Accelerated Publications

A 2-Deoxyribonolactone-Containing Nucleotide: Isolation and Characterization of the Alkali-Sensitive Photoproduct of the Trideoxyribonucleotide d(ApCpA)

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Received August 30, 1989; Revised Manuscript Received October 3, 1989

ABSTRACT: It has been reported that ACA sequences in DNA are mutagenic hot spots in UV-induced mutagenesis and are sites of an alkali-sensitive lesion produced by UV irradiation. In order to characterize the UV-induced lesion of an ACA site, chemically synthesized trideoxyribonucleotide d(ApCpA) was irradiated with UV light and the alkali-sensitive photoproduct was isolated. The structure of this photoproduct was characterized as the trinucleotide containing 2-deoxyribonolactone at the internal residue by 2D DQF-COSY, FT-IR, FAB-MS, and chemical properties. It is known that this lesion is also produced by γ-irradiation, neocarzinostatin, the 1,10-phenanthroline–copper complex, and hydrogen peroxide and is highly mutagenic because of its resistance to cleavage by certain apurinic/apyrimidinic (AP) endonucleases. Thus, 2-deoxyribonolactone may be one of the lethal DNA lesions induced by UV irradiation to organisms and one of the intermediates of UV-induced DNA strand breaks because the DNA strand is cleaved at this site with β- and subsequent δ-elimination mechanisms.

It is well-known that UV[1] irradiation of organisms is closely related to mutagenesis and carcinogenesis and that this effect is due to DNA damage. In order to elucidate the mechanism of UV-induced mutagenesis and carcinogenesis, it is essential to clarify the structure of UV-induced DNA lesions and the effects of these lesions on DNA functions (replication, transcription, stability, etc.). A number of these DNA photoadducts have been characterized. The main UV-induced DNA lesions are cyclobutane–pyrimidine dimers (Fisher & Johns, 1976a), 6–4′-(pyrimidin-2′-one)–pyrimidines (6–4 photoproducts) (Wang, 1976), pyrimidine photohydrates (Fisher & Johns, 1976b), and DNA strand breaks (Schulte-Frohlinde et al., 1985; Rahn & Patrick, 1976). A recent report suggests the formation of a new photoproduct as indicated by the alkali-induced DNA strand breaks at ACA sequences in the SV40 DNA fragment after UV irradiation (Bourre et al., 1987). This lesion was detected with piperidine treatment at 11 of the 12 ACA sites. In addition, it was found that the ACA sequence is a mutation hot spot in UV-induced mutagenesis (Miller, 1985; LeClerc et al., 1984). The above findings reveal the formation of an unknown photoproduct at the ACA sites and are suggestive of the mutagenic potency of this photolesion. The structure of the photolesion at the ACA site has not been characterized.

In this paper, we report on the isolation of the alkali-sensitive photoproduct of the trideoxyribonucleotide d(ApCpA), its extensive characterization by ^{1}H NMR, FT-IR, and FAB-MS, and its chemical properties.

Experimental Procedures

Materials and Methods. d(ApCpA) (Na^+ salt) was synthesized by a modified phosphotriester method (Uesugi et al., 1981) and characterized by ^{1}H NMR and FAB-MS. 5′-dAMP and 3′-dAMP were purchased from Yamasa Shoyu Co. and Sigma Chemical Co., respectively. ^{1}H NMR spectra were obtained with a JEOL GX-500 spectrometer. The chemical shifts were measured relative to internal *tert*-butyl alcohol (1.231 ppm downfield from DSS). FT-IR spectra were obtained with a Perkin-Elmer 1720X spectrometer in the absorbance mode and with a spectral resolution of 2 cm^{-1}. A fast atom bombardment (FAB) mass spectrum was recorded with a JEOL JMS-DX 302 mass spectrometer on the disodium salt dissolved in a glycerol matrix.

Analytical HPLC. High-performance liquid chromatography (HPLC) was carried out on a column (150 × 3.7 mm) of μBondasphere C18-100 Å (Waters Associates) with a Shimadzu LC-6A system. A linear gradient of acetonitrile in 50 mM ammonium acetate (pH 7.0) was used with UV detection at 254 nm.

Photoreaction of d(ACA). A 300-W high-pressure mercury lamp (Eikosha EHB-WI-300) equipped with a Vycor filter as a light source was used. d(ApCpA) solutions (300 A_{260} units; nucleotide concentration at 1 A_{260} unit/mL in 20 mM sodium phosphate buffer, pH 4.0) in quartz reaction tubes were placed 3.5 cm from the light source. After the solution was bubbled with N_2 for 10 min, photolysis was carried out at 27–28 °C for 1.5 h. The crude reaction mixture was applied to a DEAE-cellulose column (1.1 × 21 cm) preequilibrated with 20 mM ammonium acetate, pH 5.0, and elution was carried out with a linear gradient of ammonium acetate (20–400 mM, total 400 mL). The fractions containing the pure product were concentrated under reduced pressure, and the resulting syrup was desalted by applying it to a C18 column (1.1 × 13 cm). After the column was washed with 40 mL of

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[1] Abbreviations: UV, ultraviolet; NMR, nuclear magnetic resonance; FAB-MS, fast atom bombardment mass spectrometry; FT-IR, Fourier transform infrared; HPLC, high-performance liquid chromatography; DSS, sodium 1-(trimethylsilyl)propane-3-sulfonate; DQF-COSY, double-quantum-filtered homonuclear scalar-correlated spectroscopy; d(ApCpA) or d(ACA), deoxyadenylyl-(3′–5′)-deoxycytidylyl-(3′–5′)-deoxyadenosine.

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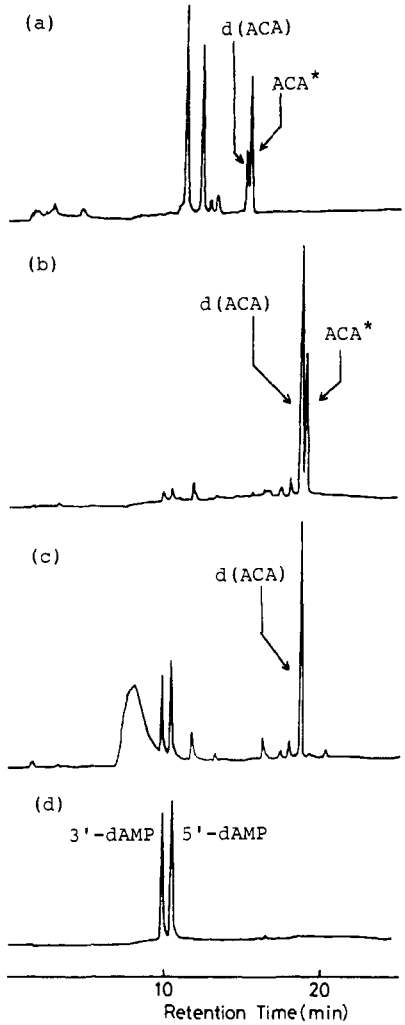

FIGURE 1: HPLC profiles of the reaction mixture detected at 254 nm (a) immediately after irradiation of d(ACA) for 1.5 h at pH 4.0, (b) after 1 day of the sample of (a) in the dark, and (c) after 1 M piperidine treatment of the sample of (b), and (d) mixture of 5′-dAMP and 3′-dAMP. Elution was carried out with a linear gradient of CH_3CN (5–13%) during 20 min for (a) or CH_3CN (0–7.5%) during the initial 10 min and then (7.5–11%) during the subsequent 10 min for (b), (c), and (d) in 50 mM ammonium acetate. Flow rate 0.8 mL/min.

distilled water, the product was eluted with 60 mL of 20% CH_3CN and the solvent was evaporated. Finally, the sample was converted to the sodium form by using a Dowex 50W-X4 (Na^+ form) ion-exchange column. The product, checked by reversed phase HPLC, was more than 95% pure and the isolated yield, estimated by absorbance at 260 nm, was 14.7%.

NMR Sample Preparation. d(ACA), 120 A_{260} units, and its photoproduct, 100 A_{260} units, were dissolved in 0.5 mL of D_2O containing 0.1 M NaCl and 50 mM sodium phosphate, pH 5.0 (isotope effects were not corrected for) and lyophilized. Following this, each sample was lyophilized three times from D_2O followed by dissolution in "100%" D_2O.

RESULTS

Photoreaction of d(ApCpA). UV irradiation of d(ApCpA) afforded three main products at pH 4.0 (Figure 1a), but two of them reversed to the starting material after 1 day in the dark (Figure 1b). At neutral pH (pH 6–7), these two products were produced more efficiently. Thus, we tentatively assigned them to the photohydrates of the cytosine residue because these observations are in agreement with the properties of such a product (Fisher & Johns, 1976b). The remaining unknown

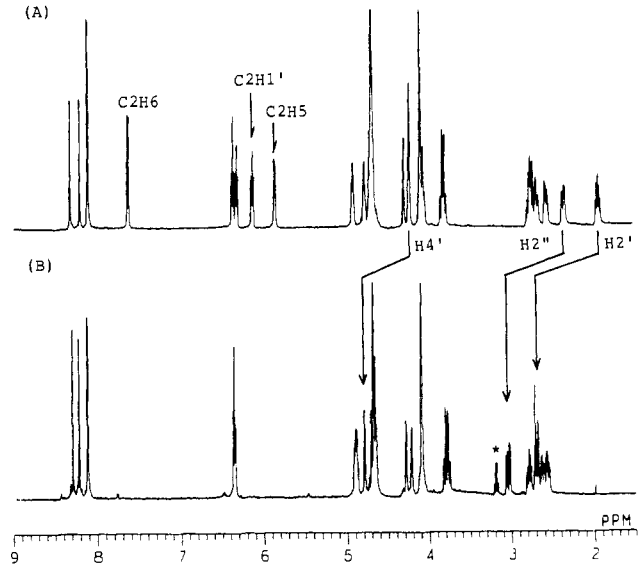

FIGURE 2: 500-MHz ^1H NMR spectra of d(ApCpA) (120 A_{260} units/0.5 mL) (A) and ACA* (100 A_{260} units/0.5 mL) (B) in D_2O containing 0.1 M NaCl and 50 mM sodium phosphate, pH 5.0 (uncorrected pH meter reading) at 30 °C. The chemical shifts were measured downfield from DSS. The arrows indicate the downfield-shifted proton signals of the internal residue. The asterisk indicates the signal of contaminated triethylamine.

product was produced more efficiently at acidic pH (this product hereafter called ACA*). Therefore, the preparative experiments for this reaction were carried out at pH 4.0. Since ACA* was not produced when a Pyrex filter was used in place of the Vycor filter, irradiation at around 254 nm is essential for this photoreaction.

*Characterization of ACA**. The structure of ACA* was characterized in the following manner. Treatment of ACA* with 1 M piperidine afforded 3′- and 5′-dAMP as determined by reversed-phase HPLC (Figure 1c). This behavior of ACA* is identical with that of apurinic or apyrimidinic nucleotides (Vodička & Hemminki, 1988). This reveals that ACA* is alkali-sensitive and that only the internal deoxycytidine residue is damaged, as the deoxyadenosine residue of neither the 3′- nor 5′-side is modified.

Figure 2 shows the 500-MHz ^1H NMR spectra of d(ApCpA) and ACA*. The proton signals in the sugar region of both nucleotides were completely assigned by DQF-COSY. In Figure 2B, the base protons (H-5, H-6) and the 1′ proton of the sugar moiety derived from the deoxycytidine residue disappear, and relatively large downfield shifts of H-2′, H-2″, and H-4′ signals are observed: 0.76, 0.70, and 0.57 ppm, respectively, with respect to the parent trinucleotide (Figure 2A). The chemical shifts for the protons of both 5′-dA and 3′-dA residues are little altered. These data suggest that the internal residue of ACA* is apyrimidinic and contains an electron-withdrawing modification at the 1′-position of the sugar moiety.

The FT-IR spectrum of this apyrimidinic trinucleotide shows the characteristic band of a γ-lactone at 1773 cm^{-1} (Figure 3a, lower) but no such band in the case of d(ApCpA) (Figure 3a, upper). The above evidence suggests the structure of the apyrimidinic site to be 2-deoxyribonolactone (Figure 4). This structure was also confirmed by the positive-ion fast atom bombardment (FAB) mass spectrum, which showed $M^{2-} + Na^+ + 2H^+$, $M^{2-} + 2Na^+ + H^+$, and $M^{2-} + 3Na^+$ at m/z 781, 803, and 825, respectively (Figure 3b). This structure is also consistent with the results of alkali hydrolysis (Goyne & Sigman, 1987).

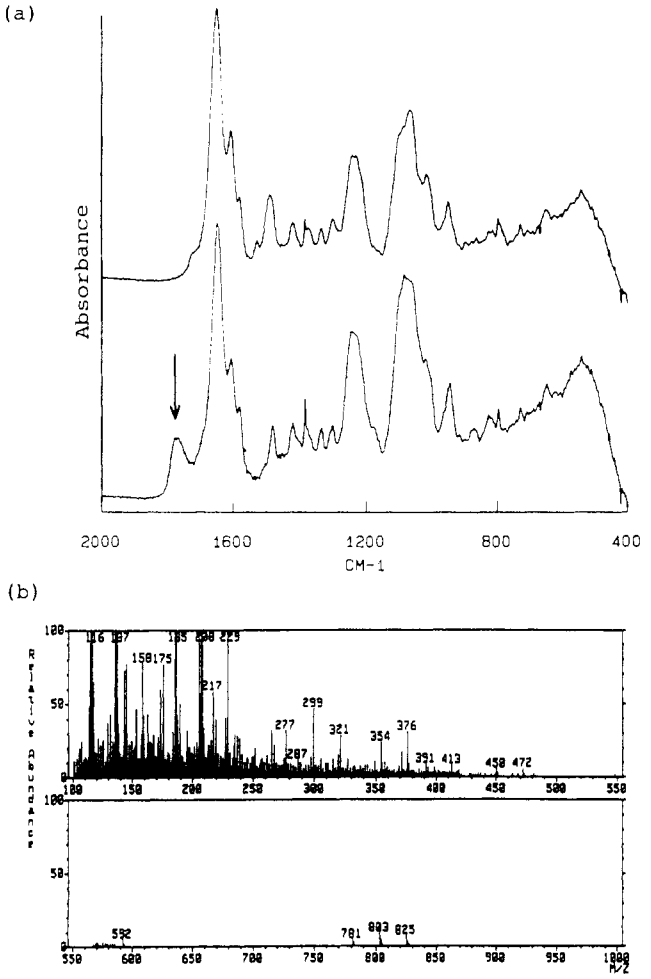

FIGURE 3: (a) Absorbance-mode FT-IR spectra of d(ApCpA) (upper) and ACA* (lower). The arrow indicates the band at 1773 cm^{-1} derived from the γ-lactone. The spectra were recorded in the 2000–400-cm^{-1} region with KBr pellets. (b) Positive-ion FAB-MS spectrum of ACA* in a glycerol matrix.

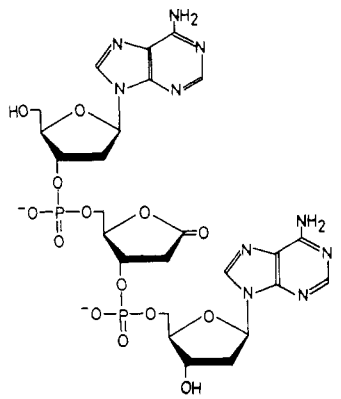

FIGURE 4: Structure of ACA* produced by UV irradiation of d-(ApCpA).

Discussion

The detailed mechanism of this photoreaction is not clear. The efficient production of ACA* at acidic pH (pH 4–5) suggests that the protonation of the cytosine residue may play a important role in this reaction. This reaction proceeds through an oxidative process, but it is not a simple oxidative depyrimidination because a cytosine base is not detected with HPLC in the reaction mixture. Thus, the cytosine residue may form an important active species, such as the pyrimidine radical, by electron transfer from both adenine rings (Daniels, 1976) because the protonated cytidine is the best electron acceptor among nucleosides (Montenary-Garestier et al., 1976).

The mechanism of the DNA strand breaks, one of the well-known UV-induced DNA lesions, is not understood. The number of DNA strand breaks has been measured by an alkaline sucrose gradient sedimentation or by a neutral one after alkali treatment and depends upon the length of time that it is exposed to alkali (Rahn & Patrick, 1976). The lesion at the ACA site described here could be cleaved by α-deprotonation with alkali or biomolecular bases, followed by β-elimination and subsequent δ-elimination (Goyne & Sigman, 1987; Rhaese & Freese, 1968; Bailly & Verly, 1988). Therefore, 2-deoxyribonolactone nucleotide may be one of the relatively stable intermediates in UV-induced DNA strand breaks. In fact, the rate constant for induction of sites of strand breaks in UV-irradiated poly(U) increases with decreasing pH within the pH range of 3.5–8.4 (Schulte-Frohlinde et al., 1985) as we found for d(ACA).

2-Deoxyribonolactone is also produced by γ-irradiation (Cadet & Têoule, 1975), neocarzinostatin (Kappen & Goldberg, 1989), the 1,10-phenanthroline–copper complex (Goyne & Sigman, 1987), and hydrogen peroxide (Rhaese & Freese, 1968). This should not be a major lesion of ACA sites in UV-irradiated organisms because it is produced less efficiently at physiological pH. However, its biological significance will be determined not only by its abundance but also by its susceptibility to cellular DNA repair systems. Since the 2-deoxyribonolactone residues in DNA cannot base pair, there is a possibility that this lesion could cause a base substitution (Povirk & Goldberg, 1985, 1986) or a frame-shift (Cuniasse et al., 1989) mutation. It has been reported that this lesion is highly mutagenic (Povirk & Goldberg, 1985, 1986; Povirk et al., 1988) and is resistant to cleavage by endonuclease IV, endonuclease VI (exonuclease III), and endonuclease III of *Escherichia coli* by a factor of 5–10 over typical apurinic sites in depurinated DNA (Kappen & Goldberg, 1989; Povirk & Goldberg, 1985). This lesion may be one of the lethal DNA lesions induced by UV irradiation.

In conclusion, the alkali-sensitive product of d(ApCpA) induced by UV irradiation was isolated and fully characterized as the 2-deoxyribonolactone-containing nucleotide by DQF-COSY, FT-IR, FAB-MS, and its chemical properties. The effects of neighboring bases and modification of the internal cytidine residue on this photoreaction are currently under investigation.

Acknowledgments

We are grateful to K. Ikai (Takara Shuzo Co., Ltd.) for measuring of FAB mass spectra.

Registry No. d(ApCpA), 70419-16-8; AcA*, 123673-82-5.

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Anisotropic ^2H NMR Spin-Lattice Relaxation in L_α-Phase Cerebroside Bilayers†

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Received August 21, 1989; Revised Manuscript Received October 5, 1989

ABSTRACT: A series of ^2H NMR inversion recovery experiments in the L_α phase of the cerebroside *N*-palmitoylgalactosylsphingosine (NPGS) have been performed. In these liquid crystalline lipid bilayers we have observed substantial anisotropy in the spin-lattice relaxation of the CD_2 groups in the acyl chains. The form and magnitude of the anisotropy varies with position in the chain, being positive in the upper region, decreasing to zero at the 4-position, and reversing sign at the lower chain positions. It is also shown that addition of cholesterol to the bilayer results in profound changes in the anisotropy. These observations are accounted for by a simple motional model of discrete hops among nine sites, which result from the coupling of two modes of motion—long-axis rotational diffusion and gauche-trans isomerization. This model is employed in quantitative simulations of the spectral line shapes and permits determination of site populations and motional rates. These results, plus preliminary results in sphingomyelin and lecithin bilayers, illustrate the utility of T_1 anisotropy measurements as a probe of dynamics in L_α-phase bilayers.

Solid-state ^2H NMR[1] has contributed substantially toward understanding the phase behavior and dynamic structure of lipid bilayers, the fundamental structural unit of membranes. To date, the most complete descriptions of lipid dynamics have been of the gel phase, because in this phase lipid molecules execute motions that are within the range of intermediate exchange rates for ^2H NMR ($10^{-7} < \tau_c < 10^{-3}$ s). The spectra are therefore sensitive to changes in the motional rates and mechanisms, and a detailed description of the dynamic processes can be extracted from simulations of the quadrupole echo line shapes and their dependence on temperature and pulse separation (Wittebort et al., 1987; Blume et al., 1982; Huang et al., 1980; Meier et al., 1983, 1986).

Although the L_α or liquid crystalline phase of lipid bilayers is an excellent model for many biological membranes, it has been less well characterized because the motions in this phase occur at rates which fall into the fast-limit regime ($\tau_c < 10^{-7}$ s), where the ^2H line shapes are insensitive to variations in the rate (Spiess & Sillescu, 1981; Wittebort et al., 1987). Thus, quadrupole echo spectra of liquid crystalline bilayers are motionally narrowed, axially symmetric Pake patterns with the splittings between the perpendicular edges of the powder patterns, $\Delta\nu_{Q\perp}$, dependent not on the rate of motion but rather on the averaged orientation of the C-D bond with respect to the axis of motion (Charvolin et al., 1973; Seelig & Seelig, 1974). As the splitting is essentially the only variable feature in these line shapes, they are generally interpreted in terms of order parameters, S_{CD}, which indicate the average orientation of the C-D segments with respect to the bilayer normal through the relation (Seelig & Seelig, 1974; Seelig, 1977)

$$\Delta\nu_{Q\perp} = \frac{3}{4}\frac{e^2qQ}{h}S_{CD} \quad (1a)$$

$$S_{CD} = (1/2)(3\overline{\cos^2\theta} - 1) \quad (1b)$$

† This research was supported by the National Institutes of Health (GM-25505 and RR-00995).

[1] Abbreviations: CHOL, cholesterol; DMPC, 1,2-dimyristoyl-*sn*-phosphatidylcholine; DPPC, 1,2-dipalmitoyl-*sn*-phosphatidylcholine; DPPE, 1,2-dipalmitoyl-*sn*-phosphatidylethanolamine; efg, electric field gradient; NMR, nuclear magnetic resonance; NPGS, *N*-palmitoylgalactosylsphingosine; NPSM, *N*-palmitoylsphingomyelin; PC, phosphatidylcholine; SUV, small unilamellar vesicles.

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