Sources and Mechanisms of Carbon Monoxide Production by Irradiation

A.S. Leaflet R1985

E. J. Lee, postdoctoral research associate
D. U. Ahn, Professor of Animal Science Department

Summary and Implications

The productions of CO in all samples were irradiation-dose dependent. Glycine, asparagine, and glutamine were the major sources of CO production among amino acids, and glyceraldehydes, pyruvate, and α-ketoglutarate were the major sources of CO among glycolysis intermediates. Phosphatidyl choline, phosphatidyl ethanolamine, and lysophosphatidyl choline produced the greatest amounts of CO among the phospholipids. The amounts of CO produced from these sources were significant, and the production of gas compounds via radiolytic degradation appears to be closely related to the structure of molecules.

Introduction

Color is a major sensory attribute determining consumer acceptance of meat. Consumers expect the color of uncured cooked light meats such as oven roasted poultry breast meat or poultry breast rolls to be white. Therefore, if those meats show pink or red color, consumers suspect that they are contaminated or undercooked. The pink pigment formed in irradiated raw and cooked turkeys was characterized as CO-myoglobin (CO-Mb). A considerable amount of carbon monoxide (CO) was produced by radiolysis of organic components in irradiated frozen meat and poultry. CO has a very strong affinity to heme pigments and thus easily forms CO-Mb complex, which increases the intensity of red meat and blood color significantly. The affinity of CO to heme pigments is significantly influenced by the valence of heme iron and the oxidation-reduction potential (ORP) of meat determines the status of iron in heme pigments. Irradiation generates favorable conditions for CO-Mb complex formation, which intensifies the redness of heme pigments.

Although CO-Mb was considered as the major pigment responsible for pinking in irradiated meat, no attempt has been made to elucidate the sources and mechanisms of CO production by irradiation.

The objectives of this study were to determine the sources of CO production and to elucidate the mechanisms of CO production in meat by irradiation.

Materials and Methods

Model systems were prepared with various components generally found in meat: fatty acids and oils (oleic acid, linoleic acid, linolenic acid, phosphatidyl choline, corn oil, and fish oil), carbohydrates (glucose, fructose, starch, and glycogen), glycolysis and TCA cycle intermediates (glucose-6-phosphate, acetone, pyruvate, lactate, α-ketoglutarate, citrate, oxaloacetate, glyceraldehydes, adenosine-5'- triphosphate, and 3-phosphate glycerol), nucleic acids (adenine, guanine, cytosine, uracil, and thymine), amino acid monomers (glycine, leucine, threoine, lysine, histidine, tyrosine, tryptophan, glutamate, aspartate, asparagine, cysteine, and methionine), amino acid homopolymers (glycine, leucine, threonine, lysine, tyrosine, glutamate, aspartate, asparagine, glutathione, and met-gly-met-met), and proteins (albumin and hemoglobin).

For fatty acids and oils, oil-in-water emulsion systems were prepared by blending 0.8 g of fatty acid or oil with 80 mL deionized distilled water. Phosphatidylcholine, phosphatidylethanolamine, or lysophosphatidyl choline was evaporated from chloroform to a thin film on the wall of a 40-mL sample vial. The vial was placed under a stream of nitrogen to remove any chloroform. Phospholipid liposome systems were prepared by hydrating each phospholipid with 10 mL water by gently shaking for 15 min. The milky suspension was then vortex-mixed to disperse the phospholipid before use. For water-soluble compounds, an aqueous solution of each component (10 mg/mL) was prepared. Four 5-mL portions of 1% solutions were transferred to scintillation vials and irradiated at 0, 2.5, 5, or 10 kGy using a Linear Accelerator.

To identify the gaseous compounds produced by irradiation, a gas chromatograph equipped with a flame ionization detector (FID) and a Nickel catalyst were used. All samples were microwaved for 10 s at full power. Ten minutes after microwave heating, the headspace gas of each sample (200 μL) was withdrawn using an airtight syringe and injected into a splitless inlet of a GC. A Carboxen-1006 Plot column (30 m x 0.32 mm id) was used. Helium was used as a carrier gas at a constant flow of 1.8 mL/min and oven conditions were set at 120 °C. To quantify the amount of gases released, peak areas (pA*second) were converted to the concentration (ppm) of gas in the sample headspace (15 mL) using CO_2 concentration (330 ppm) in air.

Data were analyzed using the generalized linear model procedure of SAS software. Student-Newman-Keul's multiple range test was used to determine significant differences between the mean values of treatments.

Results and Discussion

The production of CO in samples prepared with fatty acid, phospholipid, or plant oil were irradiation-dose dependent (Table 1). Ionizing radiation is known to generate hydroxyl radicals in aqueous or oil emulsion systems. The

hydroxyl radical is the most reactive oxygen species. It can initiate lipid oxidation by abstracting a hydrogen atom from a fatty acyl chain of a polyunsaturated fatty acid (PUFA) and form a lipid radical. After the initial cleavage at the weakest bond of fatty acids or their esters by irradiation, a variety of compounds are formed by the subsequent chemical reactions. The scission at the acyl-oxygen bond among typical compounds accounts for the formation of a major aldehyde, CO, and alcohol or water and that of the alkyl-oxygen bond generated free fatty acid, CO_2, the C_{n-1} alkane, and, possibly, some short-chain hydrocarbons. Phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), and lysophosphatidyl choline (LPC) produced the highest amounts of CO among fatty acids and oils.

The proposed mechanism of CO and CO_2 production from phosphoglycerides is that hydroxyl radical generated by high-energy radiation breaks the ester bonds between fatty acids and glycerol first, and then the -CO- group or carboxylic group of fatty acids is further degraded to produce either CO or CO_2 gas. A glycerol backbone, 2 fatty acid chains, or a fatty acid and a hydrogen, a phosphate, and a choline or ethanolamine, are the common denominators of PC, PE, and LPC. The susceptibility of certain bonds to radiolytic degradation or any other chemical reactions is decided by bond strength or bond dissociation enthalpy. The bond strength in a molecule is dependent its upon component atoms and the bond strength of a double bond is greater than that of a single bond because of electron localization. This, in turn, weakens the bond strength of adjacent atoms with single bonds. Because of the C=O double-bond in the carboxyl end of a fatty acid, the ester bonds of fatty acids to the glycerol backbone are the most susceptible to radiolytic degradation in phosphoglycerides.

Triglycerides such as corn oil and fish oil produced much lower amounts of CO than phosphoglycerides because triglycerides are nonpolar and have no direct contact with water molecules, while phosphoglycerides have a polar end which allows direct contact with water. This provides an important explanation why phosphoglycerides in a liposome system (aqueous) produce greater amounts of CO than triglycerides and free fatty acids in an oil-in-water emulsion system. Irradiation produces hydroxyl radicals by splitting water molecules and the half-life of the free radicals are very short (10^{-6} s). Therefore, the hydroxyl radicals produced by irradiation cannot travel far, and the chemical reaction should be instantaneous and site-specific.

The productions of CO in glucose, fructose, and glycogen were irradiation dose-dependent and the major sources of CO in carbohytrates were fructose and glycogen (Table 2). Among glycolysis intermediates, glyceraldehyde was the major source of CO production, and large amounts of CO gas were also detected in glucose-6-phosphate (G-6-P), pyruvate, and adenosine-5-triphosphate (A-5-P) by irradiation. The nucleic acids adenine, guanine, cytosine, uracil, and thymine were more stable than other compounds and produced only limited amounts of CO gas (Table 3). Only very small amount of CO was generated from nucleic acids. Small amounts of CH_4 were detected only when nucleic acids were irradiated at 10 kGy.

Large amounts of CO gas were detected in asparagine, glutamine, and glycine. This indicated that amino acids that contained amide as a side chain produced a large amount of CO gas after irradiation. Amino acid homopolymers generated higher amount of CO than others (Table 4). Asparagine homopolymer was the major source of CO among amino acid homopolymers and the amount of CO in asparagine homopolymer was 10 times higher than those of asparagine monomer. Large amounts of CO were also produced from albumin and hemoglobin upon irradiation. The results with amino acid homopolymers generally agreed with those of amino acid monomer.

Conclusion

Asparagine homopolymer, glyceraldehydes, and phospholipids produced the greatest amounts of CO gas by irradiation. The amounts of CO produced from these sources were large enough to react with most of the heme pigments present in light meats such as poultry breast and pork loin, and the production of CO via the radiolytic degradation was closely related to the chemical structure of molecules.

Table 1. The production of CO from fatty acids and oils by irradiation [1,2]

	0 kGy	2.5 kGy	5 kGy	10 kGy	S.E.M.[3]
	---------- *Unit (ppm [4])* ----------				
Oleic acid	18.98 ^{y}	20.93 ^{z}	20.39 ^{z}	16.81 ^{z}	3.04
Linoleic acid	12.58 ^{by}	26.57 ^{az}	25.81 ^{ayz}	20.07 ^{abz}	2.49
Linolenic acid	26.57 ^{y}	22.78 ^{z}	30.37 ^{yz}	37.96 ^{z}	6.07
Phosphatidyl choline	28.20 ^{dy}	276.90 ^{cw}	399.68 ^{bv}	472.13 ^{ax}	10.30
Phosphatidyl ethanolamine	24.73 ^{cy}	130.69 ^{by}	177.87 ^{ax}	185.79 ^{ay}	7.16
Lysophosphatidyl choline	63.45 ^{cx}	195.77 ^{bx}	207.48 ^{bw}	608.46 ^{aw}	14.32
Corn oil	34.38 ^{y}	47.72 ^{z}	31.78 ^{yz}	37.20 ^{z}	4.01
Fish oil	22.56 ^{by}	37.42 ^{az}	48.05 ^{ay}	36.66 ^{az}	3.58

[1] Different letters (a-d) within a row with the same sample indicate statistically significant difference ($P < 0.05$).
[2] Different letters (v-z) within a column with the same irradiation dose indicate statistically significant difference ($P < 0.05$).
[3] S.E.M., standard error of the means. n = 4.
[4] Gas concentration in headspace (19 mL) from 5 mL 1% sample solution.

Table 2. The production of CO from carbohydrates and glycolysis intermediates by irradiation [1,2]

	0 kGy	2.5 kGy	5 kGy	10 kGy	SEM [3]
	---------- *Unit (ppm [4])* ----------				
Glucose	15.08 ^{c}	37.42 ^{bcy}	57.48 ^{aby}	85.47 ^{ax}	9.76
Fructose	13.67 ^{d}	53.47 ^{cx}	86.55 ^{bx}	116.92 ^{ax}	8.79
Starch	13.77 ^{c}	40.46 ^{ay}	33.95 ^{bz}	32.00 ^{by}	1.95
Glycogen	13.88 ^{d}	54.23 ^{cx}	74.62 ^{bx}	115.51 ^{ax}	4.77
Glucose-6-phosphate	46.64 ^{cx}	87.64 ^{by}	114.43 ^{by}	171.15 ^{aw}	9.54
Acetone	17.79 ^{by}	27.98 ^{bz}	26.36 ^{by}	45.88 ^{axyz}	3.36
Pyruvate	14.43 ^{by}	37.96 ^{bz}	46.64 ^{by}	91.97 ^{axy}	10.30
Lactate	27.44 ^{bxy}	25.27 ^{bz}	22.02 ^{by}	38.50 ^{ayz}	3.15
α-Ketoglutarate	45.88 ^{bx}	73.75 ^{aby}	68.87 ^{aby}	99.57 ^{ax}	9.22
Citrate	22.02 ^{xy}	24.40 ^{z}	20.39 ^{y}	22.02 ^{yz}	3.47
Oxaloacetate	14.53 ^{dy}	29.83 ^{cz}	41.21 ^{by}	63.77 ^{axyz}	3.47
Glyceraldehyde	36.12 ^{dxy}	168.44 ^{cx}	345.23 ^{bx}	740.24 ^{av}	36.98
Glyceraldehyde-3-phosphate	22.56 ^{abxy}	24.73 ^{az}	15.51 ^{by}	21.15 ^{abyz}	2.06
Adenosine-5-triphosphate	14.43 ^{dy}	28.74 ^{cz}	42.62 ^{by}	91.43 ^{axy}	3.80

[1] Different letters (a-d) within a row with the same sample indicate statistically significant difference ($P < 0.05$).
[2] Different letters (v-z) within a column with the same irradiation dose indicate statistically significant difference ($P < 0.05$).
[3] S.E.M., standard error of the means. n = 4.
[4] Gas concentration in headspace (19 mL) from 5 mL 1% sample solution.

Table 3. The production of CO from nucleic acids by irradiation [1,2]

	0 kGy	2.5 kGy	5 kGy	10 kGy	SEM [3]
	---------- *Unit (ppm [4])* ----------				
Adenine	11.28	16.05	16.16 [y]	15.73 [z]	2.39
Guanine	13.12 [b]	19.31 [ab]	22.34 [abxy]	28.20 [ayz]	3.04
Cytosine	14.64 [c]	24.19 [b]	30.91 [bx]	44.25 [ay]	2.71
Uracil	15.73 [b]	27.98 [b]	29.61 [bxy]	69.74 [ax]	3.80
Thymine	12.58 [b]	22.23 [ab]	16.81 [aby]	29.83 [ayz]	3.80

[1] Different letters (a-d) within a row with the same sample indicate statistically significant difference ($P < 0.05$).
[2] Different letters (x-z) within a column with the same irradiation dose indicate statistically significant difference ($P < 0.05$).
[4] Gas concentration in headspace (19 mL) from 5 mL 1% sample solution.

Table 4. The production of CO from protein, amino acid monomers, and amino acid homopolymers by irradiation [1,2]

	0 kGy	2.5 kGy	5 kGy	10 kGy	SEM [3]
	---------- *Unit (ppm [4])* ----------				
Glycine	15.51 [cyz]	49.53 [by]	52.39 [bx]	72.13 [ax]	3.90
Leucine	15.73 [byz]	17.68 [bz]	15.51 [bz]	26.36 [ayz]	1.95
Threonine	20.07 [bcxyz]	31.45 [abyz]	30.37 [abyz]	41.21 [ay]	3.90
Lysine	19.52 [xyz]	28.20 [yz]	21.69 [yz]	26.36 [yz]	3.25
Histidine	31.45 [w]	22.02 [z]	22.23 [yz]	31.78 [yz]	2.93
Tyrosine	23.10 [bwxyz]	47.72 [ay]	38.29 [axyz]	38.83 [ayz]	4.23
Tryptophan	25.27 [wxyz]	22.56 [z]	20.93 [yz]	23.32 [z]	2.82
Glutamate	23.64 [wxyz]	31.45 [yz]	20.39 [yz]	32.00 [yz]	4.99
Aspartate	27.66 [wx]	20.39 [z]	40.13 [xy]	38.29 [yz]	7.27
Asparagine	26.03 [dwxyz]	80.26 [cx]	97.61 [bw]	123.10 [aw]	3.47
Glutamine	15.18 [cy]	48.27 [by]	55.31 [bx]	72.99 [ax]	5.31
Cysteine	26.90 [awxy]	18.44 [bz]	19.85 [byz]	32.32 [ayz]	1.95
Methionine	21.48 [bwxyz]	21.15 [bz]	20.61 [byz]	37.42 [ayz]	2.39
Albumin	22.02 [dxy]	95.77 [cyz]	164.86 [by]	227.22 [aw]	9.54
Hemoglobin	39.91 [dxy]	101.95 [cy]	188.50 [by]	259.00 [av]	11.71
Glycine homopolymer	15.18 [dy]	46.64 [cz]	60.20 [bz]	77.87 [az]	3.47
Threonine homopolymer	46.42 [dx]	76.25 [cyz]	91.43 [bz]	146.42 [ax]	2.60
Lysine homopolymer	26.36 [cxy]	52.93 [byz]	68.33 [bz]	112.26 [ay]	6.62
Tyrosine homopolymer	21.48 [bxy]	50.43 [ayz]	47.72 [z]	65.94 [z]	6.94
Glutamate homopolymer	32.86 [bxy]	70.28 [ayz]	67.03 [az]	77.01 [az]	3.15
Aspartate homopolymer	24.19 [cxy]	78.63 [byz]	77.33 [bz]	122.34 [axy]	4.77
Asparagine homopolymer	31.24 [dxy]	473.97 [cx]	823.21 [bx]	962.04 [au]	24.08
Glutathione	47.18 [abx]	43.17 [bz]	48.81 [abz]	56.18 [az]	2.60
Met-Gly-Met-Met	13.56 [dy]	77.55 [cyz]	101.74 [bz]	144.25 [ax]	4.34

[1] Different letters (a-d) within a row with the same sample indicate statistically significant difference ($P < 0.05$).
[2] Different letters (t-z) within a column with the same irradiation dose indicate statistically significant difference ($P < 0.05$).
[4] Gas concentration in headspace (19 mL) from 5 mL 1% sample solution.