4-Oxo-2-hexenal, a Mutagen Formed by ω -3 Fat Peroxidation, Causes DNA Adduct Formation in Mouse Organs

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Received March 28, 2005 and accepted August 29, 2005

Abstract: To identify mutagens formed in a model reaction of lipid peroxidation, linolenic acid methyl ester and hemin were reacted with dG. As a result, a 4-oxo-2-hexenal-dG adduct (dG*) was identified in the model reaction mixture. The 4-oxo-2-hexenal (4-OHE) showed mutagenic activity in the *Salmonella typhimurium* strains TA100 and TA104. After 4-OHE was orally administered to mice, dG*, 4-OHE-dC- and 4-OHE-5-methyl-dC adducts were detected in esophageal, stomach and intestinal DNA. In the vapor phase released from the methyl linolenate-hemin model system, and in the smoke released during the broiling of fish, 4-OHE was detected by GCMS. The 4-OHE seems to be produced by the auto-oxidation of ω -3 polyunsaturated fatty acids. These results provide a warning to workers dealing with ω -3 fats, who may be exposed to this volatile mutagen.

Key words: 4-oxo-2-hexenal, ω -3 polyunsaturated fatty acids, DNA adduct

It is important to identify environmental mutagens and to remove them to prevent cancer in industry workers. We have been interested in the mutagens produced by lipid peroxidation^{1, 2)}, because they may be formed during the extraction and purification of edible oils, or by frying foods with vegetable-oil. Many low molecular-weight mutagens are released into the atmosphere by oil factories, the food industry, and restaurant kitchens. Various lipid peroxide compounds are produced by the oxidative fragmentation of fats catalyzed by iron and heat or light. Our study focused on the identification of mutagens in the lipid peroxide products formed from ω -3 polyunsaturated fatty acids, such

as linolenic acid.

It is well known that many mutagens react with DNA components, particularly with deoxyguanosine (dG)³⁾. To identify the mutagens formed in a model reaction of lipid peroxidation, linolenic acid methyl ester and hemin were reacted with dG in phosphate buffer (pH 7.4), as an emulsion for 72 h at 20°C. The dG-mutagen adducts were isolated by HPLC, and their structures were determined by mass-, UV-, ¹H- and ¹³C-NMR spectra. As a result, the 4-oxo-2-hexenal-dG adduct (dG*) was identified in the model reaction mixture. M+Na, m/e 384.1256 (384.1284 calcd. for C₁₆H₁₉N₅NaO₅); UV λ max in H₂O, 228, 280 nm. The structure was confirmed by comparison with an authentic dG* sample prepared from synthetic 4-oxo-2-hexenal (4-

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Fig. 1. Structures of DNA adducts formed by 4-oxo-2-hexenal.

OHE) and dG, and by X-ray crystallography of the 4-OHE-9-ethylguanine-adduct. The 4-OHE was mutagenic in the *Salmonella* strains, TA100 and TA104, without S9 mix. Its specific mutagenic activities against TA100 and TA104 were 78 and 67 revertants/ μg , respectively. The mutagenicity of 4-OHE has not been reported thus far. It also induced apoptosis in human HL-60 cells. This is reasonable because DNA damage is a common trigger for apoptosis induction in mammalian cells⁴⁾.

The 4-OHE reacts not only with dG but also with dC and 5-methyl-dC to yield adducts (dG*, dC*, 5-methyl-dC*). The structures of dC* and 5-methyl-dC* were determined mainly by mass-, ¹H- and ¹³C-NMR- spectra. dC*: M+Na, m/e 344.1192 (344.1222 calcd. for C₁₅H₁₉N₃NaO₅); UV λ_{max} in H₂O, 227 nm. 5-methyl-dC*: M+Na, m/e 358.1372 (358.1379 calcd. for C₁₆H₂₁N₃NaO₅); UV λ_{max} in H₂O, 278 nm. The structure of dC* was confirmed by X-ray crystallography. After the reaction of 4-OHE with double stranded calf thymus DNA, the yield of these DNA modifications were dC* > 5-methyl-dC* > dG*. The more efficient formation of dC* than dG* in DNA may be due to

the fact that the amino group of dC in DNA is located in the major groove, while that of dG is in the minor groove and is more sterically hindered. As a prelude for testing its genotoxicity *in vivo*, 4-OHE (3 mg) was orally administered to mice, and after 24 h, the DNA was isolated from the esophagus, stomach, intestine, liver and kidney. The DNA was enzymatically digested to deoxynucleosides, and was subjected to LC/MS/MS analyses. Particularly, in the esophagus, stomach and intestine DNA, dC* was detected in the range of 2.0, 5.5 and 1.1 adducts / 10⁷ dC, respectively. Lower amounts of 5-methyl-dC* and dG* were also detected in these organ DNAs.

It is important to confirm the formation of 4-OHE in a model system of lipid peroxidation, as well as during the heat processing of edible vegetable oil, and during cooking. From ethylacetate traps (extracts) of the vapor phase released from the heated methyl linolenate-hemin model system, and the smoke released during the broiling of fish, 4-OHE was detected by GCMS. Commercial perilla oil, which is rich in linolenic acid (40–50 $\mu\text{g/g}$), the edible part of broiled fish (5–170 $\mu\text{g/g}$), and various fried foods (5–50 $\mu\text{g/g}$) also

contained high levels of 4-OHE. It was present at an especially high concentration (70–170 $\mu\text{g/g}$) in broiled saury. However, 4-oxo-2-nonenal⁵⁾, a cognate longer carbon chain derivative, was only detected in an one order of magnitude lower range in these food products. This may be due to the fact that the major natural ω -3 polyunsaturated fatty acids, such as linolenic acid, DHA and EPA, the precursor of 4-OHE, are more easily oxidized than ω -6 fatty acids, such as linoleic acid.

Workers involved in the manufacturing of vegetable oils, such as colza oil and soybean oil, which contain linolenic acid, will be exposed to 4-OHE during the crude oil extraction and purification. The same is true for workers manufacturing instant cup noodles and snack foods, and producing EPA and DHA from fish oil. Kitchen workers involved in frying foods or broiling fish in restaurants may also be exposed to 4-OHE. Since 4-OHE induces DNA adduct formation in experimental animal organs, it is possible that 4-OHE is an important human carcinogen. Further studies on the carcinogenicity of 4-OHE and its detection in working environments will be required.

Acknowledgement

We would like to thank Dr. T. Nohmi of the National Institute of Health Sciences, Tokyo, for providing us the *S. typhimurium* strains for the mutagenicity test.

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