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High Resolution NMR Studies of Chromomycin - Oligonucleotide Interactions: Structure and Sequence Specificity.

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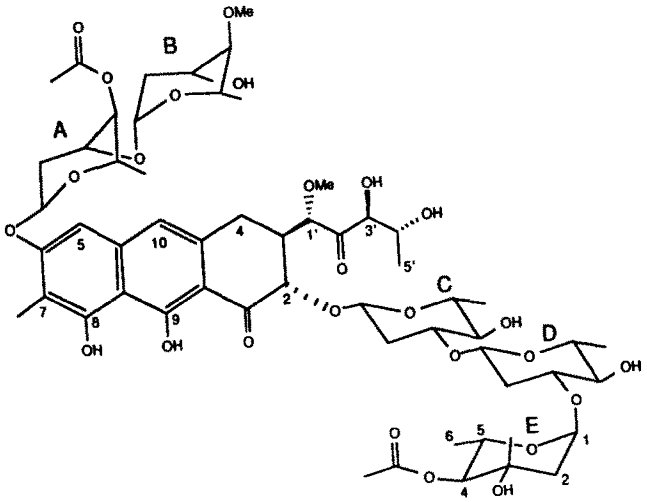
Chromomycin A_3 is a member of the aureolic acid antitumor antibiotics which binds to DNA in the presence of divalent cations with a preference for GC base pairs. It is a potent inhibitor of DNA dependent RNA polymerase as well as a clinically useful anticancer agent (1). We have analyzed the interaction between chromomycin A_3 and several oligonucleotides using the techniques of one dimensional and two dimensional Nuclear Magnetic Resonance (NMR).



Structure of Chromomycin A_3

Our initial studies (2) focused on the complex formed between this drug and the self-complementary hexamer d(ATGCAT)$_2$. Spectrophotometric binding studies demonstrated that this hexamer mimicked large DNA as a drug binding site, both in terms of equilibrium as well as kinetic behavior. One dimensional nuclear Overhauser effect experiments (NOE) showed that the 9-OH proton of the drug chromophore was close to one of the G-C imino protons. Also, the chromophore 7-methyl group was shown to lie close to either the GH8 or TH6 proton of the DNA base bairs or both. Two dimensional NOE experiments revealed additional drug-DNA contacts between several protons on the B sugar of the drug and the GH8 and TH6 protons as well as contacts between the drug aliphatic side chain methoxy group and the internal AH8 proton. A contact was also observed between the drug chromophore methyl group and the internal thymine sugar 1' proton. The complex remained stable upon heating to temperatures as high as $45^{\circ}C$, with resonances from one GC imino proton and the drug chromophore 9-OH proton remaining sharp.

The drug-oligonucleotide contacts described above can be used to develop a model of the drug binding in the major groove of the DNA, with the hydroxyl edge of the chromophore proximal to the DNA and the aromatic proton edge distal to the DNA. Additional drug-drug contacts suggest that both the alphatic and disaccharide side chains are folded back towards the aromatic proton edge of the drug chromophore.

The interaction between chromomycin A_3 and the octamer $d(ACCGGGT)_2$ was also examined. This sequence was shown to be a good binding site for the drug by DNA footprinting studies carried out by others (3). In this case, two different but thermodynamically equivalent complexes were observed. The pattern of drug-oligonucleotide contacts seen here was similar to that found for the $d(ATGCAT)_2$ complex, also suggesting binding in the major groove. The drug-octamer remained stable up to temperatures exceeding 60^oC.

Experiments carried out on the complex formed between chromomycin A_3 and the hexamer $d(ATCGAT)_2$ indicated a very weak type of complex with very little effect on the DNA proton chemical shifts. This shows that there is a strong effect of base sequence on the binding of chromomycin A_3. Finally, experiments have also been performed on the drug complexed with the octamer $d(ATAGCTAT)_2$. In this case, significant effects were seen at both the GC imino protons and the internal AT imino protons, with drug contacts to both of them.

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