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Interaction of 4-Nitroquinoline 1-Oxide with Deoxyribonucleic Acid and Synthetic Polydeoxyribonucleotides

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SUMMARY

Native calf thymus DNA and, to a lesser extent, poly dG:dC produced marked changes in the absorption spectrum of 4-nitroquinoline 1-oxide (4-NQO) as determined by difference spectrum measurements. Poly d(A-T) or denatured calf thymus DNA caused minor changes in the absorption spectrum of 4-NQO, using difference spectrum methods.

The addition of sodium chloride from 0.5 mM to 1.0 M decreased the effect of native DNA on the difference spectrum of 4-NQO by approximately 4-fold. The interaction was partially dependent upon ionic parameters, which suggested the importance of charged sites in the interaction of 4-NQO with DNA.

Urea (6 M) abolished the effect of DNA on the difference spectrum of 4-NQO. The effect of urea may indicate some role of hydrogen bonding in the formation of the DNA–4-NQO complex or in the alteration of hydrophobic interactions resulting in disruption of the DNA–4-NQO complex.

Strand separation (T_m) of native DNA was significantly stabilized by the addition of 4-NQO.

Studies on the binding of native DNA with 4-NQO resulted in a nonlinear curve, consistent with the involvement of more than one site of interaction in the formation of the DNA-4-NQO complex.

The integrity of the double-helical conformation of DNA and the presence of G-C pairs appeared to be essential for maximal complex formation. The binding of 4-NQO to DNA evidently is complex and cannot be described by a single model of complex formation.

INTRODUCTION

4-Nitroquinoline 1-oxide is one of a large group of quinoline 1-oxides, of which several are potent carcinogens and others are noncarcinogenic (1, 2). Certain other derivatives possess carcinostatic activity (3) and exert profound effects on nucleolar morphology (4), ATP (5), and RNA and protein synthesis (6).

By means of flow dichroism measure-

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ments, 4-NQO[3] has been shown to complex with native DNA so that the quinoline ring is oriented parallel to the base pairs (7). The interaction with DNA was dependent primarily upon the presence of purines (adenosine and/or guanosine) at the polymer level and on the presence of a double-helical conformation (7, 8). Several investigations have been carried out with respect to base specificity on the nucleoside level. These have demonstrated similar degrees of preferential complex formation with both guanosine and adenosine, with little interaction with cytidine and thymidine (8–11). The present study was conducted to investigate base specificity by comparing the interaction of double-stranded synthetic polydeoxynucleotides and native and denatured DNA with 4-NQO.

The possible base specificity of the interaction between 4-NQO and DNA is of particular interest in view of the interaction of DNA with other quinoline derivatives of biological significance, such as chloroquine (12) and quinine (13). These compounds complex with DNA; they exhibit enhanced interaction with increasing guanosine-cytosine content and little complex formation with denatured DNA (12, 13).

METHODS

4-NQO was obtained from the Beacon Chemical Company, and purified by recrystallization from hot ethanol. Highly polymerized calf thymus DNA, type I, was obtained from the Sigma Chemical Company. The polydeoxyribonucleotides were synthesized using purified DNA polymerase (fraction IV) prepared by the method of Richardson (14). Poly d(A-T) was synthesized by the method of Schachman *et al.* (15), and poly dG:dC, by the method of Radding *et al.* (16). The DNA or polydeoxyribonucleotides were dissolved in sodium chloride–sodium citrate buffer, at approximately 2 mg/ml, with gentle intermittent agitation for 48 hr at 4°. The insoluble material was removed by centrifugation at 10,000 × *g* for 20 min at 4°. The interaction of the calf thymus DNA, poly d(A-T), and poly dG:dC was verified by increases in hyperchromicity of 38%, 25%, and 28%, respectively, at 260 mμ in sodium chloride–sodium citrate buffer.

Denatured DNA was prepared by heating native DNA for 5 min in a boiling water bath, followed by rapid cooling in ice water (17). The final concentrations of DNA and synthetic polymers were based on their percentage phosphorus content (18) and expressed in milligrams per milliliter.

Spectrophotometric studies with a Beckman DK-2A spectrophotometer at maximum sensitivity were performed in matched 1-cm quartz cuvettes in sodium chloride–sodium citrate buffer. Specific concentrations and conditions of the measurements are given in the pertinent figures.

Absorbance changes at 260 mμ during heating were recorded with a Beckman DK-2A spectrophotometer equipped with a heating cuvette holder and melting curve (T_m) analyzer. Temperatures inside the cuvettes were monitored using a platinum thermistor and calibrated temperature bridge. Absorbance changes as a function of temperature were recorded with a Hewlett-Packard *xy* plotter to record the internal temperature and optical density simultaneously. The median strand-separation temperature (T_m) was computed by the procedure of Mandel and Marmur (19).

Experiments involving the effect of urea on the difference spectrum of 4-NQO were performed in the presence of 6 M urea in both sample and reference cells, which also contained sodium chloride–sodium citrate buffer.

The binding of 4-NQO to DNA was studied with the methods developed by Scatchard (20) and Scatchard, Coleman, and Shen (21) in studies dealing with the interaction of ligands with polymeric molecules. The application of these techniques to the interaction of chloroquine with DNA has been demonstrated by Cohen and Yielding (12).

[3] Abbreviations used are: 4-NQO, 4-nitroquinoline 1-oxide; poly d(A-T), double-helical polymer consisting of two chains of alternating adenosine and thymidine residues; poly dG:dC, double-helical polymer consisting of one chain of deoxyguanosine residues and one chain of deoxycytidine residues; sodium chloride–sodium citrate buffer, 0.5 mM sodium chloride–0.5 mM sodium citrate, pH 7.0; T_m, melting temperature.

The computational methods used to study the binding of 4-NQO to DNA were identical with those of Cohen and Yielding (12). The changes in the absorption spectrum of the ligand (4-NQO) as a result of binding to a polymeric molecule (DNA) were measured. The fraction of the bound 4-NQO (α) is given by the relationship $\alpha = (D_1 - D)/(D_1 - D_2)$, where D_1 and D_2 are the absorbances of the free and bound 4-NQO, respectively, and D is the absorbance of the mixture. If T_P and T_L represent the molar concentrations of DNA (nucleotide phosphorus content) and 4-NQO, respectively, then $\bar{\nu} = \alpha T_L/T_P$ and $c = T_L - \alpha T_L$, where c is the free 4-NQO concentration in moles per liter and $\bar{\nu}$ is the number of 4-NQO molecules bound per DNA molecule. If a constant 4-NQO concentration is maintained, and the value of D is determined at a fixed wavelength (417 mμ for 4-NQO), with corrections for volume changes, a plot of $\bar{\nu}/c$ against c may be obtained. The association constant (K) was calculated as follows: $K = \bar{\nu}/[(n - \bar{\nu})c]$, where n is the maximum number of binding sites per DNA molecule. The values of n and K were obtained by extrapolation (12). Native calf thymus DNA was added in increments to a solution of 2×10^{-4} M 4-NQO in sodium chloride–sodium citrate buffer in 1-cm cuvettes (initial volume, 1.5 ml). Spectral measurements were carried out at 417 mμ. Corrections for volume changes were applied, and the plot of $\bar{\nu}/c \times 10^3$ against c was constructed by the procedure of Scatchard, Coleman, and Shen (21).

RESULTS

Figure 1 illustrates the alterations in the difference spectrum of 2×10^{-3} M 4-NQO in the presence of 0.4 mg/ml of native calf thymus DNA in sodium chloride–sodium citrate buffer. Native calf thymus DNA induced the most profound alterations in the difference spectrum of 4-NQO, while poly dG:dC exhibited a significant but decreased interaction with 4-NQO based upon alterations in the difference spectrum. Poly d(*A–T*) and denatured DNA had the least influence upon the difference spectrum of 4-NQO. DNA and synthetic polydeoxyribonucleotides altered the difference spectrum of 4-NQO in the following sequence of effectiveness: native DNA > poly dG:dC > poly d(A-T) > denatured DNA.

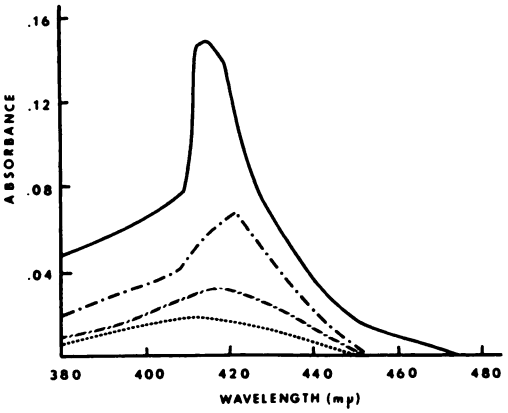

FIG. 1. *Difference spectrum of 4-NQO in the presence of DNA and synthetic polydeoxyribonucleotides*

Difference spectrum measurements were carried out at 20° in 1-cm cuvettes. The sample cell contained 0.4 mg/ml of the deoxyribonucleotide and 2×10^{-3} M 4-NQO. The reference cuvette contained 2×10^{-3} M 4-NQO. Curves, from top to bottom, represent native calf thymus DNA, poly dG:dC, poly d(A–T), and denatured calf thymus DNA. The solvent in all instances was 0.5 mM sodium chloride–0.5 mM sodium citrate, pH 7.0.

The difference spectrum of 4-NQO at 417 mμ was observed to be a function of the polydeoxynucleotide concentration. The initial concentration of 4-NQO was 2.0 ml of 2×10^{-3} M quinoline in sodium chloride–sodium citrate buffer. The effect of native DNA on the difference spectrum of 4-NQO diminished above a level of 0.4 mg/ml, reflecting the impending saturation of binding sites. The difference spectrum was also increased by increasing the levels of poly dG:dC and, to a lesser extent, poly d(A-T). Denatured DNA had very little effect on the difference spectrum of 4-NQO (Fig. 2).

Many compounds which interact with DNA exert a stabilizing effect on the thermal denaturation of DNA (T_m); actinomycin D (22), proflavine (23), chloroquine (12), and quinine (13) are representative ligands. Figure 3 illustrates the increased T_m point of 25 μg/ml of native calf thymus DNA in 4×10^{-6} M 4-NQO observed at 260 mμ in

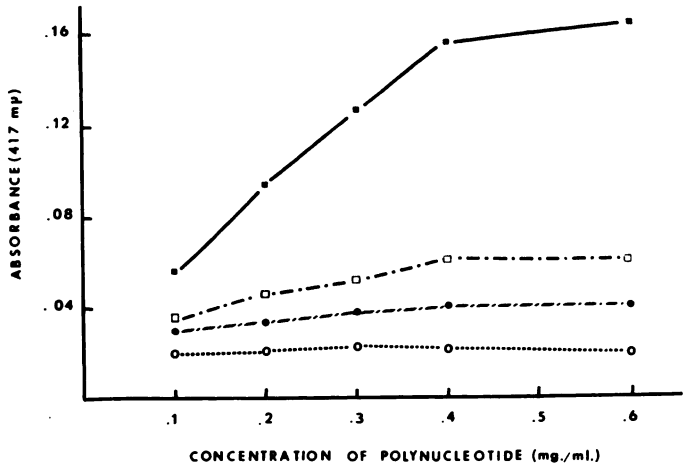

FIG. 2. *Difference spectrum of 4-NQO at 417 mμ as a function of polynucleotide concentration*
Polynucleotides were examined for alterations in the absorbance spectrum of 4-NQO at 417 mμ. The sample cuvette contained the polynucleotide in the presence of 2×10^{-3} M 4-NQO. The reference cuvette contained 2×10^{-3} M 4-NQO. The solvent was 0.5 mM sodium chloride–0.5 mM sodium citrate, pH 7.0 ■, native calf thymus DNA; □, poly dG:dC; ●, poly d(A − T); ○, denatured calf thymus DNA.

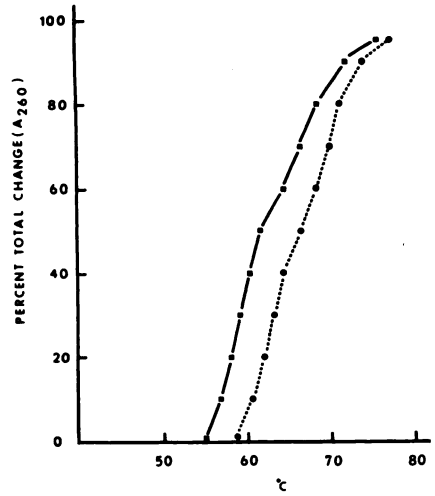

FIG. 3. *Melting curve (T_m) of native calf thymus DNA, and effect of 4-NQO*
Native calf thymus DNA (25 μg/ml) in 0.5 mM sodium chloride–0.5 mM sodium citrate had a T_m point of 61.7°. Native calf thymus DNA (25 μg/ml) in the presence of 4×10^{-6} M 4 NQO exhibited an increased T_m point of 66.5°. The reference cuvette contained 4×10^{-6} M 4-NQO. Both cuvettes contained sodium chloride–sodium citrate buffer. ■, native calf thymus DNA; ●, native calf thymus DNA (25 μg/ml) in the presence of 4×10^{-6} M 4-NQO.

sodium chloride–sodium citrate buffer. The reference cuvette contained 4×10^{-6} M 4-NQO in sodium chloride–sodium citrate buffer to compensate for the absorbance of 4-NQO at 260 mμ. The T_m point of DNA in sodium chloride–sodium citrate buffer was 61.7° and was increased to 66.5° in the presence of 4×10^{-6} M 4-NQO (Fig. 3). The increments above the control T_m point of poly dG:dC and poly d(A-T) were 3.8° and 0.8°, respectively, at the same polydeoxynucleotide concentration and 4-NQO level as for native DNA.

The effect of 6 M urea on the difference spectrum of 4-NQO in the presence of the polydeoxynucleotides and native and denatured DNA is presented in Fig. 4. Urea (6M) does not disrupt the double-helical configuration of DNA (24), but the addition of urea resulted in a drastic reduction in the effect of native DNA on the difference spectrum of 4-NQO. Denatured DNA, poly dG:dC, or poly d(A-T) in the presence of urea was virtually without influence on the difference spectrum of 4-NQO. The effects of urea on the binding of ligands to DNA are complex but might indicate some involvement of hydrogen bonding in the DNA–4-NQO interaction. A highly polar 1-oxide

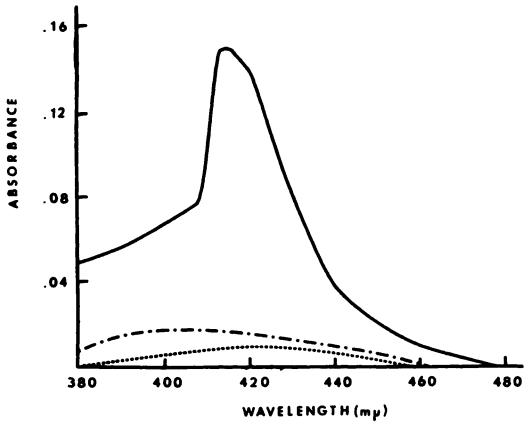

FIG. 4. *Effect of urea (6 M) on difference spectrum of 4-NQO in the presence of DNA and polydeoxyribonucleotides*

The sample cuvette contained native or denatured calf thymus DNA (0.4 mg/ml) in 0.5 mM sodium chloride–0.5 mM sodium citrate buffer. Both the sample and reference cuvettes contained 2×10^{-3} M 4-NQO. In the studies involving urea, both the sample and reference cuvettes contained 0.5 mM sodium chloride–0.5 mM sodium citrate, 2×10^{-3} M 4-NQO, and 6 M urea, pH 7.0. The sample cuvette contained native or denatured DNA (0.4 mg/ml). ——, native calf thymus DNA; –·–·, denatured DNA; - - - - -, native or denatured DNA, poly d(A–T), or poly dG:dC in the presence of urea.

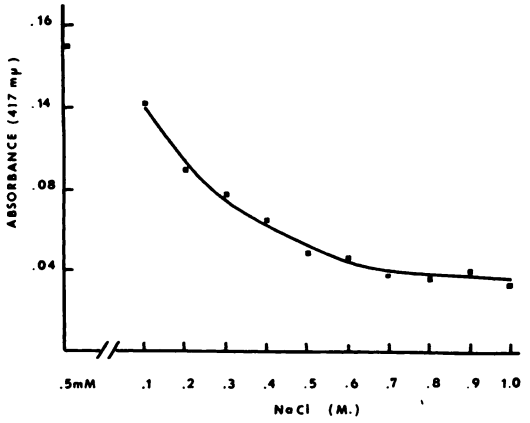

FIG. 5. *Effect of sodium chloride on difference spectrum of the 4-NQO–DNA complex*

The sample cuvette contained 0.5 mM sodium chloride–0.5 mM sodium citrate, 0.4 mg/ml of native calf thymus DNA, and 2×10^{-3} M 4-NQO. The reference cuvette contained sodium citrate buffer with 2×10^{-3} M 4-NQO. The reference and sample cuvettes were adjusted to the sodium chloride concentration given on the abscissa.

group present in 4-NQO could, in theory, participate in a hydrogen bond at several sites available for hydrogen bonding in the nucleic acids.

The addition of higher concentrations of sodium chloride to the 0.5 mM sodium chloride–0.5 mM sodium citrate buffer, pH 7.0, decreased the effect of native DNA on the difference spectrum of 4-NQO at 417 mμ. Sodium chloride was added to both the reference and sample cuvettes, and the absorbance values were corrected for volume changes.

Difference spectrum measurements at 1 M sodium chloride showed a 4-fold decrease in the interaction of 4-NQO with native DNA, as compared with values obtained in 0.5 mM sodium chloride–0.5 mM sodium citrate buffer (Fig. 5). The effect of increased sodium chloride may reflect competition between 4-NQO and inorganic ions for charged sites on the DNA molecule.

Figure 6 summarizes a plot of $\bar{\nu}/c \times 10^3$ against $\bar{\nu}$ for the conditions described in the legend. Since the existence of a linear plot would indicate only one site of interaction (12, 21), the nonlinearity of the plot is indicative of the presence of at least two types of binding sites (20) for 4-NQO on the native DNA molecule. Type I sites were weakly reactive ($K \simeq 0.9 \times 10^3$ M^{-1}, $\bar{\nu} = 0.65$), while type II sites were strongly reactive ($K \simeq 8 \times 10^3$ M^{-1}, $\bar{\nu} = 0.30$). This nonlinearity of the isobestic curve is reminiscent of the interaction of the quinoline derivatives (12) and quinine (13) with polynucleotides.

DISCUSSION

The interaction of DNA and 4-NQO has been well documented (7, 8). The significance of the interaction with DNA of the carcinogenic derivatives of 4-NQO studied in relationship to the process of carcinogenesis is obscure. The noncarcinogenic derivatives studied did not appear to interact to a significant degree with native DNA in flow dichroism studies (7, 8). Evidence has been presented that a metabolic reduction product (4-hydroxylaminoquinoline 1-oxide) (25) of 4-NQO is the proximal carcinogen, while

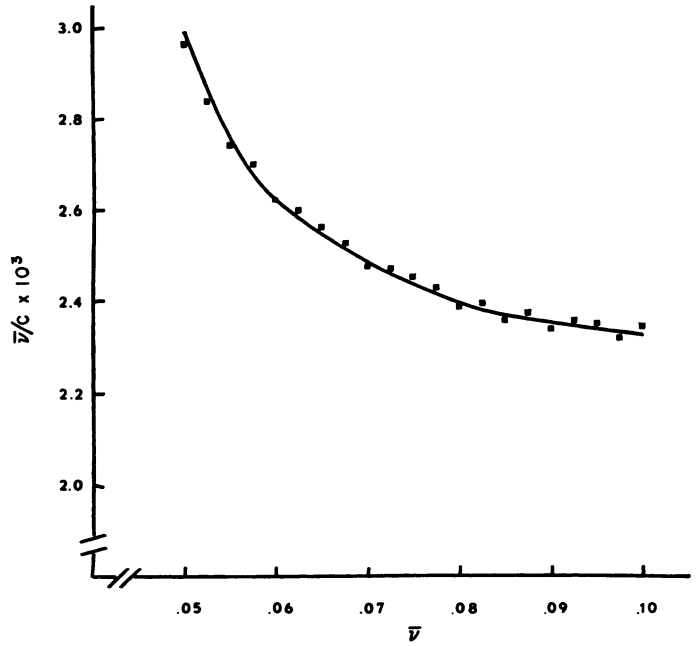

FIG. 6. *Binding of 4-NQO to native calf thymus DNA*

$\bar{\nu}$ represents the number of ligands (4-NQO) bound per polymer molecule, and c is the concentration of the free ligand (moles per liter). The spectrophotometric measurements were carried out in cuvettes of 1.0-cm path length at 25°. The system contained, in an initial volume of 2 ml, 5×10^{-5} M 4-NQO and 0.5 mM sodium chloride–0.5 mM sodium citrate, pH 7.0. Native DNA was dissolved in the sodium chloride–sodium citrate buffer. The plot of $\bar{\nu}/c \times 10^3$ against $\bar{\nu}$ is clearly nonlinear.

recent reports have postulated that the *O,O'*-diacetyl derivative is the proximal carcinogen (26), based upon covalent bonding of a portion of the quinoline derivative with deoxyguanosine in DNA. It has been pointed out that it is not presently possible to exclude 4-NQO itself from direct involvement in the process of carcinogenesis (27).

Few studies have been directed toward elucidating the base specificity possibly involved in the interaction at the polymer level. One study (28) implicated guanosine as the primary site of interaction, as determined by cesium chloride analytical ultracentrifugation of poly d(A-T) and poly dG:dC. Another study (29) implicated adenosine as the preferential site of complex formation, based upon thin-layer chromatography, although poly dG:dC was not investigated. Studies of the interaction at the deoxyribonucleoside level have questioned the role of adenosine without the involvement of guanosine in the formation of the DNA–4-NQO complex (10).

Based upon difference spectral analysis, the polydeoxyribonucleotide dG:dC exhibited less interaction with 4-NQO than did native DNA, although the degree of hypochromicity may not exactly parallel the extent of interaction. This discrepancy has been observed in studies of the interaction of chloroquine with DNA (12). Native DNA exhibited greater interaction with 4-NQO, in agreement with the results of ultraviolet flow dichroism studies (7). However, these flow dichroism studies were not extended to poly d(A-T) and poly dG:dC.

The important role of guanosine in complex formation between DNA and 4-NQO is reminiscent of those reported for actinomycin D (30), chloroquine (12), and quinine (13), the latter two compounds containing a quinoline ring with extensive side chains. The substantial interaction of 4-NQO with poly A (native DNA > poly A) (7) and the somewhat greater affinity of 4-NQO for native DNA than for poly dG:dC indicates that adenosine is involved in some manner

in the interaction of 4-NQO with DNA. In view of the low affinity of 4-NQO for poly d(A-T), adenosine may act indirectly by modifying the properties of guanosine to enhance complex formation. 4-NQO interacts equally well with deoxyadenosine and deoxyguanosine, and only slightly with deoxycytidine and thymidine, on the nucleoside level (8–11). Evidently the specificity of the polymers cannot be compared quantitatively with the specificity on the nucleoside level. The specificity of actinomycin D for guanosine can be interpreted as resulting from dependence either on participation of the amino group of guanosine in hydrogen bonding (24, 31) or on the unique electron donor-acceptor properties of guanosine (32, 33). From the evidence presented in this study on 4-NQO, we cannot distinguish with certainty between these two alternative hypotheses. The planar nature of the 4-NQO molecule, its electron-acceptor properties (34), and the spectral changes, both on a nucleoside level (9, 10) and with DNA (7, 8), make intercalation an attractive hypothesis. It has been reported that DNA in the presence of proflavine does not inhibit the formation of the DNA–4-NQO complex (29); it was concluded that proflavine and 4-NQO occupy different sites. The possibility that 4-NQO can displace proflavine or exist in an equilibrium condition cannot be excluded.

The increased T_m point of DNA in the presence of 4-NQO is quite similar to the effect seen when DNA is exposed to a variety of compounds, including the acridines (23), actinomycin D (22), and various antibiotics (35).

The abolition of the complex by urea at concentrations at which DNA maintains a double-helical structure (24) may reflect altered properties of DNA and/or 4-NQO at high ionic strengths or may indicate some role of hydrogen bonding. The work of Klotz and Shikama (36) has demonstrated that urea may alter the properties of water in such a manner as to affect hydrophobic interactions. Perhaps urea disrupts the 4-NQO–DNA complex primarily by this means rather than by directly affecting hydrogen bond formation between the oxygen of the 1-oxide group and DNA. 4-NQO contains two highly polar regions; the oxygen of the 1-oxide group and the nitro group, which contains 2 negatively charged oxygen atoms and a positively charged nitrogen atom. These regions could conceivably interact with oppositely charged groups on deoxyguanosine, the ring oxygen of the deoxyribose groups, or the highly polar phosphorus and oxygen atoms in DNA. From a theoretical viewpoint the 1-oxide group could certainly participate in a hydrogen bond with the amino group in deoxyguanosine. It is interesting that both the 1-oxide group and a nitro group in position 4 of the quinoline ring are mandatory for carcinogenicity (37), and both were required in derivatives tested for complex formation (7). However, they do not constitute the sole requirement for complex formation, since other functional groups can modify binding to DNA (7). The addition of a 6-carboxy group decreases binding to DNA (7) and deoxyguanosine (11), while the addition of a 6-chloro group increases complex formation with DNA (7) and deoxyguanosine (11).

In view of the nonlinear binding curve reported in this paper, two or more sites of interaction were probably involved in complex formation between 4-NQO and DNA. Similar isobestic curves have been reported in the interaction of two quinoline derivatives, chloroquine (12) and quinine (13), with DNA. There also appears to be some ionic competition for binding sites between 4-NQO and sodium and/or chloride ions, in view of the partial dependence of the 4-NQO–DNA complex on the concentration of sodium chloride. The same effect of sodium chloride has been reported by other workers (7).

The shift to longer wavelengths in the difference spectrum of the DNA–4-NQO complex in this paper shows that the 4-NQO concentration approached a limiting value, beyond which there was no further wavelength shift. Presumably, therefore, 4-NQO does not undergo aggregation, as reported for the acridines (38).

The dependence of complex formation between DNA and 4-NQO on ionic strength suggests that ionic groups may be important for the interaction. More information is

needed to determine the relative importance of these factors and to construct a model of interaction which would explain the possible participation of hydrogen bonding and charged groups.

Intercalation of the 4-NQO molecule with DNA might be one mode of interaction, with another site involving binding of the highly polar 1-oxide and 4-nitro groups to charged sites on the exterior of the DNA helix, where phosphate groups and possibly the amino group of guanosine could participate in charge-transfer interactions and hydrogen bonding, respectively.

In view of the greater interaction of native DNA than the double-helical polymer dG:dC with 4-NQO, certain subtle factors may be present in native DNA which are not imitated by the polymer. It is also possible that the adenosine groups in DNA are directly involved in complex formation with 4-NQO or modify the properties of adjacent guanosine residues in DNA to render them more liable to complex formation with 4-NQO.

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