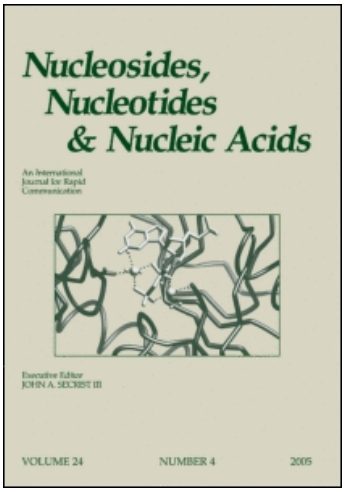

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Site-Specific ^{13}C Labeling of DNA to Deduce DNA Repair Mechanisms of Uracil-DNA Glycosyiase and UV Endonuclease V

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SITE-SPECIFIC ^{13}C LABELING OF DNA TO DEDUCE DNA REPAIR MECHANISMS OF URACIL-DNA GLYCOSYLASE AND UV ENDONUCLEASE V

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ABSTRACT

The oligodeoxynucleotide d(GCGUGCG) was synthesized with [1',3' -$^{13}C_2$]U labeling. The uracil unit was removed with uracil-DNA glycosylase to generate an abasic site and the resulting oligonucleotide was paired with the possible d(CGCNCGC) structures. One of these heteroduplexes was a substrate for UV endonuclease V. The ^{13}C NMR spectra of these heteroduplexes describe the structure of the abasic site and the mechanism of the endonuclease reaction.

INTRODUCTION

The enzymatic repair of chemical damage to DNA bases involves a complex series of events involving several enzymes. This repair is initiated by the hydrolytic excision of the modified base by a glycosylase enzyme to yield an abasic site followed by the cleavage of the phosphodiester bond(s) of the abasic site by an endonuclease.

In order to delineate the mechanisms of two such repair enzymes, uracil-DNA glycosylase and UV endonuclease V, and also to ascertain the structure of the abasic site, we have carried out site-specific ^{13}C labeling of oligodeoxynucleotides in the 1'- and 3'- carbons of the deoxyuridine unit.

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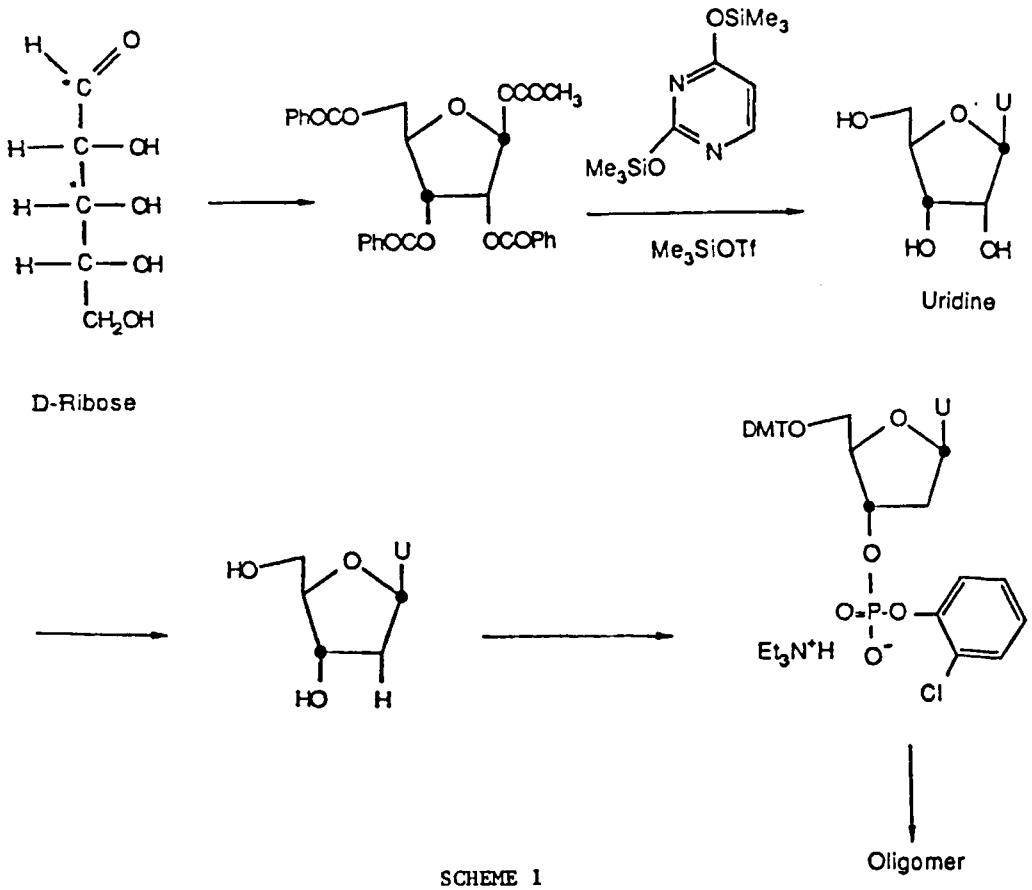


SCHEME 1

MATERIALS

[1,3 -$^{13}C_2$]-D-Ribose (Omicron Biochemicals) was converted to uridine by the method of Vorbruggen et al, and then chemically reduced to deoxyuridine by the method of Robins et al. The resultant [1',3'-$^{13}C_2$] deoxyuridine was converted to 5'-dimethoxytrityl-3'-O- chlorophenylphosphate deoxyuridine and incorporated into the oligomer, d(GCGUGCG) employing solution phase phosphotriester chemistry (scheme 1). The complementary strands (CGCNCGC), where N = A,G,T and C, were synthesized on a DNA synthesizer by using cyanoethylphosphoramidite chemistry.

Uracil-DNA glycosylase[1] from E.coli was isolated from heat induced cells of a strain of E. coli transformed with an expression plasmid containing the cloned gene for the enzyme. UV endonuclease V[2] was purified from a strain of E.coli transformed with a plasmid containing the den V gene downstream of the inducible tac promoter.

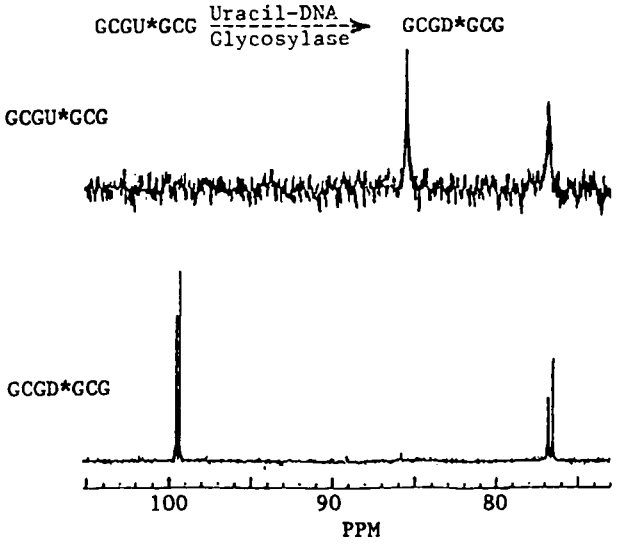


FIG. 1

RESULTS

The oligonucleotide d(GCGUGCG), designated as U strand, generated the desired abasic site on treatment with uracil-DNA glycosylase at pH 7.0 and at room temperature, to give d(GCGDGCG) where D is the deoxyribose unit. Mixing of d(GCGDGCG) (designated as D strand) with each of the possible complementary strands, d(CGCNCGC) where N = A,G,T and C resulted in duplex formation as evidenced by both the observation of imino proton spectra at 400 MHz in H_2O and aromatic and anomeric resonances which differed in chemical shift from those of the individual strands. From the temperature dependence of the imino spectra it is clear that these heteroduplexes with an abasic site are less stable than the intact A-U duplex. The ^{13}C spectrum of the D strand (Figure 1) showed the presence of both anomeric hemiacetals in a ratio of approximately 1:1 (at pH 7 and 15°). After mixing the D strand with the four "complimentary" strands, the ^{13}C resonances were not significantly altered in intensities. Thus the abasic site in each duplex is populated by approximately equal amounts of both anomers. Resonances associated with the open chain aldehyde and its hydrate could not be distinguished from the resonances associated with the natural abundance ^{13}C at other sites in the duplexes; therefore acyclic structures make structurally (but not chemically) insignificant contributions to the abasic site.

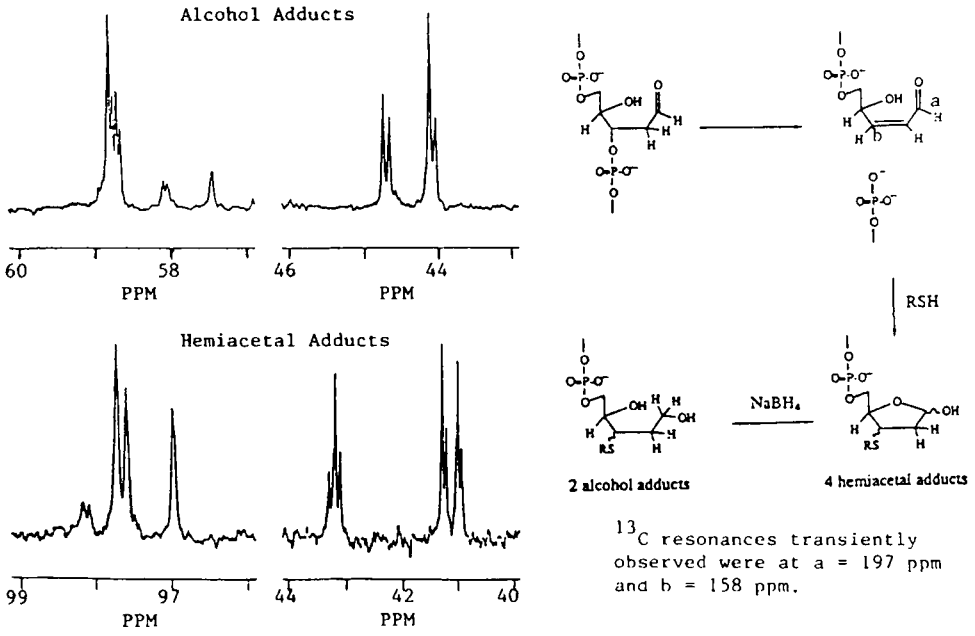
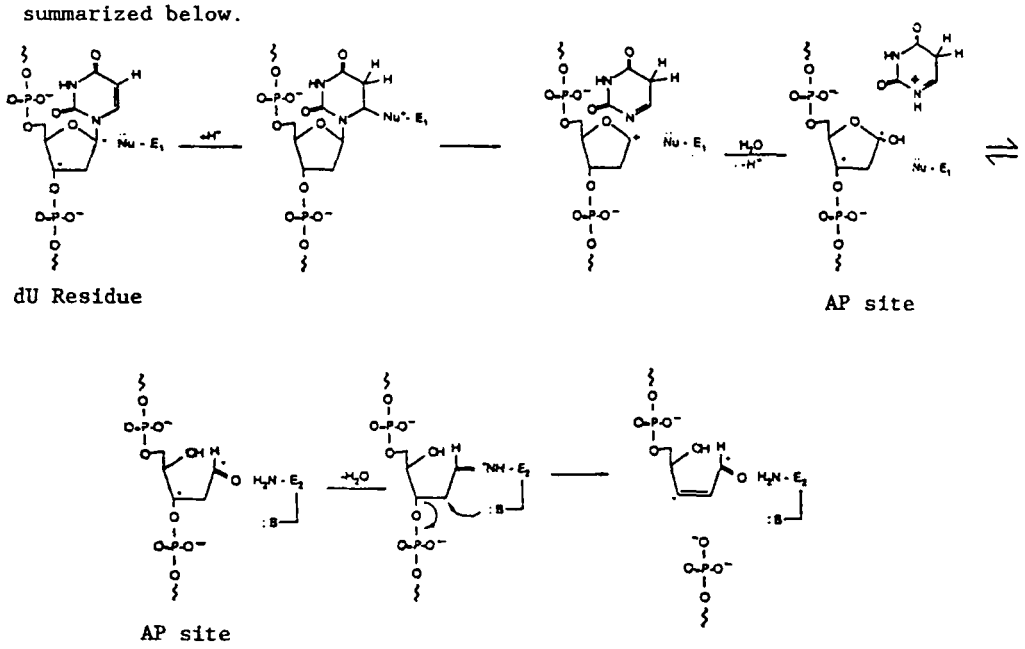


FIG. 2

The heteroduplex A-D [d(GCGDGCG)·d(CGCACGC)] is a substrate for UV endonuclease V. Before adding the enzyme, the ^{13}C resonances of the labeled carbons are seen at ca. 100 ppm (for the 1'-carbons) and at ca. 75 ppm (for the 3'-carbons). After the addition of the enzyme, these resonances diminished in intensity while resonances at 197 ppm (-CHO) and 158 ppm (β-olefinic) appeared transiently showing the formation of α,β-unsaturated aldehyde. Since the reaction mixture contained thiols (employed to preserve the enzymes) the product from cleavage underwent Michael addition[3] followed by cyclization to give cyclic hemiacetals as shown by signals at 97 ppm for the 1'-carbons and at 42 ppm for the 3'-carbons. Reduction of these cyclic adducts with $NaBH_4$ yielded open chain compounds carrying sulfur addendum at 3'-carbon (44 ppm) and primary alcohol at 1'-carbon (59 ppm) resulted (Figure 2). All these evidences clearly indicate that the mechanism of UV endonuclease V involves a β-elimination.

A possible mechanism showing the action of both these enzymes are summarized below.



dU Residue

AP site

AP site

Proposed mechanism for the uracil-DNA glycosylase (top) and the UV endonuclease V (bottom) reactions.

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1. Manoharan, M.; Ransom, S.C.; Mazumder, A.; Gerlt, J.A.; Wilde, J.A.; Bolton, P.H. J. Am. Chem. Soc. 1988, 110, 1620-1622 and references therein.

2. Manoharan, M.; Mazumder, A.; Ransom, S.C.; Gerlt, J.A.; Bolton, P.H. J. Am. Chem. Soc. 1988, 110, 2690-2691 and references therein.

3. For a similar reaction at a chemically generated abasic site, see Vasseur, j.-J.; Rayner, B.; Imbach, J.-L.; Verla, S.; McCloskey, J.A.; Lown, J.W.; Chang, D.K. Nucleosides & Nucleotides. 1987, 6, 467-468.