Cross-Link Dimer Formation of the Acetaldehyde-Derived Cyclic 1,N^2-Propano-2′-deoxyguanosine Adduct Using Electrochemical Oxidation

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The electrochemically oxidative lesion of the acetaldehyde-derived cyclic propano adduct 2 of 2′-deoxyguanosine 1 was identified as the cross-linked dimer 4 of adduct 2. Cross-link formation is explained by the nucleophilic preference of the exocyclic amino group in 2 to the carbocation 3 electrogenerated by 1-proton and 2-electron transfers. Dimer formation was also detected in duplex DNA during exposure to acetaldehyde followed by electrochemical oxidation. The dimer has been deduced to be an intrastrand cross-link generated specifically in the G–G sequence in duplex DNA, which is expected to contribute to acetaldehyde-mediated genotoxicity.

Key words cross-link dimer formation; cyclic propano adduct; 2′-deoxyguanosine; electrochemical oxidation; duplex DNA

A recent and exciting prospect in the area of the chemistry of oxidative DNA damage lies in understanding the mechanism for formation of interstrand and intrastrand cross-links, which are expected to interfere with DNA replication and contribute to genotoxicity.[1,2] Recently, our attention has been drawn to the reactivity and characteristics of the acetaldehyde-derived cyclic 1,N^2-propano adduct **2** of 2′-deoxyguanosine **1** in terms of genotoxic, mutagenic, and carcinogenic effects of acetaldehydes arising from exogenous and endogenous sources such as smoking and drinking.[3—5] The formation of **2** has been detected in calf thymus DNA and cultured human leukemia (HL)-60 cells during exposure to acetaldehyde.[6] Recently, it is reported that the analog of adduct **2**, produced by the reaction of **1** with α,β-unsaturated aldehydes such as acrolein and crotonaldehyde, undergoes ring opening, changing to the ring-opened aldehyde when placed opposite deoxycytosine in duplex DNA. This is followed by condensation with a neighboring **1**, involving the exocyclic amino group on the complementary strand, to form imine-linked bis-nucleoside, which in turn cyclizes to the pyrimidopurinone-type bis-nucleoside.[7—9] On the other hand, it is well recognized that **2** is susceptible to oxidation and easily causes oxidative degradation of the purine moiety.[3] In fact, the less negative oxidation potential of **2** than that of **1** was inferred from cyclic voltammetric measurements[10] and B3LYP/6-31+G(d) calculations.[11] This situation inspires a search for cross-linking as an oxidative lesion of **2**. We used controlled potential electrooxidation to find an oxidative lesion initiated by 1-electron transfer as a mimic of biological electron transfer. Here, we identify the novel cross-linked dimer of **2** formed by the electrochemical electron-drawing of **2** without oxidative degradation of the guanine moiety and show actual formation of the cross-linked dimer in calf thymus DNA.

Controlled potential electrolysis for **2** was performed on a carbon felt electrode set at 1.1 V *vs.* Ag/AgCl in phosphate buffer (pH 7), and an aliquot of the solution was injected into an HPLC. A prominent peak corresponding to the oxidative lesion of **2** was observed as it increased over electrolyzed time, as shown in Fig. 2a. The lesion was isolated and subjected to electrospray ionization (ESI)-MS measurements. A molecular ionic peak of $m/z=705$ ($[(2M-2H)+H]^+$, M being the molecular weight of **2**) was observed with a fragment peak of $m/z=573$ ($[(2M-2H-\text{ribose})+H]^+$), which is expected to generate the dimer of **2**. Dimer formation is deduced from MS/MS spectra showing a fragment peak of

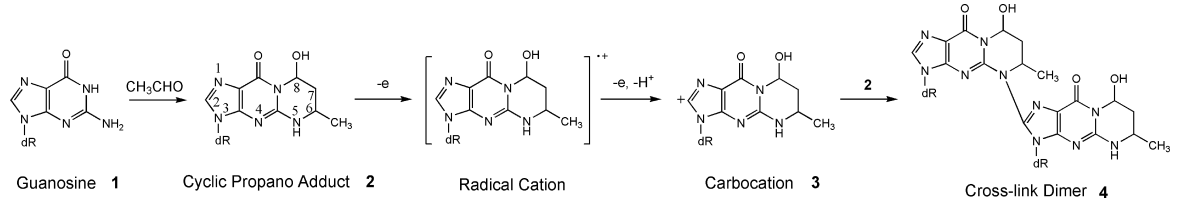

Fig. 1. Plausible Mechanism for Formation of the Cross-Link Dimer of Acetaldehyde-Derived Propano Adduct of 2′-Deoxyguanosine

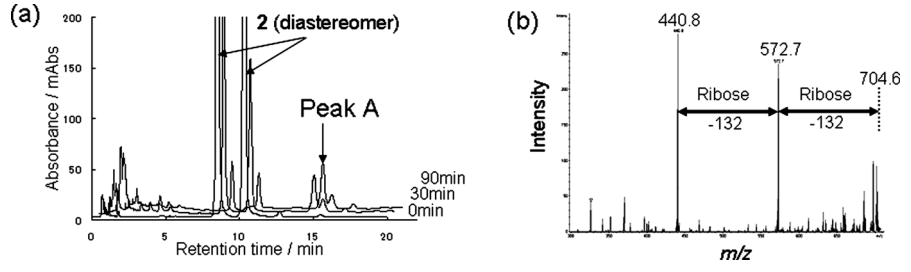

Fig. 2. HPLC Elution Profile for the Electrolyzed Solution of **2** (a), and MS/MS Spectra for the Molecular Ionic Peak ($m/z=705$) on the ESI-MS Spectra of the Isolated Oxidative Lesion of **2** (b)

HPLC separation was performed on an ODS column with a mobile phase consisting of water and methyl alcohol (linear gradient: 10—23% methanol over 20 min) at a flow rate of 0.3 ml min^{-1}.

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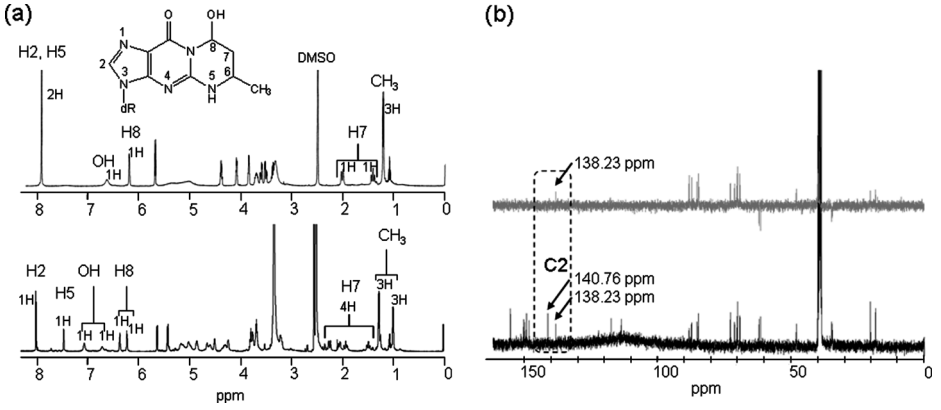

Fig. 3. ^{1}H-NMR Spectra of **2** (Upper) and the Oxidative Lesion of **2** (Lower) in DMSO-d_6 (a), and ^{13}C-NMR (Lower) and DEPT135 Spectra (Upper) of the Oxidative Lesion of **2** in DMSO-d_6 (b)

Addition of D_2O is accompanied by the disappearance of the peaks assigned to H5 and OH in the ^{1}H-NMR spectra. The signals at 138.2 and 140.8 ppm in Fig. b were assigned to the C2 carbons in the dimer with the aid of HMBC spectral measurements and comparison to the ^{13}C-NMR spectra for monomer.

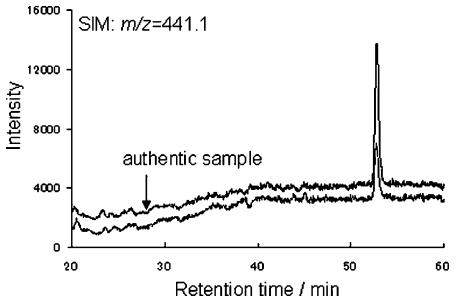

Fig. 4. HPLC Elution Profile for the Electrolyzed Solution of Double-Stranded DNA Containing the Acetaldehyde-Derived Adduct **2**, Observed with an ESI-MS Detector Set in a Selected Ion Monitoring (SIM) Mode of *m*/*z* 441.1 Corresponding to the Molecular Ion of the Dimer of the Cyclic Propano Adduct of Guanine

See text for sample preparation details. The chromatogram of an authentic sample of the dimer is overlaid on the chromatogram.

m/*z*=441, corresponding to the dimer of the cyclic propano adduct of guanine resulting in the elimination of riboses, as shown in Fig. 2b. High-resolution ESI-MS results (*m*/*z*=727.24117, $[(2M-2H)+Na]^+$) provide the chemical formula for the lesion as $C_{28}H_{36}N_{10}O_{12}$. ^{1}H- and ^{13}C-NMR spectrometry was used to obtain unambiguous evidence for the structure of the dimer. It is found that two resonances for the equivalent protons in two monomers linked with each other are detected except for the 8-ppm signal in monomer **2** assigned to H2 and H5, as shown in Fig. 3a. The exemplary case is given by the methyl protons observed at 1.1 and 1.3 ppm in the dimer. This means that the dimer does not have the symmetric structure linked by the homo-coupling. The dimer cross-linked at C2 and N5 is deduced from the 1H-signals at 7.5 and 8.1 ppm observed for the dimer, assigned to H5 and H2, respectively, from the effect of D_2O addition and in comparison to the spectra for monomer. Further evidence for the C2–N5 linking in the dimer was obtained from ^{13}C-NMR spectra. The presence of the quaternary C2 carbon in the dimer has been inferred from the elimination of the signal at 140.76 ppm assigned to one of C2 carbons in DEPT135 spectra, as shown in Fig. 3b. On the basis of product analyses with MS and NMR spectra, it has been concluded that the oxidative lesion of **2** is the cross-linked dimer **4** illustrated in Fig. 1. In addition, 2-dimensional NMR spectra (double quantum filtered-correlation spectroscopy (DQF-COSY) and heteronuclear multiple bond connectivity (HMBC)) were also measured (data not show) to confirm the assignments of ^{1}H- and ^{13}C-NMR spectra and the structure of the cross-linked dimer, the correlations on the spectra being consistently explained by the structure of **4**. On the basis of HPLC analyses, the apparent conversion ratio of **2** to **4** at the stationary state attained for 30 min was estimated as 9.2% at a nucleoside level.

The initial electrode reaction has been deduced to involve a 1-electron step resulting in a radical cation, which on further rapid oxidation involving a 1-proton and 1-electron step gives the carbocation **3**. Two-electron oxidation is consistent with the electrochemical results.[10] The cross-linking of **4** has been rationalized in terms of a nucleophilic reaction of **2** and **3**, which involves a series of organic electrochemical reactions of carbocations against nucleophiles. Plausible mechanism for formation of **4** was shown in Fig. 1. B3LYP/6-31+G(d) calculation results have supported this mechanism from the point of view of the highest occupied molecular orbital (HOMO)-lowest unoccupied molecular orbital (LUMO) interaction between **3** and **2**.

Cross-link dimer formation in duplex DNA was examined by the following procedure. Duplex DNA containing **2** was prepared by exposing calf thymus DNA to acetaldehyde.[12] The duplex DNA solution was electrolyzed on a platinum mesh electrode, followed by depurination with hydrochloric acid after dialysis. The purine bases were analyzed with LC/MS; the results are shown in Fig. 4. The cross-linked dimer of cyclic propano guanine was detected in the double-stranded DNA. This implies that the formation of **4** occurs efficiently in sterically crowded duplex DNA.[13] Simple molecular modeling suggested that a preference for interstrand cross-linked dimer formation was unfavorable because of the long distance between active sites of formation. The **2**–**2** sequence in the same DNA strand prefers to form **4** with intrastrand cross-linking. It is reported that the electron-loss center in duplex DNA ultimately ends up at guanine residues *via* hole migration through the DNA duplex.[14,15] Likewise, oxidative stress in duplex DNA is localized at a **2**–**2** step (low oxidation potential site) acting as a trap in long-range hole

migration through the DNA duplex, the resulting carbocation **3** formed, being susceptible to fast and efficient electron transfer conjugated with deprotonation. The formation of cross-link dimer **4** has been rationalized in terms of nucleophilic reaction between the C2 position of **3** and the N5 position of a vicinal base **2** on the same DNA strand. Intrastrand cross-link formation was expected to involve disruption of Watson–Crick hydrogen bonding at one or both of the tandem cross-linked C–G base pairs.

In conclusion, the propano adduct **2** induced by acetaldehyde is easily oxidized to form a cross-linked dimer. The formation of carbocation **3** plays an important role in the formation mechanism. In double-stranded DNA, dimer formation can be explained by considering sequence-specific cross-link dimer formation in the G–G sequence as oxidative damage of the propano adduct **2**. The intrastrand cross-link is expected to interfere with DNA replication and contribute to acetaldehyde-mediated genotoxicity.

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10) The anodic peak potentials in the cyclic voltammograms of **2** and **1**, recorded in pH 7 phosphate buffers on a GC electrode, are 1.04 and 1.08 V *vs.* Ag/AgCl, respectively. The number of electrons for the irreversible anodic oxidation of **2** was estimated as 2.
11) B3LYP/6-31+G(d) ionization potentials are 5.82 and 5.98 eV for **2** and **1**, respectively, on the basis of Koopman's theorem.
12) Calf thymus DNA (4.0 mg), L-arginine (9.0 mg) and acetaldehyde (100 μl) were dissolved in 1.2 ml phosphate buffer (pH 8), and the reaction mixture was incubated at 70 °C for 4 h, followed by dialysis. The reaction mixture was freeze-dried, followed by dissolution in 10 ml of 0.1 M phosphate buffer, and supplied to the electrochemical treatment. The presence of L-arginine in the reaction mixture strongly accelerates the conversion of **1** to **2** in DNA.[4)] The conversion ratio of **1** to **2** was estimated as 61%.
13) The conversion ratio of **1** to **4** in calf thymus DNA was estimated as 0.0008% on the assumption that the DNA consisted of four bases at the same rate. Formation of **4** in double stranded DNA is much lower than in a nucleoside level. This is due to the fact that the cross-linking formation is strongly dependent on the base sequence in DNA.
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