Formation of Malonaldehyde and Acetaldehyde from the Oxidation of 2′-Deoxyribonucleosides

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2′-Deoxyribonucleosides, ribonucleosides, nucleobases, deoxyribose, and ribose were oxidized with Fenton's reagent. Malonaldehyde (MA) formed was derivatized with *N*-methylhydrazine to *N*-methylpyrazole, and acetaldehyde formed was derivatized with cysteamine to 2-methylthiazolidine. The resulting nitrogen-containing derivatives were quantitatively analyzed using gas chromatography with a nitrogen−phosphorus detector. MA and acetaldehyde were found in 2-deoxy-D-ribose and 2′-deoxyribonucleosides but not in ribonucleosides, nucleobases, and D-ribose. Amounts of MA formed from four deoxynucleosides were in the following order: 2′-deoxyguanosine > 2′-deoxycytidine > 2′-deoxyadenosine ≥ thymidine. Amounts of acetaldehyde formed from four deoxynucleosides were in the following order: 2′-deoxycytidine > thymidine > 2′-deoxyadenosine ≥ 2′-deoxyguanosine. The results suggest that the formation of MA and acetaldehyde requires a deoxy group on carbon 2′ of a ribose moiety.

Keywords: *Malonaldehyde; nucleosides; acetaldehyde; gas chromatography*

INTRODUCTION

Malonaldehyde (MA) and acetaldehyde are the major reactive carbonyl compounds resulting from oxidation biological membranes (Vaca et al., 1988) and animal blood plasma (Miyake and Shibamoto, 1998). These reactive aldehydes readily form an adduct with proteins (Chio and Tappel, 1969) and DNA (Vaca et al., 1988) and, consequently, produce cytotoxicity and genotoxicity (Canonero et al., 1990) including carcinogenicity (Emerit et al., 1985). Therefore, it is important to know the exact amount of MA and acetaldehyde formed from biological substances, such as DNA, upon oxidation to assess adverse effects caused by oxidation.

Analysis of these reactive aldehydes in biological samples such as DNA is extremely difficult because they are highly reactive and readily bind to proteins. The most widely used assay for DNA oxidation is a thiobarbituric acid (TBA) method that measures total products reacted with TBA. Consequently, the results obtained with this method are not always specific to the product of interest (Esterbauer and Zollner, 1989). Moreover, this method requires both high acidity (pH 2) and elevated temperature (95 °C), resulting in possible alteration of the samples. Recently, we have developed several gas chromatography (GC) methods for the analysis of reactive carbonyl compounds, including formaldehyde, acrolein, acetaldehyde, and MA, in foods, cigarette smoke, and biological samples (Miyake and Shibamoto, 1993, 1995; Ebeler et al., 1997). Saturated aldehydes such as acetaldehyde were reacted with cysteamine to form corresponding thiazolidines. β-Dicarbonyl compounds such as MA were reacted with *N*-methylhydrazine to form corresponding pyrazoles. The derivatizations were conducted at room temperature under neutral or weak basic conditions. These stable S- and/or N-containing heterocyclic derivatives were analyzed by GC with a highly sensitive and selective nitrogen−phosphorus detector (NPD).

In this study, GC was used to investigate the formation of MA and acetaldehyde from low molecular weight analogues of DNA, such as deoxyribonucleosides, upon oxidation.

EXPERIMENTAL PROCEDURES

Materials. Deoxyribonucleosides (2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyadenosine, and thymidine), nucleobases, ribonucleosides, D-ribose, 2-deoxy-D-ribose, 1,1,3,3-tetraethoxypropane, and butylated hydroxytoluene were purchased from Sigma (St. Louis, MO). Acetaldehyde (99% grade), 2-methylpyrazine, 2,4,5-trimethylthiazole, MA bis(diethyl acetal), *N*-methylhydrazine, potassium chloride, hydrogen peroxide, ferrous chloride, and cysteamine hydrochloride were obtained from Aldrich (Milwaukee, WI). A standard stock solution of 2-methylpyrazine was prepared by adding 10 mg of 2-methylpyrazine to 1 mL of dichloromethane. A standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 10 mg of 2,4,5-trimethylthiazole to 1 mL of dichloromethane. Authentic *N*-methylpyrazole was synthesized according to the method previously reported (Umano et al., 1988); 2-methylthiazolidine was synthesized according to the reported method (Yasuhara and Shibamoto, 1989a,b). Sodium malonaldehyde was synthesized according to methods previously described (Lacombe et al., 1990). All other chemicals, reagents, and solvents were of analytical grade.

Oxidation of Deoxyribonucleosides and 2-Deoxy-D-Ribose with Fenton's Reagent. An aqueous solution (5 mL) containing varying amounts of deoxyribonucleosides (20, 16, 12, 8, or 4 μmol) or 2-deoxy-D-ribose (12, 8, 4, 3, 2, or 1 μmol), 0.25 mmol Trizma buffer (pH 7.4), 15 μmol of ferrous chloride, 1.7 mmol of hydrogen peroxide, and 0.75 mmol potassium chloride was incubated at 37 °C for 18 h. Oxidation of the samples was stopped by adding 50 μL of 4% butylated hydroxytoluene in methanol. The sample tubes were covered with aluminum foil during incubation to exclude photooxidation.

Analysis of Acetaldehyde as 2-Methylthiazolidine. Acetaldehyde formed in oxidized samples was analyzed using

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10.1021/jf990109u CCC: $18.00
Published on Web 06/29/1999

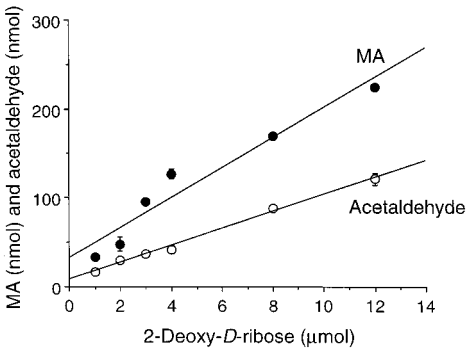

Figure 1. Quantities of MA and acetaldehyde formed from various amounts of 2′-deoxyribose oxidized with Fenton's reagent.

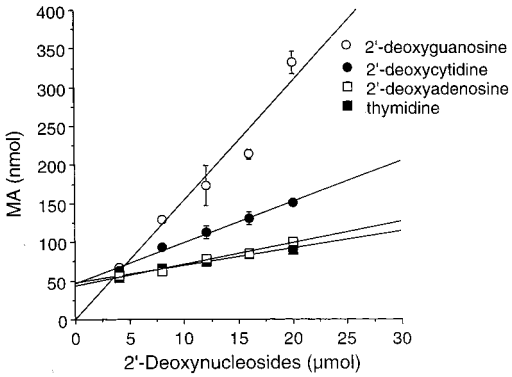

Figure 2. Quantities of MA formed from various amounts of 2′-deoxynucleosides oxidized with Fenton's reagent.

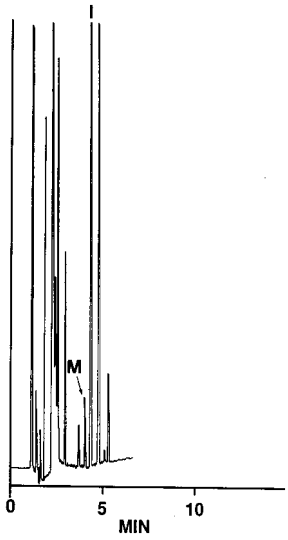

Figure 3. Typical GC of an extract from oxidized 2′-deoxynucleosides treated with *N*-methylhydrazine. M, malonaldehyde; I, internal standard (2-methylpyrazine).

a method previously reported (Miyake and Shibamoto, 1993). To the above oxidized samples was added 400 μL of cysteamine hydrochloride solution (1.2 M). The pH of the samples was immediately adjusted to 8−9 with 6 M NaOH. The solutions were stirred for 1 h at room temperature. After the pH of the reaction solutions was adjusted to 7 with 5 N HCl, the solutions were extracted with 10 mL of dichloromethane using a liquid−liquid continuous extractor for 3 h. The extracts were dried over anhydrous sodium sulfate for 1 h. After removal of the sodium sulfate, the volume of each extract was brought up to exactly 1 mL with dichloromethane. A standard solution of 2,4,5-trimethylthiazole (4 μL) was added to each extract as an internal standard prior to GC analysis. The experiment was replicated three times.

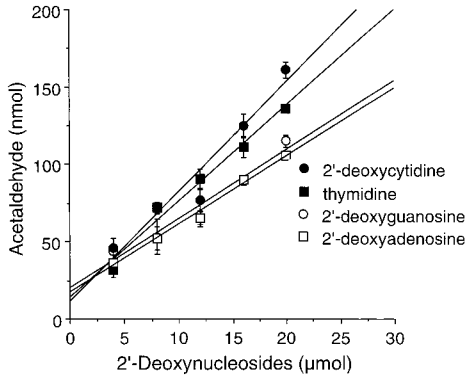

Figure 4. Quantities of acetaldehyde formed from various amounts of 2′-deoxyribonucleosides oxidized with Fenton's reagent.

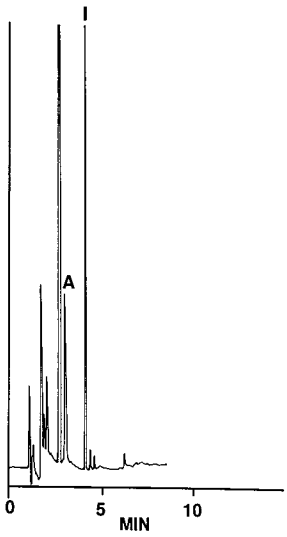

Figure 5. Typical GC of an extract from oxidized 2′-deoxynucleosides treated with cysteamine. A, acetaldehyde; I, internal standard (2,3,5-trimethylthiazole).

Analysis of MA as *N*-Methylpyrazole. In a separate experiment, *N*-methylhydrazine (50 μL) was added to the above-described samples. The solutions were stirred for 1 h at room temperature. After the pH of the reaction solutions was adjusted to 7 with 5 N HCl, the solutions were extracted with 10 mL of dichloromethane using a liquid−liquid continuous extractor for 3 h. The extracts were dried over anhydrous sodium sulfate for 2 h. After removal of the sodium sulfate, the volume of each extract was adjusted to exactly 1 mL with dichloromethane. A standard solution, 2-methylpyrazine (4 μL), was added to each extract as an internal standard prior to GC analysis. The experiment was replicated three times.

GC Analysis. A Hewlett-Packard (HP) model 5890A GC equipped with an NPD and a Spectra Physics SP 4290 integrator was used. A 30 m × 0.25 mm i.d. ($d_f = 1$ μm) DB-1 bonded-phase fused silica capillary column (J&W Scientific, Folsom, CA) was used for quantitative analysis of 2-methylthiazolidine, and a 30 m × 0.25 mm i.d. ($d_f = 1$ μm) DB-Wax bonded-phased fused silica capillary column (J&W Scientific) was used for quantitative analysis of *N*-methylpyrazole. The detector and injector temperatures were 250 °C. The linear velocity of the helium carrier gas was 30 cm/s with a split ratio of 21:1. The oven temperature was programmed from 70 to 180 °C at 4 °C/min and held for 10 min. An HP model 5890 series II GC interfaced to an HP 5971 mass spectrometer was used to confirm the 2-methylthiazolidine and *N*-methylpyrazole. The GC conditions were the same as described above. The mass spectra were obtained by electron impact ionization at 70 eV at an ion source temperature of 250 °C.

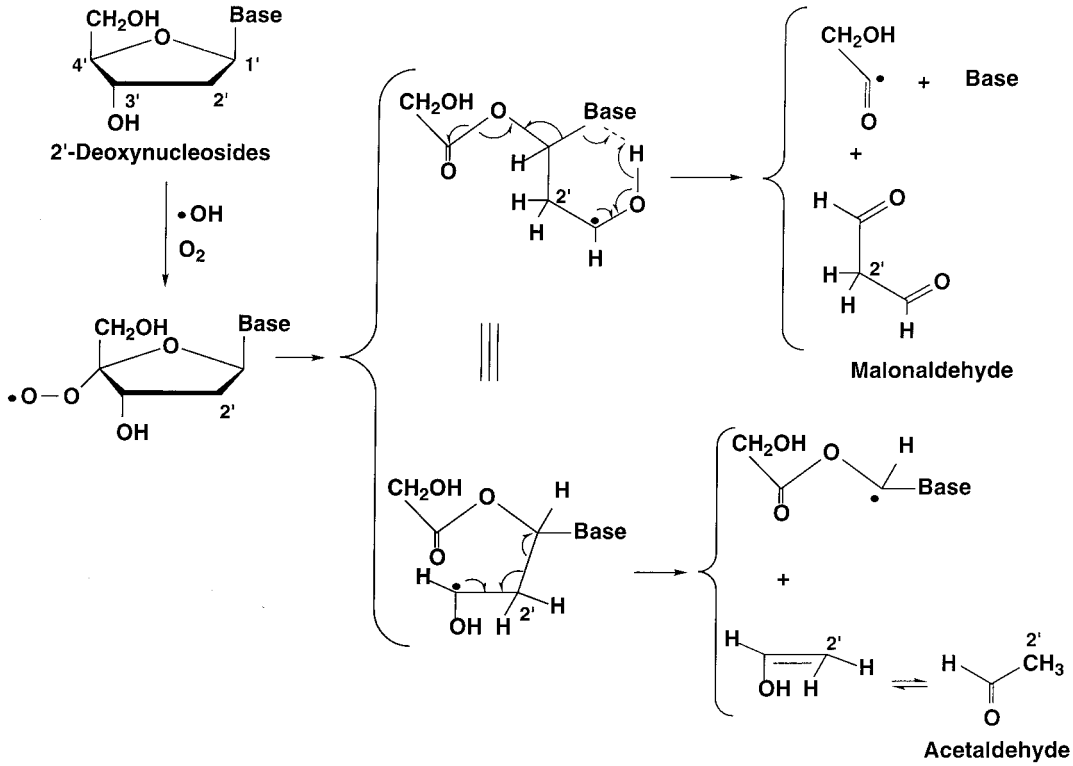

Figure 6. Hypothesized formation mechanisms of MA and acetaldehyde from 2′-deoxyribose moiety upon oxidation.

RESULTS AND DISCUSSION

Fenton's reagent was used to oxidize samples in the present study because a hydroxyl radical (HO•) is extremely reactive and is thought to cause cellular damage such as modifications in nucleic acid bases and DNA strand scission (Halliwell and Gutteridge, 1984).

The quantities of MA and acetaldehyde formed from various amounts of 2′-deoxyribose upon oxidation are shown in Figure 1. Formation of MA ($r^2 = 0.94$) or acetaldehyde ($r^2 = 1.0$) was linearly related to the amount of 2′-deoxyribose oxidation mixture. The quantities of MA formed from various amounts of 2′-deoxynucleosides upon oxidation are shown in Figure 2. The GC calibration curve for *N*-methylpyrazole showed a linear relationship between peak area ratio (*N*-methylpyrazole/2-methylpyrazine) and the concentration of *N*-methylpyrazole (nanomoles). The lowest detection level of MA by an NPD was 7.8 pg. A typical chromatogram of an extract from oxidized 2′-deoxynucleosides is shown in Figure 3. The amounts of MA recovered from four deoxynucleosides were in the order 2′-deoxyguanosine > 2′-deoxycytidine > 2′-deoxyadenosine ≥ thymidine. For example, when 16 μmol of 2′-deoxyguanosine, 2′-deoxycytidine, 2′-deoxyadenosine, and thymidine each was oxidized, 213.8 ± 6.67, 130.6 ± 7.76, 85.06 ± 2.21, and 84.46 ± 2.46 nmol of MA were formed, respectively. The values are mean ± standard deviation ($n = 3$). Assuming that a certain amount of MA formed from a deoxyribose moiety is trapped by the nucleoside base, this result suggests that the order of reactivity of deoxynucleosides toward MA is thymidine ≥ 2′-deoxyadenosine > 2′-deoxycytidine > 2′-deoxyguanosine.

Figure 4 shows the quantities of acetaldehyde formed from various amounts of 2′-deoxynucleosides. A linear relationship between peak area ratio (2-methylthiazolidine/2,4,5-trimethylthiazole) and the concentration of 2-methylthiazolidine (nanomoles) was also obtained. The lowest detection level of acetaldehyde by an NPD was 7.1 pg. A typical GC of an extract from oxidized 2′-deoxynucleosides is shown in Figure 5. The amounts of acetaldehyde formed from the four deoxynucleosides were 2′-deoxycytidine > thymidine > 2′-deoxyadenosine ≥ 2′-deoxyguanosine. When 16 μmol each of 2′-deoxycytidine, thymidine, 2′-deoxyguanosine, and 2′-deoxyadenosine was oxidized with Fenton's reagent, 125.1 ± 7.56, 111.3 ± 6.87, 90.37 ± 1.44, and 89.87 ± 3.19 nmol of acetaldehyde were formed, respectively. The values are mean ± standard deviation ($n = 3$).

Oxidation of 2′-deoxyribonucleosides and 2′-deoxyribose led to the formation of MA and/or acetaldehyde, whereas the oxidation of ribonucleosides, nucleobases, and ribose did not. This suggests that the absence of the hydroxyl group on the 2′ carbon of the sugar moiety is key in the formation of MA and acetaldehyde. These results strongly suggest that MA and acetaldehyde are produced from oxidation of a deoxyribose moiety of deoxyribonucleosides. Takeshita et al. (1978) hypothesized that DNA strand scission is initiated by free radical attack on carbon 4′ of the deoxyribose moiety of DNA, followed by cleavage of the bond between carbons 3′ and 4′ of the sugar ring. This cleavage yields MA. The hypothesized formation mechanisms of MA and acetaldehyde formed from a 2′-deoxyribose moiety are shown in Figure 6.

There have been only a few reports on oxidative degradation of DNA. For example, investigation of the degradation of nucleic acids with ozone in aqueous solution demonstrated that the sites of ozone attack were primarily on the base moieties of DNA (Ishizaki et al., 1984). The reaction between bleomycins (a group of related glycopeptide antibiotics) and DNA reportedly produced several reactive carbonyl compounds (Sasuville et al., 1978). This implies that some carbonyl compounds are produced from DNA oxidized by radical

species. However, there are no published reports about direct measurement of reactive carbonyl compounds, such as MA and acetaldehyde, formed from DNA upon oxidation.

The GC method used in the present study determined the specific levels of MA, which may be one indicator of damage to proteins, nucleic acids, and other nucleophiles.

The most widely used method to determine MA has been the TBA assay. In this method, TBA gives a red complex with certain carbonyl compounds, including MA, which is measured by colorimetry. The TBA method is not specific for MA and often overestimates MA levels (Bird et al., 1983; Hirayama et al., 1983) because some other carbonyl compounds react with TBA to produce absorbance spectra similar to that of the TBA−MA complex (Marcuse and Johansson, 1973; Witz et al., 1986). However, the TBA method is a simple and useful method to examine oxidation of certain substances such as foods.

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Received for review February 11, 1999. Revised manuscript received May 10, 1999. Accepted May 16, 1999.

JF990109U