Mechanism of UV Endonuclease V Cleavage of Abasic Sites in DNA Determined by ^{13}C Labeling

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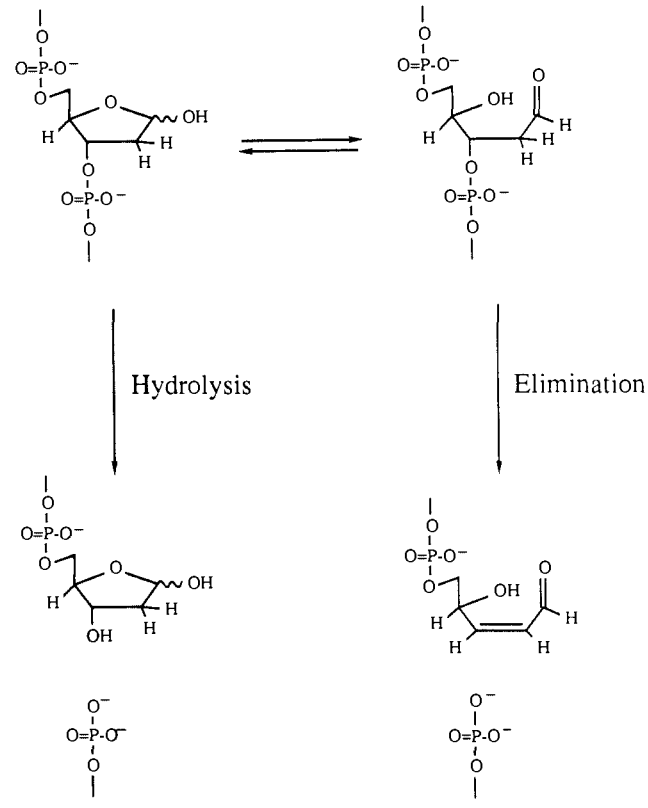
The enzymatic repair of chemical and photochemical damage to the bases of DNA involves hydrolysis of the glycosidic bond to the modified base followed by cleavage of the phosphodiester backbone on the 3'- and 5'-sides of the resulting abasic site.[2,3] Frequently, a single enzyme catalyzes both an *N*-glycosylase reaction and an endonuclease reaction resulting in cleavage of the phosphodiester bond on the 3'-side of the abasic site. Both UV endonuclease V from bacteriophage T_4 (UV endo V) and endonuclease III from *Escherichia coli* (endo III) catalyze *N*-glycosylase and the 3'-abasic endonuclease reactions. Speculation regarding the mechanism of the 3'-abasic endonuclease reaction has focussed on the potential for β-elimination of the 3'-phosphate of the abasic site rather than actual hydrolysis of the phosphodiester bond (Scheme I).[4] Recently, endo III was shown to labilize the 1- and/or 2-hydrogens of the abasic site during its conversion to product, and the sugar phosphate liberated by enzymatic degradation of the product differed from deoxyribose-5-phosphate; these observations were suggested to be evidence for the β-elimination mechanism.[5] In this communication we report the use of ^{13}C NMR spectroscopy to directly and definitively characterize the carbohydrate fragment derived from the abasic site in the reaction catalyzed by UV endo V; the observed α,β-unsaturated aldehyde establishes that the reaction proceeds by the β-elimination mechanism.

We recently described the chemical synthesis of d(GCGUGCG) in which the deoxyuridine moiety is labeled with ^{13}C in the 1'- and 3'-carbons[6] and its enzymatic conversion to isotopically labeled d(GCGDGCG) containing ^{13}C in the 1- and 3-carbons of the aldehydic abasic site (D).[7] The heteroduplex formed by mixing d(GCGDGCG) with d(CGCACGC) is a substrate for UV endo V as judged by both HPLC and gel electrophoresis.[8]

The ^{13}C NMR spectra recorded before and 1.75, 5.25, 12.25, and 24 h after the addition of UV endo V to the isotopically labeled heteroduplex are shown in Figure 1.[12] Prior to the addition of the enzyme (spectrum A), the ^{13}C resonances associated with the substrate were those previously described for the mixture of hemiacetals, i.e., two resonances associated with the anomeric carbons at ca. 100 ppm and two degenerate resonances associated with the 3-carbons at ca. 75 ppm.[7] After the addition of enzyme (spectra B through E), these resonances diminished in intensity and single resonances at 197.5 and 157.6 ppm transiently appeared; these can be assigned to the aldehydic and β-vinyl carbons, respectively,[13] of the α,β-unsaturated aldehyde obtained from the β-elimination mechanism. Thus, these ^{13}C NMR data demonstrate directly that the product of the degradation of the abasic site by UV endo V is the α,β-unsaturated aldehyde obtained by β-elimination of the 3'-phosphate (Scheme I).[14]

Scheme I



The spectra in Figure 1 also demonstrate that the enzymatic product is subsequently converted to a mixture of adducts by addition of nucleophiles to the α,β-unsaturated aldehyde. The resonances at ca. 97 ppm and at ca. 42 ppm that appear can be associated with the 1- and 3-carbons, respectively, of adducts formed by the addition of two nucleophiles to the α,β-unsaturated aldehyde.[15] Since the reaction mixture contained thiols,[17] it is

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(8) UV endo V was purified according to a modification of the published procedure[9] from a strain of *E. coli* transformed with a plasmid containing the denV gene downstream of the inducible tac promoter.[10]
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(11) The single strand d(GCGUGCG) was 5'-end labeled with [γ-^{32}P]-ATP and polynucleotide kinase, treated with uracil-DNA glycosylase, and mixed with d(CGCACGC). After incubation with UV endo V, the reaction mixture was electrophoresed on a 20% polyacrylamide gel; the extent of reaction was assessed after autoradiography of the dried gel.
(12) The incubation was carried out in the NMR probe at 17 °C in a solution of 10 mM sodium cacodylate, pH 7.0, containing 1 mM heteroduplex, 0.1 M NaCl, and 0.1 mM EDTA.
(13) The ^{13}C NMR chemical shifts of the aldehydic and β-vinyl carbons of 2-butenal are 193.4 and 153.9 ppm, respectively: Bremser, W.; Ernst, L.; Franke, B. *Carbon-13 NMR Spectra Data*; Verlag Chemie: New York, 1979.
(14) Since both the single strand d(GCGDGCG) and the heteroduplex containing the abasic site are stable under the conditions of the reaction in the absence of UV endo V, it is unlikely that the hydrolysis mechanism is correct but obscured by rapid elimination of water from its predicted product (Scheme I) to generate the observed α,β-unsaturated aldehyde.
(15) Since addition is possible on either face of the double bond and after the addition reaction the 1-carbon of the saturated aldehyde exists as an anomeric mixture of cyclic hemiacetals, four adducts should result from the addition of a nucleophile to the α,β-unsaturated aldehyde. Therefore, a total of eight ^{13}C NMR resonances should be observed for each labeled carbon if two nucleophiles add to the double bond. Seven (partially superimposed) resonances are observed for the labeled 3-carbons at ca. 42 ppm; a total of three broadened signals are observed for the 1-carbons at ca. 97 ppm, and this number may be explained by chemical shift degeneracy or, perhaps, relatively rapid equilibration of the hemiacetals. After reduction of the anomeric hemiacetals with $NaBH_4$, two diastereomeric adducts should be derived from addition of each nucleophile; four resonances are observed at ca. 44 ppm for the 3-carbons of the adducts, and four resonances characteristic of primary alcohols[16] are observed at ca. 59 ppm. These data are reproduced in the ^{13}C NMR spectra contained in the Supplementary Material.

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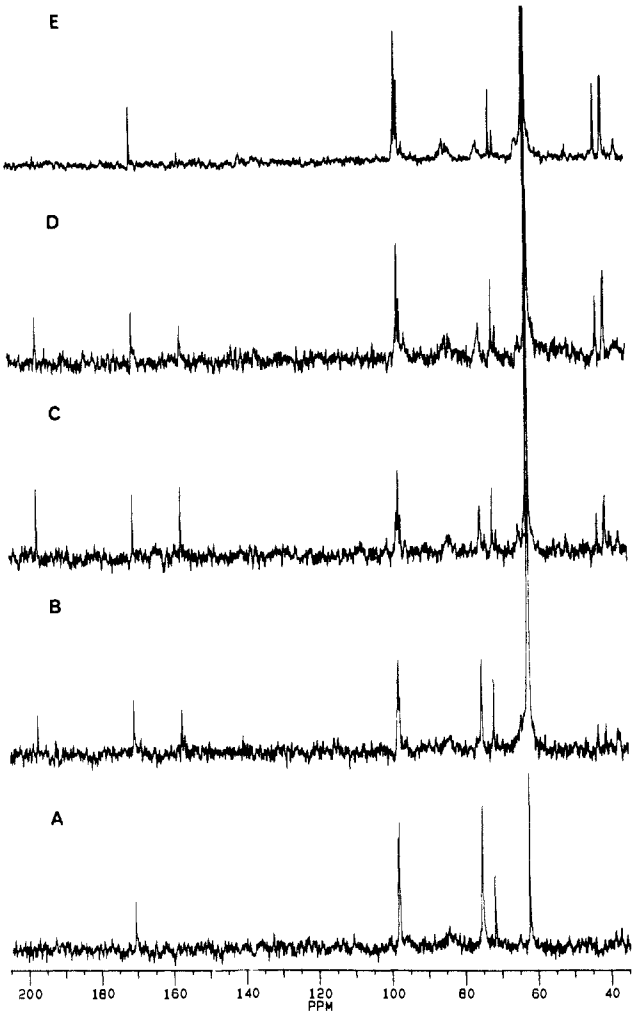

Figure 1. ^{13}C NMR spectra at 100 MHz illustrating the progress of the reaction catalyzed by UV endo V. Spectrum A was recorded before the addition of enzyme, and spectra B through E were recorded 1.75, 5.25, 12.25, and 24 h after the addition of enzyme. The resonance at 72 ppm is associated with the 2-carbon of the glycerol present in the storage buffer for uracil-DNA glycosylase, the resonance at 62 ppm is associated with the primary alcohol carbons of both the glycerol and the ethylene glycol present in the storage buffer for UV endo V, and the resonance at 171 ppm is associated with (ammonium) formate contaminating the heteroduplex; these resonances serve as convenient intensity standards during the course of the reaction.

likely that the adducts we have detected are derived from addition of these to the α,β-unsaturated aldehyde.[18] While the formation of such adducts does not alter our conclusion that the mechanism of the UV endo V catalyzed reaction involves a β-elimination mechanism, it does provide an explanation for the previously reported observation that more than one product is obtained from the action of endo III on abasic sites.[5]

Our observations on the cleavage of abasic sites in DNA catalyzed by UV endo V and those previously reported for the cleavage catalyzed by endo III[5] illustrate that the enzymatic cleavage of phosphate ester bonds can proceed by C–O as well as P–O bond scission. Further studies of the mechanism of the β-elimination reaction catalyzed by UV endo V are in progress.

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Supplementary Material Available: ^{13}C NMR spectra of the adducts formed by addition of nucleophiles to the α,β-unsaturated aldehyde enzymatic product (1 page). Ordering information is given on any current masthead page.

(16) The ^{13}C NMR chemical shifts of the 1-carbons of ethanol, 3-methylbutanol, and 3,3-dimethylbutanol are 57.0, 60.2, and 58.9 ppm, respectively: *Carbon-13 NMR Spectra Data*; Verlag Chemie: New York, 1979.

(17) The uracil-DNA glycosylase is stored in a buffer containing dithiothreitol, and UV endo V is stored in a buffer containing 2-mercaptoethanol.

(18) The ^{13}C NMR chemical shift of carbon-1 in 1-(methylthio)cyclohexane is 44.9 ppm: Eliel, E. L.; Kandasamy, D. *J. Org. Chem.* **1976**, *41*, 3899–3904.

The Amido–Ammine Bridging Ligand ($H_5N_2^-$)

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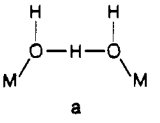
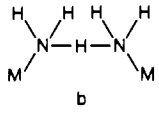
The role of very strong O–H–O hydrogen bonds in coordination chemistry, especially in the hydrolysis of metal ions, has been revealed in recent years.[2,3] An H_2O ligand of one metal atom may interact with an OH ligand of another metal atom by means of a very short (<2.50 Å) and centered hydrogen bond to form an ($H_3O_2^-$) bridging ligand (a) between the metal atoms. The



a

central role of this ligand in the structure of hydroxoaqua and related complexes has been reviewed recently.[3] The mechanism and steric course of *olation* (formation of μ-(OH) bridges between metal atoms) was shown to be controlled by the configuration of the H_3O_2 bridges in the reacting species.[4] The exceptional acidity of chromium(III) diols such as $[(H_2O)_4Cr(OH)_2Cr(H_2O)_4]^{4+}$ was ascribed to stabilization of the deprotonated species by formation of an H_3O_2 bridge (in addition to the two OH bridges).[5,3] This assumption was recently confirmed by a single-crystal X-ray study.[6]

An ammine analogue of a di-μ-(OH) metal complex, the di-μ-amidobis[tetraammineplatinum(IV)] ion, $[(NH_3)_4Pt(NH_2)_2Pt(NH_3)_4]^{6+}$, was shown to be very acidic,[7] with a pK_1 = 1.9, compared to $[Pt(NH_3)_6]^{4+}$ with pK_1 = 7.0. This remarkable acidity was first ascribed to deprotonation of a bridging amido ligand, but an NMR study ruled out this possibility[7,8] and supported the conclusion that deprotonation occurred at a *terminal* ammine ligand, located above the $Pt(NH_2)_2Pt$ plane, i.e., in a cis position relative to the two amido bridges.[8] Stabilization of such a deprotonated species may be achieved by strong hydrogen bonding of the terminal amido nitrogen with the ammine ligand of the second Pt atom located above the $Pt(NH_2)_2Pt$ plane, i.e., by formation of an ($H_5N_2^-$) bridging ligand (b), which is the ammine analogue of ($H_3O_2^-$).



b

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