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ANALYSIS OF DNA AND DNA-LEXITROPSIN INTERACTION USING MULTINUCLEAR NMR AND MOLECULAR DYNAMICS

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^{13}C-nmr spectroscopy of DNA oligomers shows that C4 of T and C2 and C6 of G are sensitive indicators of the presence of Watson-Crick hydrogen bonds. These carbons, attached to heteroatoms involved in H-bonds, are predicted to have the largest deshielding according to molecular orbital calculations of H-bonding effects [1]. The three carbons undergo shielding increases of about 1 ppm upon duplex melting, contrary to the deshielding expected from the loss of ring current shielding. Several other carbons show similar, though smaller effects.

Netropsin was added to $[d(GGTATACC)]_2$ to explore the effects of hydrogen bonding on the chemical shifts of the base carbons. 0.6 ppm Deshielding was observed at C4 of one A, thought to be involved in a strong H-bond from netropsin to AN3. A 1.4 ppm shielding increase at T(5)C2 is consistent with disruption of a H-bond between the carbonyl oxygen and water without formation of a strong H-bond to netropsin.

Netropsin analogs, called lexitropsins, have altered binding specificity. Lex371, is a singly charged analog with N-methyl imidazole substituted for N-methyl pyrrole, which binds preferentially to the sequence d(CCGT) + d(ACGG) as judged by DNase I footprinting experiments on a restriction fragment of pBR322 DNA [J. Lown & J. Dabrowiak, private communication]. The MNDO optimized configuration of Lex371 was docked to this sequence using real-time molecular graphics. The structure of the complex was then refined using the molecular dynamics program CHARMM (M. Karplus). The refined structures indicate the likelihood of H-bonds from the GN2H amino proton to the imidazole (IMD) nitrogens, as suggested by [2,3]. However, the details of the interaction are different in that the lexitropsin amide adjacent to the IMD also binds the N3 of the same G base (see Figure below). This H-bond "clamp" may be a significant feature in the sequence specificity of this category of lexitropsins.

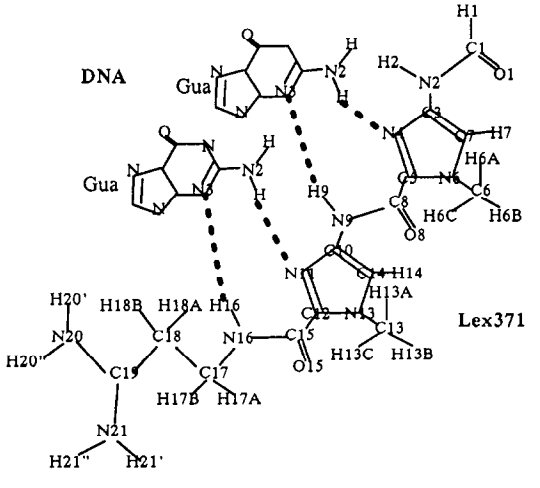

Fig.1 Lex371 and Guanine H-bond clamp

A comparison was made of the energy-minimized structures of Lex371 with the preferred sequence and a sequence that Lex371 does not protect from DNase I in the pBR322 fragment.

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          1 2 3 4 5 6 7 8 9 10
On1 = 5'G A T C C G T T A G3'
        * *
          1 2 3 4 5 6 7 8 9 10
On2 = 5'G A A C C C T T A G3'
        * *
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The stars indicate the substitutions made between On1 and On2 and the underlined bases show the preferred and non-preferred sequences in the pBR fragment. In the energy-refined structures, Lex371 clamps the G-bases bound to C(4) and C(5) in both complexes, and in neither structure is there an interaction between IMD and a base at chain position 6. This observation, together with the following results on contributions to the total interaction energy and groove widening, suggest that simple predictions of H-bond "reading" by lexitropsins may not be accurate. The net interaction energy is 11 kcal lower for the On1 complex than for the On2 complex, but the changes are significant in the van der Waals and electrostatic contributions as well as in the H-bonding interactions. Lex371 binding causes substantial widening of the minor groove in both duplexes, causing the distance between the nearest phosphates in the minor groove to increase an average of 1.2 Å in On1 while only 0.5 Å in On2. NOESY measurements on the complex with the preferred sequence are in progress in Prof. Lown's lab. When these are combined with energy minimization and real-time graphics, a complete picture of the Lex371 complex should become possible.

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