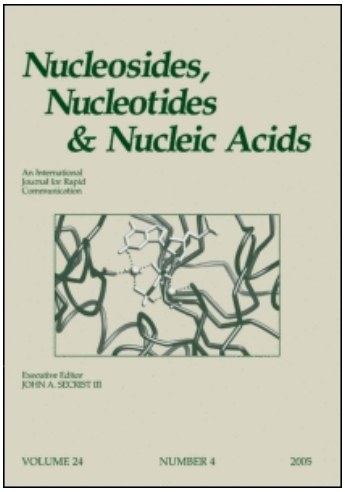

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NMR Structure of an Oligonucleotide Containing A Base Pair 3-(2-Hydroxyethyl) Deoxyuridine-Adenine

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NMR STRUCTURE OF AN OLIGONUCLEOTIDE CONTAINING A BASE PAIR 3-(2-HYDROXYETHYL) DEOXYURIDINE-ADENINE.

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ABSTRACT: It has been shown that ethylene oxide reacts with dC at the N3 position to produce a potentially mutagenic lesion, 3-(2-hydroxyethyl) deoxyuridine (3-HE-dU). In this article, we report NMR and Molecular Mechanic studies of a duplex containing the 3-HE-dU base with an adenine in front of the lesion which is in the sequence, 5'-GCAAGTC(3-HE-dU)AAAACG.

Ethylene oxide belongs to the class of aliphatic epoxides which are highly reactive compounds known to be effective mutagens and carcinogens [1-2]. It is widely used as an intermediate in the chemical industry. It was classified by the international Agency for Research on Cancer as a human carcinogen [3]. Ethylene oxide is a direct acting alkylating agent which reacts with DNA[1]. For example, it has been shown that ethylene oxide reacts with dC at the N3 position to produce a potentially mutagenic lesion, 3-(2-hydroxyethyl) deoxyuridine (3-HE-dU) as shown in FIG. 1. This modified base is chemially stable in DNA [4] and no repair activity has been found in prokaryotes or eukaryotes. In the absence of proofreading, dA could be incorporated opposite the lesion by DNA polymerases [5]. Since 3-HE-dU is derived from dC, the initial G·C base pair is mutated to an A·T base pair. We have incorporated the 3-HE-dU base in an oligonucleotide, a 14-mer, to study the 3-HE-dU·A base pair which is at the origin of the mutation. NMR spectra were recorded and treated as described in detail previously [6]. Model building and minimizations were done as explained previously [7-8]. This study will help us to understand the mutagenic aspect of 3-HE-dU and what determines the insertion by the polymerase.

FIG. 2 shows the H8/H6-H2'/H2" region of a NOESY spectrum recorded in ^2H_2O. The typical connectivities predicted for a right-handed B-type DNA duplex can be followed all along both strands. At the mispair site the intra and internucleotide interactions are well resolved. These observations demonstrate that the 3-HE-dU·A base pair is stacked into the

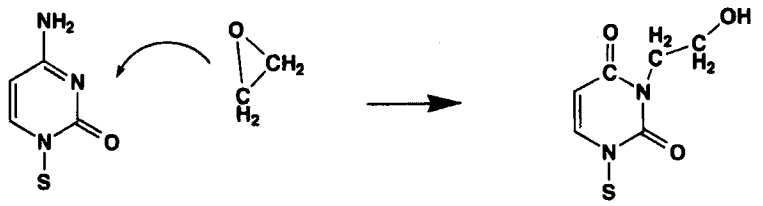

FIG.1: The formation of the 3-(2-hydroxyethyl) deoxyuridine.

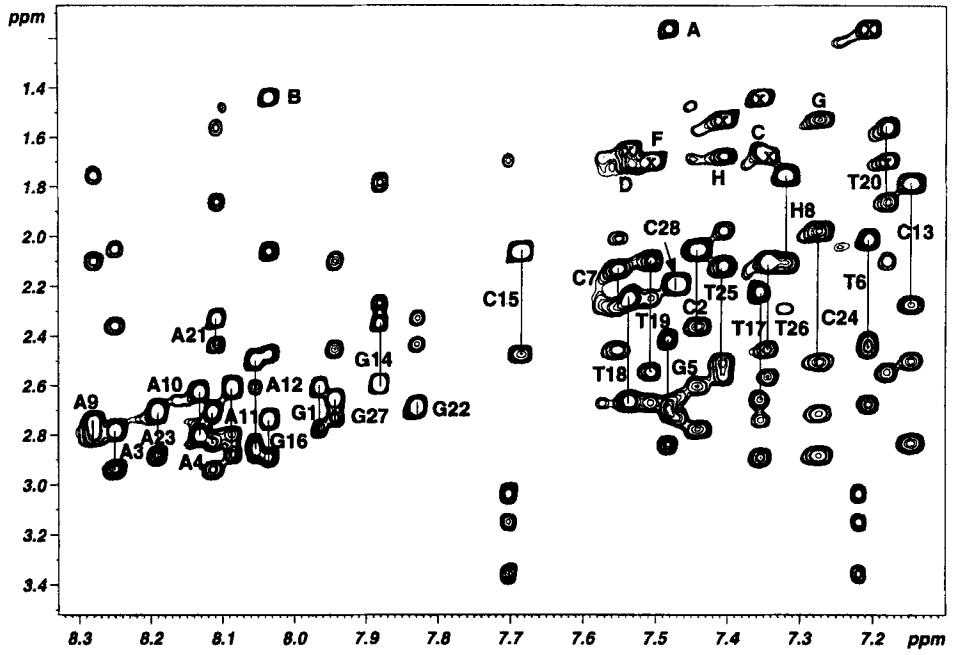

FIG. 2: Expanded contour plot of the H8/H6-H2'/H2" region of a NOESY spectrum (110 ms mixing time, 20°C) in ^2H_2O. Peaks labelled A to H correspond to the H8/H6(n)-CH_3(n+1) peaks.

helix which adopts a global B-DNA structure. We could identified all the expected resonances for Watson-Crick base pairs of the duplex. At this stage, three resonances at 3.03, 3.15 and 3.35 ppm remain unassigned. The resonance at 3.03 ppm corresponds to two protons and the two others each to one proton. As we have already identified all the non exchangeable protons of the oligonucleotide except those of the CH_2-CH_2 of the 3-HE-dU base, they must belong to them. The CH_2-CH_2 protons of 3-HE-dU show principaly interactions with the two H2 protons of the adenine 9 and 21.

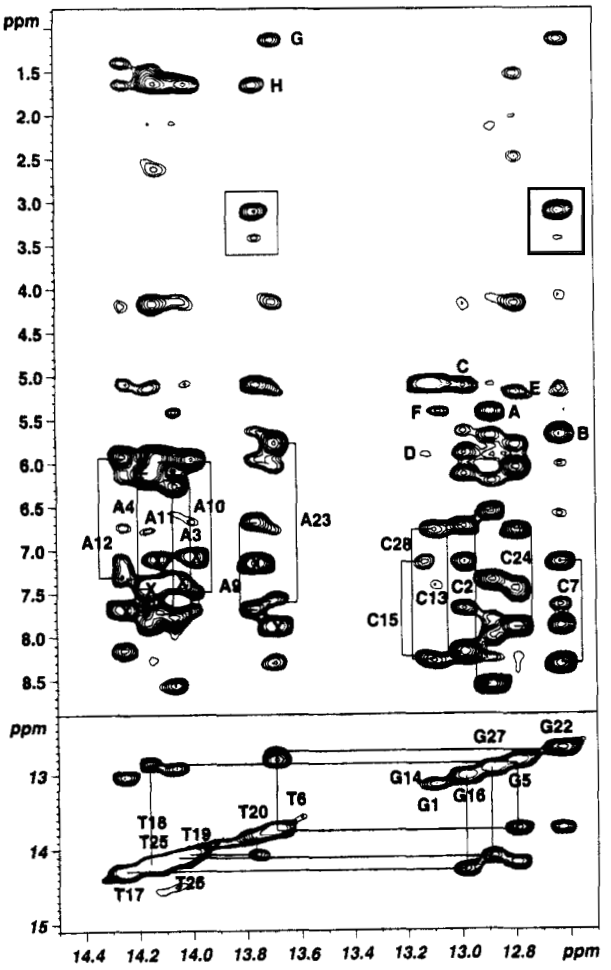

FIG. 3: Expanded contour plot of a NOESY spectrum (250 ms mixing time, 5°C) in H_2O. The upper part shows the interactions the imino protons and the amino/H5/H2 protons. The amino protons of the cytidines and the adenines belonging to the Watson-Crick base pairs are connected by a continuous line. The lower part shows the interactions between the imino protons.

FIG. 3 shows two regions of the 250ms NOESY spectrum recorded in H_2O. In the lower part, we can follow the imino-imino connectivities expected for non terminal Watson-Crick base pairs. In the upper part, the imino proton of G22 shows interactions with the H2 of A23 and A21 demonstrating that the adenines are inside the helix. The G22 and T20 imino protons show interactions with the CH_2-CH_2 chain of the modified base.

The NMR results demonstrate firstly that we observe only one species or one family of similar conformations in solution, secondly that the 3-HE-dU·A base pair stacks inside the

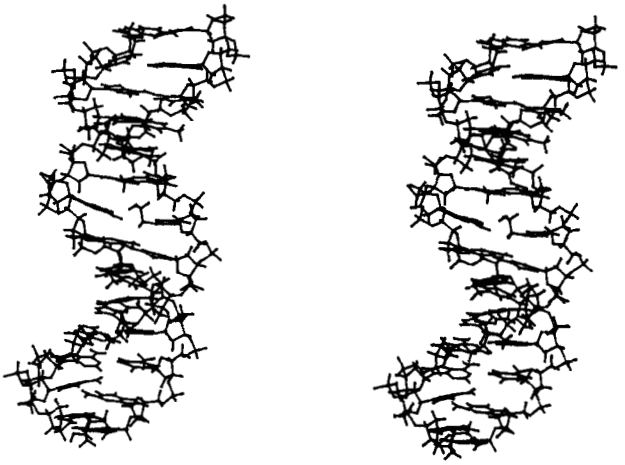

FIG. 4: Stereo view of the duplex after minimization.

helix which is globally in a B-form. The two base pairs C7·G22 and A9·T20 around the 3-HE-dU·A mispair are well formed. The CH_2-CH_2 chain of the modified base must be inside the helix as it presents mainly interactions with protons in the center of the helix, the two H2 protons of A9 and A21 and the two imino protons of T20 and G22. The duplex model has been built in a B-DNA form as shown by NMR. We have generated a set of 256 structures by varying the angles of the CH_2-CH_2-OH chain to explore all the disponible space inside the helix. The best familly of structures was selected by considering firstly both the NMR distance violations and the energy of the structures and secondly we have analysed all the remaining structures which show hydrogen bonding potential for the hydroxyl group of the 3-HE-dU base. The best pairing involved the N1 of A21 and the H9 of the 3-HE-dU base. One example of the best structures is presented in FIG. 4.

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