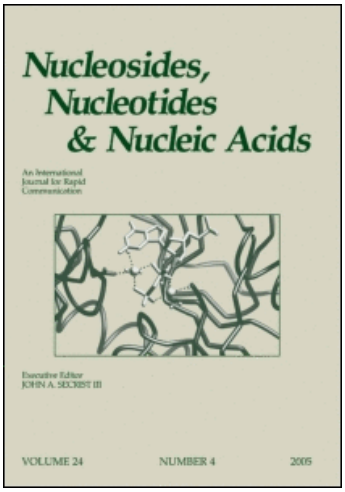

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Interaction of the Deoxy-Oligonucleotide Duplex d(CGCGATCGCG)$_2$ and Anti-Herpes Virus Active Indolo [2,3-*b*]-quinoxaline Derivatives

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INTERACTION OF THE DEOXY-OLIGONUCLEOTIDE DUPLEX d(CGCGATCGCG)$_2$ AND ANTI-HERPES VIRUS ACTIVE INDOLO [2,3-*b*]-QUINOXALINE DERIVATIVES

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ABSTRACT: *Ellipticine (1) and the derivatives 2,3-dimethyl-6(2-dimethyl-aminoethyl)6H-indolo-(2,3-b)quinoxaline (2) and 6-(2-dimethylaminoethyl)6H-indolo-(2,3-b)quinoxaline (3) intercalate in the duplex d(CGCGATCGCG)$_2$ with slow exchange kinetics. (1) and (2) show a non-specific interaction. (3) shows an AT specific interaction.*

The plant alkaloid ellipticine (5,11-dimethylpyrido[4,3-*b*]-carbazole, 1) and the related compounds (2), and (3) are active against herpes simplex virus type 1 [1,2]. Light absorption, linear dichroism and fluorescence spectroscopy have given direct evidence that ellipticine and compound (3) bind by intercalation [3,4]. In the case of (3) significant binding specificity for alternating AT sequences has been observed.

We used 500 MHz ^{1}H n.m.r. spectroscopy to study the mode and site specific interactions of these compounds with the decadeoxyribonucleotide d(CGCGATCGCG)$_2$. From the spectral changes at 2°C in 0.01 M phosphate buffer (pH 7.0) compounds (1) and (2) showed no base preferences, indicated by the appearance of a complexity of new imino proton resonances. (3) showed a primary binding between the AT base pairs with a minor secondary binding between the AT and adjacent GC4 base pairs. Here we observed saturation transfer between the new and original imino proton resonances. The presence of both the original and new imino proton resonances following drug titration suggests a slow chemical exchange *i.e* drug residence time in the duplex of greater than 1ms. A doubling of the new imino proton resonances indicates the loss of the two fold symmetry of the duplex.

Clearly the drugs bind by intercalation, as the new imino proton resonances appear *ca.* 1.0 p.p.m. upfield shifted from those of the original imino protons. Since the ring-current shifts on the imino protons from the drugs, are larger than those from neighbouring base pairs. We also observed slight upfield shifts for the aromatic protons of drug (3), when titrated with the oligonucleotide with subsequent severe line broadening at 25°C in 0.01 M deuterated phosphate buffer (pD 7.0).

Through pH titrations monitored by optical absorbance and fluorescence spectroscopy in 0.05 M phosphate buffer at 25°C the drugs showed two pKa's. Ellipticine was predominantly uncharged at pH 7.0 *i.e.* $pKa_1 < 1.0$ and pKa_2 5.8 whereas, (2) and (3) carried at least two charges *i.e.* $pKa_1 < 1.0$ and pKa_2 8.0 and 9.0 respectively. Here pKa_2 corresponds to the protonation of the ring N-2 for ellipticine and one or both of the ring nitrogens for (2) and (3). The aliphatic side chain amino group is probably protonated throughout the acidic and lower alkaline pH range. Thus, since drug (3) carries several positive charges it is likely that it enters the duplex via the minor groove. The minor grooves of AT sequences are sites of location of the deepest molecular electrostatic potential in the DNA. We also observed drug-induced non-uniform broadening of the resonances (in intermediate chemical exchange), in the spectral region of the non-exchangeable aromatic proton resonances of the duplex at 25°C in 0.01 M deuterated phosphate buffer (pD 7.0). Significant line broadening of the aromatic protons of the AT bases was notable also the sugar H1' of adenine. This again suggests that drug (3) enters the duplex via the minor groove. Severe line broadening in both the oligonucleotide and drug proton resonances made it impossible to derive NOE relationships between the drug and the oligonucleotide.

The observed AT specificity and formation of a stable intercalated complex for (3) may be attributed to the presence of positive charges in its structure and the aliphatic side chain. There may be an initial electrostatic attraction to the AT minor groove. A tight steric fit, helped by the side chain acting as an anchor may account for the formation of a more stable complex compared to ellipticine which is uncharged and does not have a side chain. For drug (2) the observed nonspecificity may be attributed to the formation of an unstable intercalated complex. Thus the side chain alone can not account for the formation of a stable complex.

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