**Proton magnetic resonance studies of ultraviolet-irradiated
apurinic acid**

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ABSTRACT

In apurinic acid, a single-stranded polydeoxyribonucleotide easily obtained upon depurination of DNA, the proton resonances arising from thymine and cytosine are readily observable in aqueous solution at 25°C. Two methyl thymine resonances, centered at 1.88 ppm and separated by 0.045 ppm, are observed. We attribute the downfield methyl resonance to thymines with no pyrimidine nearest neighbors and the upfield methyl resonance to thymines having pyrimidine neighbors in the 3' and/or 5' positions. Upon ultraviolet irradiation, the upfield methyl and thymine H-6 resonances decrease in amplitude and two methyl resonances appear at 1.63 and 1.52 ppm, corresponding, respectively, to cytosine-thymine and thymine-thymine cyclobutane dimers. Photoreversal eliminates these two minor methyl resonances from the pmr spectrum. We conclude that apurinic acid provides a suitable model system for pmr studies of chemically modified pyrimidine bases in DNA.

INTRODUCTION

The purpose of this report is to describe the use of apurinic acid as a simple model system for investigating the pmr behavior of thymine and cytosine residues of DNA. Previous magnetic resonance work in this area (1) has focused primarily on the pmr behavior of single-stranded DNA at temperatures above 90°C, due to the fact that below this temperature anisotropic dipolar broadening of the resonance occurs (2) as a result of the establishment of a semi-rigid base-stacked conformation.

We have found that in apurinic acid, a single-stranded polydeoxyribonucleotide that is easily obtained upon depurination of DNA, the proton resonances arising from the constituent thymine and cytosine bases are readily observable in neutral aqueous solution at 25°C; hence, studies on cytosine and thymine bases in a DNA-like environment (i.e., still attached to the sugar-phosphodiester backbone) are possible. We present here, by way of illustration, a pmr study of the photochemical changes that occur upon ultraviolet irradiation of apurinic acid.

EXPERIMENTAL SECTION

Preparation of Apurinic Acid. Apurinic acid was prepared from salmon sperm DNA (Calbiochem, A grade) by dissolving 200 mg of DNA in 100 ml of 66% formic acid and maintaining the solution at 37°C for 18 hr. This procedure results in a depurinated DNA of about 57,000 daltons (3). Removal of free purines and formic acid was accomplished by exhaustive dialysis against distilled water, and the apurinic acid was recovered by lyophilization. Before depurination of the DNA solution, a small amount of Escherichia coli DNA labeled with tritiated thymidine was added as a radioactive tracer.

Ultraviolet Irradiation. Apurinic acid was irradiated with a low-pressure mercury lamp (UVSL-25 Mineralight; Ultra-Violet Products, Inc.), which emits mainly at 254 nm. To enhance the rate of photodimerization, the apurinic acid solution was adjusted to pH 9, and stoichiometric amounts of Ag^+ were added before irradiation. Complexing of Ag^+ with DNA has been shown to enhance the rate of photodimerization of thymine 20- to 30-fold (4). After irradiation, the apurinic acid was complexed with CN^- and dialyzed against distilled water to remove the Ag^+ . The final product was recovered by lyophilization. The yield of photodimers was determined as described previously (5), using 98% formic acid at 175°C to hydrolyze the irradiated material and separating the products by paper chromatography with a butanol-water-acetic acid (80:30:12) solvent.

Proton Magnetic Resonance Measurements. Samples of apurinic acid were dissolved in D_2O . The dry residue from the formic acid hydrolysis of apurinic acid was dissolved in deuterated trifluoroacetic acid. Spectra were obtained with either a JEOL PS-100 spectrometer operated in the external-lock, field-swept mode or a Bruker HX-270 spectrometer. Line positions were measured relative to external 0.1 M 3-trimethylsilylpropane carboxylate- d_6 in D_2O (adjusted to pD 7.0) or to external tetramethylsilane in trifluoroacetic acid, as appropriate. The chart sweep was calibrated against a sample containing six known resonance lines (6).

RESULTS AND DISCUSSION

The complete 100-MHz pmr (7) spectrum of apurinic acid is shown in Figure 1A along with a portion of the 270-MHz spectrum in the region of the thymine methyl resonance. The assignments were made by analogy to DNA (1, 2), deoxyuridine (8), thymidine (9), and cytidine (9). Whereas two thymine methyl resonances separated

Figure 1. 100-MHz proton magnetic spectra of (A) apurinic acid before UV irradiation and (B) apurinic acid after UV irradiation to give a maximum decrease in the absorbance at 260 nm. Spectra were recorded at 27°C in D_2O at pD 7.0. Polymer concentrations were 60 mg/ml. Chemical shifts are expressed relative to external 3-trimethylsilylpropane carboxylate- d_6 . Shown in the inserts are 270-MHz spectra for the thymine methyl regions.

by 0.13 ppm are observed in a single-stranded DNA, the methyl resonances in apurinic acid are centered at 1.88 ppm and differ by only 0.045 ppm. This difference, which disappears at 80°C, is the same (in ppm) at both 100 and 270 MHz, indicating that it arises from chemical shift differences of a conformational origin, such as ring current shifts associated with base-base interactions. [The splitting due to spin-spin coupling between the thymine H-6 and methyl protons is slightly less than 2 Hz (1) and is neglected.] A similar chemical shift difference of 0.06 ppm was observed by McDonald

Figure 2. Decrease in the thymine methyl resonance (●) and increase in the photoproduct methyl resonance (O) (peaks "a" and "b" together) for apurinic acid as a function of UV irradiation time. Also shown is the concomitant decrease in the optical absorbance at 260 nm (▲) due to photoproduct formation. Equal transverse relaxation times were assumed for the various polymer resonances after UV irradiation. Peak heights were normalized to the H-2' resonance in each case.

et al. (1) between the single thymine methyl resonances of pdT and dT-dT. We conclude, therefore, that the basis for our observation of two thymine methyl resonances in apurinic acid is that the thymine methyl groups contained in this polymer are situated in two different types of environment — "isolated" (no pyrimidine in either the 3' or 5' neighbor position) and "surrounded" (at least one pyrimidine neighbor).

Irradiation of apurinic acid resulted in the appearance of two new methyl thymine peaks at 1.63 and 1.52 ppm ("a" and "b" in Figure 1B), and their intensity increased as a function of irradiation time (Figure 2). This increase was accompanied by a decrease in the intensity of the original thymine methyl resonance and a parallel decrease in the optical absorbance of the apurinic acid at 260 nm (Figure 2). There was also a significant decrease in the amplitude of the thymine H-6 resonance (~38%), but no intensity change could be discerned in either the H-5 or H-6 resonance of cytosine. Studies performed at 270 MHz showed clearly (see inserts in Figures 1A and 1B) that the upfield methyl

resonance of thymine (1.86 ppm) decreases more rapidly in amplitude upon irradiation than does the downfield resonance (1.90 ppm). It would thus appear that thymine residues with either a 3' or 5' cytosine or thymine neighbor are preferentially converted to photoproducts.

Evidence that peaks "a" and "b" correspond to photodimers was obtained from their photoreversibility in situ. Apurinic acid which has been extensively irradiated at pH 7 was reirradiated at pH 12. At this pH, thymine has lost the N-3 proton and dimerization is prevented, but dimers already present can be monomerized. Hence, once a dimer is split it cannot reform, and extended irradiation reverses all of the dimers. The pmr spectrum of a sample that had undergone photoreversal was identical in all respects to that of unirradiated apurinic acid. This result indicated that the minor methyl resonances in apurinic acid are photoreversible and correspond to thymine-containing dimers of the cyclobutane type.

In order to compare peaks "a" and "b" with the cyclobutane dimers known to be formed by thymine, irradiated apurinic acid was hydrolyzed to its components with formic acid (a treatment that results in the conversion of Cyt $\diamond$ Thy to Ura $\diamond$ Thy), and a portion of the hydrolysate was subjected to paper chromatographic analysis. Table 1 shows the percentages of thymine present in the form of Thy $\diamond$ Thy and Ura $\diamond$ Thy determined by this

TABLE 1

Distribution of thymine in ultraviolet-irradiated apurinic acid after formic acid hydrolysis

Method of analysis	Product (%)				
	Thymine	Ura $\diamond$ Thy	"a _h "	Thy $\diamond$ Thy	"b _h "
Paper chromatography	67	7	—	26	—
pmr	64	—	8	—	28

The values for chromatography were obtained only on the basis of the radioactivity found in the thymine and two dimer spots. Similarly, the values for pmr were obtained only on the basis of the amplitudes of the thymine methyl and the peak "a_h" and "b_h" resonances which were measured in deuterated trifluoroacetic acid and had chemical shifts of 1.95, 1.73 and 1.62 ppm, respectively. Equal transverse relaxation times were assumed in determining the amplitudes.

procedure. The rest of the hydrolysate was then subjected to pmr analysis. Two minor methyl peaks ("a_h" and "b_h") were observed, corresponding to peaks "a" and "b" in the pmr spectrum of the intact polymer (Figure 1B). [Because of the low solubility of the isolated bases in D₂O, deuterated trifluoroacetic acid was used as a solvent for the pmr measurements; hence, it was not possible to make a positive correlation of peaks "a_h" and "b_h" with the peaks found for the intact polymer in D₂O (Figure 1B).] The percentages of thymine in peaks "a_h" and "b_h" as shown in the second column of Table 1, are nearly the same as the percentages of thymine in the photoproducts Ura < Thy and Thy < Thy, respectively. The ratio of Thy < Thy and Ura < Thy determined chromatographically is 3.8, in close agreement with the value 3.5 obtained for the ratio of peak "a_h" to "b_h". In addition, the chemical shift for peak "b_h" (1.62 ppm) is in good agreement with the value for isolated cis-syn Thy < Thy obtained by Hollis and Wang (10) in this solvent. Since the spectrum of the hydrolysate closely resembles that of intact apurinic acid, it is reasonable to assume that, in the latter, resonance "a" corresponds to Cyt < Thy.

It should be emphasized that the pmr data in Table 1 are based on apurinic acid derived from salmon sperm, whereas the chromatographic results are based on radioactively labeled E. coli apurinic acid. Since the yield of thymine-containing dimers increases with increasing (A+T) content (11), the slight difference between the dimer yields determined by the two methods may result from the higher (A+T) content of salmon sperm DNA [(A+T)/(G+C) = 1.3] than of E. coli DNA [(A+T)/(G+C) = 1.0].

We have not been able to detect the appearance of a new resonance in the pmr spectrum of apurinic acid corresponding to the decrease in intensity of the thymine H-6 resonance. This finding is not unexpected, since previous work (10) demonstrated that the cyclobutane ring protons of Thy < Thy resonate at approximately 4 ppm, which is within the broad envelope of the 3', 4', and 5' resonances of the deoxyribose protons. We were also unable to detect changes in the cytosine H-5 and H-6 resonances due to the formation of Cyt < Thy. Since Cyt < Thy is formed in much smaller amounts than Thy < Thy (see Table 1), it appears likely that the present pmr analytical techniques are not sufficiently sensitive to changes in the apurinic acid spectra resulting from this small number of altered cytosine residues.

We conclude on the basis of the results presented here that apurinic acid provides a good model system for observing by pmr the molecular changes that occur in thymine when DNA is modified either photochemically or by some other means. Although the

changes we have observed were made directly on apurinic acid, it is reasonable to assume that chemical modification of the thymine residues in DNA itself could be similarly studied after depurination of the DNA to form apurinic acid, provided that the modified bases were stable to the mild formic acid treatment necessary for depurination.

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- 7 Abbreviations: pmr, proton magnetic resonance; ppm, parts per million; Cyt $\diamond$ Thy, cytosine-thymine dimer; Thy $\diamond$ Thy, thymine-thymine dimer; Ura $\diamond$ Thy, uracil-thymine dimer.
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