at which point the secondary structure of poly(G) is markedly altered with the four-stranded complex being disrupted. Further CPD binding may continue to occur at the N-7 position, but, since N-7 is not now involved in a hydrogen-bonding interaction and exists in quite a different environment, the binding affinity is considerably reduced. Therefore, although the binding behavior mimics the model containing formally two sets of equivalent and independent sites, the data are also consistent with *initially* one set of n equivalent sites which is altered drastically in character at $\bar{v} \simeq 0.25$. In this analysis, for the initial CPD-poly(G) interaction, the magnitude of the first interaction constant, K_1, is equal to or greater than ca. 2×10^4, significantly greater than the K values for the CPD interaction with either single-stranded poly(A) or poly(C). The magnitude of K_2, representative of the interaction at $\bar{v} \geq 0.25$, and, of course, a weaker interaction, is about an order of magnitude smaller, with $K_2 \simeq 3 \times 10^3$. The magnitude of K_2 is comparable to the CPD binding affinity observed in the poly(C) and the poly(A) single-stranded systems.

It should be clear that other alternate binding schemes may reasonably be proposed which will also be consistent with the binding profile. Binding of CPD at N-7 has been suggested since it is clear that CPD binding occurs at N-7 in 5′-GMP,

Table II. Binding Results for Aquated *cis*-$(NH_3)_2Pt^{II}$ Interaction with Nucleosides and Homopolynucleotides

	(log K_1, n)			t, °C	pH	μ	buffer
	Cyd	Ado	Guo				
nucleoside	(3.5, 1)	(3.6, 1)	(3.7, 1)	25	6.5	0.1	—
poly(N)	(3.7, 1)	(4.0, 1)	(≥4.3, 0.25)	37	7.0	0.01	phosphate

Table III. Comparative Data for Metal Species Binding to Homopolynucleotides[a]

metal species	polynucleotide	log K	n	w	ref
Mg(II)	poly(A)	4.6			14
Mn(II)	poly(A)	4.6	0.33	0	66
		4.0	0.5		67
	poly(C)	~4.3	0.7-0.8		
	poly(I)	~4.3	0.5-0.8		
Ag(I)	poly(A)	5.80	0.5	−3.7	9
	poly(C)	7.25	0.5	−6.0	
	oligo(G)	6.00	0.5	−0.9	
cis-$(NH_3)_2Pt^{II}$ (aq)					
	poly(A)	4.0	1.0	0	(this work)
	poly(C)	3.7	1.0	0	
	poly(G)	$K_1 \geq 4.3$	0.25	(+1.9)	
		$K_2 \simeq 3.5$	1		
	E. coli DNA	4.40	0.57		35

[a] Consult references for solution conditions.

in addition to the fact that N-7 in the four-stranded poly(G) is exposed on the exterior of the helix. However, since N-3 is also on the exterior of the helix, it may also provide a site for interaction. There is no support for CPD interacting with a purine at N-3, although Yamane and Davidson have proposed this possibility in studies involving Ag(I) binding to DNA.[18]

It is of interest to note that Chu et al. report that the Raman spectrum of a mixture of CPD with poly(G) at an r value of 0.6 exhibits no significant changes from that of poly(G) alone.[28] If there is not a dramatic concentration dependence on this equilibrium, the r value of 0.60 is equivalent to our $\bar{\nu}$ value of about 0.17, at which point there is about 27% CPD bound. The conditions in the Raman experiment were such that our data would indicate, and agree with the Raman data, that no disruption of the four-stranded helix had yet occurred. However, why covalently bound CPD could not be detected is unclear.

Munchausen and Rahn[32] have utilized the ^{195}Pt radioisotope to determine the saturation r values for *cis*-$(NH_3)_2$ $^{195}PtCl_2$ binding to homopolynucleotides and DNAs from different sources. They determined that r is 0.4–0.5 for poly(N) where N = A, G, and C, which is just under one-half of the $\bar{\nu}$ value obtained in our studies. They therefore conclude that the *cis*-$(NH_3)_2PtCl_2$ binds to two bases simultaneously in all the polynucleotides. It also should be noted that they report a saturation r value for the binding to *E. coli* DNA of 0.26, which is again just about one-half the $\bar{\nu}$ value of 0.57 recently reported by Roos.[35]

A comparison of the intrinsic binding constants for the CPD–poly(N) reactions indicates that the binding affinity decreases in the order poly(G) > poly(A) > poly(C). In each case, a maximum of only one CPD can bind per nucleotide. This trend in the relative magnitude of the intrinsic binding constants for the CPD–homopolynucleotides was predicted previously by Scovell and O'Connor[27] from studies on the CPD reaction with the corresponding nucleosides. Table II compares the binding constants for the CPD interaction with the nucleosides and the corresponding homopolynucleotide. The trend in the magnitude of the binding constants for the CPD–nucleoside interactions *parallels* the intrinsic binding constants obtained for the CPD–poly(N) interactions. Unfortunately, a direct comparison may suffer because experimental conditions were not identical in the two studies. Both the temperature and the salt composition are different. Significantly, the 0.01 M phosphate buffer used in the CPD–poly(N) studies can be expected to reduce the binding constant by perhaps as much as an order of magnitude.[65] Therefore, the intrinsic binding constants can be considered to be artificially low in this comparison, although the trend in the relative magnitudes of the K values should be unaffected. As has been pointed out previously,[27] this parallel behavior with binding constants at the nucleoside level is not unique to the CPD interaction, but is observed for the Hg(II) and CH_3Hg^{II} interactions as well.[52-54]

We have also pointed out the effect phosphate buffer exerts on the rate of the reaction; it is, therefore, very evident from our results that both the kinetic and thermodynamic aspects of this interaction are significantly influenced by the salt conditions in solution.

A comparison of binding data for metal species binding to homopolynucleotides is shown in Table III. The binding of both Mg(II) and Mn(II) is electrostatic and noncooperative. The covalent binding of Ag(I) to poly(A), poly(C), and oligo(G) is strong and cooperative. The results obtained from our study are included and compared to the binding data obtained in the analogous CPD–DNA studies.

Since CPD binds selectively and to a comparable extent to cytidine, adenosine, and guanosine at equilibrium,[27] it has been suggested that CPD will exhibit a thermodynamic selectivity for G·C base pairs or (G-C)-rich regions of DNA as opposed to A·T base pairs or (A-T)-rich regions of DNA. The results from these binding studies of CPD to the homopolynucleotides further support these expectations. Amacher and Lieberman[68] have recently arrived at this same conclusion from equilibrium bouyant density studies involving *cis*- and *trans*-$(NH_3)_2PtCl_2$ with synthetic polydeoxyribonucleotides. However, the suggestion is derived from their conclusion that inter- and intrastrand interactions are important in the *cis*-$(NH_3)_2PtCl_2$ interaction, but less so for the *trans*-$(NH_3)_2PtCl_2$.

Table IV lists a number of metal complexes which exhibit selectivity in binding to DNA. It has been shown that Hg(II), CH_3Hg^{II}, and OsO_4 selectively bind to thymidine and as a result bind selectively to A·T base pairs or (A-T)-rich regions of DNA. On the other hand, Cu(II) and Ag(I) bind prefer-

Table IV. Metal Species Exhibiting Selectivity for Nucleic Acid Bases in Aqueous Solution

metal species	selective base(s)
Cu(II)	Guo
Ag(I)	Guo
cis-$(NH_3)_2Pt^{II}$ (aq)	Guo, Ado, Cyd
OsO_4	Thd
CH_3Hg^{II}	Thd
Hg(II)	Thd

entially to guanine which results in these metal ions selectively binding to G·C base pairs or (G-C)-rich regions of DNA.[14] In fact, the different selectivity characteristics of Ag(I) and Hg(II) have been successfully used to analytically separate the two components from crab DNA.[53,69-71] Interestingly, CPD is unique when compared to the other ions or complexes in that the selectivity of CPD is *predicted* because it binds strongly to three bases in DNA, with little or no affinity for the fourth base, thymine. In contrast, the selectivity *observed* for Cu(II), Ag(I), Hg(II), CH_3Hg^{II}, and OsO_4 results because the ion or complex has an especially strong binding affinity for one of the bases with a markedly lower affinity for the other three bases.

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References and Notes

(1) The following abbreviations are used: CPD, aquated *cis*-$(NH_3)_2Pt^{II}$; $\bar{\nu}$, the number of moles of ligand *bound* per mol of nucleotide; *r*, the number of moles of ligand added per mol of nucleotide; en, ethylenediammine.
(2) B. Lewin, "Gene Expression", Vol. 1 and 2, Wiley, New York, N.Y., 1974.
(3) H. M. Sobell and S. C. Jain, *J. Mol. Biol.*, **68**, 21 (1972).
(4) P. H. von Hippel and J. D. McGhee, *Annu. Rev. Biochem.*, **41**, 231 (1972).
(5) M. J. Waring, *Nature (London)*, **219**, 1320 (1968).
(6) B. Festy and M. Daune, *Biochemistry*, **12**, 4827 (1973).
(7) M. Beer, S. Stein, D. Carmalt, and K. H. Mohlhenrich, *Biochemistry*, **5**, 2283 (1966).
(8) G. Corneo, E. Ginelli, and E. Polli, *J. Mol. Biol.*, **48**, 319 (1970).
(9) M. Daune, C. A. Dekker, and H. K. Schachman, *Biopolymers*, **4**, 51 (1966).
(10) R. F. Steiner and R. F. Beers, Jr., "Polynucleotides", American Elsevier, New York, N.Y., 1961.
(11) C. Schildkraut and S. Lifson, *Biopolymers*, **3**, 195 (1965), and references cited therein.
(12) M. T. Record, Jr., C. P. Woodbury, and T. M. Lohman, *Biopolymers*, **15**, 893 (1976).
(13) W. F. Dove and N. Davidson, *J. Mol. Biol.*, **5**, 467 (1962).
(14) G. L. Eichhorn, "Inorganic Biochemistry", G. L. Eichhorn, Ed., American Elsevier, New York, N.Y., 1973, Chapter 34.
(15) A. Y. Shin, *Biopolymers*, **12**, 2459 (1973).
(16) R. J. Maquire, *Can. J. Biochem.*, **54**, 583 (1976).
(17) G. L. Eichhorn, J. J. Butzow, P. Clark, and E. Tarien, *Biopolymers*, **5**, 283 (1967).
(18) T. Yamane and N. Davidson, *Biochim. Biophys. Acta*, **55**, 609, 780 (1962).
(19) D. W. Gruenwedel and N. Davidson, *J. Mol. Biol.*, **21**, 129 (1966).
(20) I. R. Miller and D. Bach, *Biopolymers*, **6**, 169 (1968).
(21) I. Krakoff, *J. Clin. Hematol. Oncol.*, **7**, 604 (1977). This and other articles in this volume summarize papers presented at the Third International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy, Dallas, Texas, Oct 18–20, 1976.
(22) H. C. Harder and B. Rosenberg, *Int. J. Cancer*, **6**, 207 (1970).
(23) J. A. Howle, H. S. Thompson, A. E. Stone, and G. R. Gale, *Proc. Soc. Exp. Biol. Med.*, **137**, 820 (1971).
(24) J. A. Howle and G. R. Gale, *Biochem. Pharmacol.*, **19**, 2757 (1970).
(25) L. P. G. Wakelin, *Biochem. Soc. Trans.*, **2**, 866 (1974).
(26) S. Mansy, B. Rosenberg, and A. J. Thomson, *J. Am. Chem. Soc.*, **95**, 1633 (1973).
(27) W. M. Scovell and T. O'Connor, *J. Am. Chem. Soc.*, **99**, 120 (1977).
(28) G. Y. H. Chu, S. Mansy, R. E. Duncan, and R. S. Tobias, *J. Am. Chem. Soc.*, **100**, 593 (1978).
(29) S. Mansy, G. Y. H. Chu, R. E. Duncan, and R. S. Tobias, *J. Am. Chem. Soc.*, **100**, 607 (1978).
(30) G. Y. H. Chu, R. E. Duncan, and R. S. Tobias, *Inorg. Chem.*, **16**, 2625 (1977).
(31) S. Wherland, E. Deutsch, J. Eliason, and P. B. Sigler, *Biochem. Biophys. Res. Commun.*, **54**, 662 (1973).
(32) L. L. Munchausen and R. O. Rahn, *Biochim. Biophys. Acta*, **414**, 242 (1975).
(33) P. Horacek and J. Drobnik, *Biochim. Biophys. Acta*, **254**, 341 (1971).
(34) J. P. Macquet and T. Theophanides, *Biopolymers*, **14**, 781 (1975).
(35) I. A. G. Roos, *Chem.-Biol. Interact.*, **16**, 39 (1977).
(36) J. A. Schellman, *Isr. J. Chem.*, **12**, 219 (1973).
(37) J. D. McGhee and P. H. von Hippel, *J. Mol. Biol.*, **86**, 469 (1974).
(38) J. M. Pascoe and J. J. Roberts, *Nature (London)*, **235**, 282 (1972).
(39) K. V. Shooter, R. Howse, R. K. Merrifield, and A. B. Robins, *Chem.-Biol. Interact.*, **5**, 289 (1972).
(40) L. L. Munchausen, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 4519 (1974).
(41) S. S. Berman and E. C. Goodhue, *Can. J. Chem.*, **37**, 370 (1959).
(42) A. Y. Shin, J. H. Heim, and G. L. Eichhorn, *Bioinorg. Chem.*, **1**, 149 (1972) and references cited therein.
(43) C. Tanford, "Physical Chemistry of Macromolecules", Wiley, New York, N.Y., 1961.
(44) I. M. Klotz and D. L. Hunston, *Biochemistry*, **10**, 3065 (1971).
(45) G. Scatchard, *Ann. N.Y. Acad. Sci.*, **51**, 660 (1949).
(46) J. Bjerrum, "Metal Amine Formation in Aqueous Solution", P. Haase & Son, Copenhagen, Netherlands, 1941.
(47) A. B. Robins, *Chem.-Biol. Interact.*, **7**, 11 (1973).
(48) J. P. Macquet and T. Theophanides, *Bioinorg. Chem.*, **5**, 59 (1976).
(49) D. M. L. Goodgame, I. Jeeves, F. L. Phillips, and A. C. Skapski, *Biochim. Biophys. Acta*, **378**, 153 (1975).
(50) R. W. Gellert and R. Bau, *J. Am. Chem. Soc.*, **97**, 7379 (1975).
(51) R. Bau, R. W. Gellert, S. M. Lehovec, and S. Louie, *J. Clin. Hematol. Oncol.*, **7**, 51 (1977).
(52) R. B. Simpson, *J. Am. Chem. Soc.*, **86**, 2059 (1964).
(53) T. Yamane and N. Davidson, *J. Am. Chem. Soc.*, **83**, 2599 (1961).
(54) D. W. Gruenwedel and N. Davidson, *Biopolymers*, **5**, 847 (1967).
(55) J. A. Howle, G. R. Gale, and A. B. Smith, *Biochem. Pharmacol.*, **21**, 1465 (1972).
(56) G. Schwarz, *Biophys. Struct. Mech.*, **2**, 13 (1976).
(57) A. D. Kelman, H. J. Peresie, and P. J. Stone, *J. Clin. Hematol. Oncol.*, **7**, 440 (1977).
(58) G. Felsenfeld and H. T. Miles, *Annu. Rev. Biochem.*, **36**, 407 (1967).
(59) H. T. Miles and J. Frazier, *Biochim. Biophys. Acta*, **79**, 216 (1964).
(60) J. Fresco and J. Massoulie, *J. Am. Chem. Soc.*, **85**, 1352 (1963).
(61) J. J. Englander, N. R. Kallenbach, and S. W. Englander, *J. Mol. Biol.*, **63**, 153 (1972).
(62) S. Arnott, R. Chandrasekaran, and C. M. Marttila, *Biochem. J.*, **141**, 537 (1974).
(63) S. B. Zimmerman, G. H. Cohen, and D. R. Davies, *J. Mol. Biol.*, **92**, 181 (1975).
(64) H. J. Hinz, W. Haar, and T. Ackerman, *Biopolymers*, **9**, 923 (1970).
(65) R. S. Reaoch, V. Weerasooriya, and W. M. Scovell, unpublished studies.
(66) A. Danchin, *Biopolymers*, **11**, 1317 (1972).
(67) J. Eisinger, F. Fawaz-Estrup, and R. G. Schulman, *J. Chem. Phys.*, **42**, 43 (1965).
(68) D. E. Amacher and M. W. Lieberman, *Chem.-Biol. Interact.*, **16**, 265 (1977).
(69) J. C. Wang, U. S. Nandi, D. S. Hogness, and N. Davidson, *Biochemistry*, **4**, 1697 (1965).
(70) N. Davidson, J. Widholm, U. S. Nandi, R. Jensen, B. M. Olivera, and J. C. Wang, *Proc. Natl. Acad. Sci. U.S.A.*, **53**, 111 (1965).
(71) U.S. Nandi, J. C. Wang, and N. Davidson, *Biochemistry*, **4**, 1687 (1965).