Nutraceuticals & Food
Vol. 7, p.184~187 (2002)

Nutraceuticals and Food

Effect of Gamma-Irradiation on the Molecular Properties of Blood Plasma Proteins

Seunghwan Lee, Seunghyun Lee and Kyung Bin Song†

Department of Food Science and Technology, Chungnam National University, Taejon 305-764, Korea

Abstract

Blood products from slaughterhouses that are not hygienically prepared for disposal or food consumption pose a human health hazard. Gamma irradiation is an effective method for sterilization of blood products, but may introduce changes in the molecular characteristics of proteins. This study evaluated the effects of irradiation on animal plasma proteins. Bovine and porcine blood was obtained from a slaughterhouse and the plasma proteins purified and lyophilized. The secondary structure and molecular weight distribution of the plasma protein solutions and powders were examined after γ-irradiation at 1, 5, 7 and 10 kGy. Gamma-irradiation affected the molecular properties of the protein solutions, but not the protein powders. Circular dichroism and sodium dodecyl sulfate-polyacrylamide gel electrophoresis studies showed that increased doses of γ-irradiation decrease the ordered structure of plasma proteins in solution, and cause initial fragmentation of the polypeptide chains and subsequent aggregation.

Key words: plasma proteins, gamma-irradiation, molecular properties, circular dichroism

INTRODUCTION

Gamma irradiation technology has been applied to food production as a non-thermal process for reducing microbial contamination and extending the shelf-life of food products (1,2). However, irradiation also induces chemical changes in biopolymers, such as proteins, including: fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals generated by the radiolysis of water (3-6). Hydroxy and superoxide anion radicals generated by γ-irradiation can modify the primary structure of proteins, resulting in distortions of secondary and tertiary structures (5). Assuming that these changes occur simultaneously, their rates depend on the chemical properties and physical state of the protein and the irradiation conditions (7). The effect of γ-irradiation on protein conformation appears to be especially dependent upon factors such as protein concentration, the presence of oxygen, and the quaternary structure of the proteins.

In general, radiation causes irreversible changes at the molecular level by breakage of covalent bonds of polypeptide chains. Exposure of proteins to oxygen radicals results in both nonrandom and random fragmentation (8). Protein fragmentation is affected by the local environment around amino acid side chains, the primary amino acid sequence of the protein, and its accessibility to oxygen radicals generated by radiation (6). In Schuessler and Schilling's model (3), bovine serum albumin (BSA) is cleaved by oxidative destruction of proline residues, yielding specific protein fragments. Irradiation may also cause aggregation and cross-linking of proteins (3,4,6,9,10), and protein solutions are subject to the formation of covalent cross-linkages between free amino acids and proteins and between peptides and proteins (4).

Blood released from slaughterhouses is not efficiently utilized and mostly discarded without proper disposal treatment, which may cause pollution of drinking water in developing countries. Animal products should be appropriately processed in slaughterhouses in compliance with hazard analysis critical control point (HACCP) programs for safety. In Korea, bovine blood is processed as Sunji and/or Soondae. Because plasma proteins are an excellent source of nutrients, preparation of plasma hydrolysates may be a good way to utilize blood as a food ingredient in sauces or as a functional food. Therefore, the objectives of this study were to evaluate the use of gamma-irradiation in the processing of plasma proteins, and to investigate the effects of irradiation on the molecular properties of plasma proteins.

MATERIALS AND METHODS

Sample preparation

Bovine and porcine blood samples collected from a

†Corresponding author. E-mail: kbsong@cnu.ac.kr
Phone: 82-42-821-6723, Fax: 82-42-825-2664

slaughterhouse (Taejeon, Korea) were immediately treated with ethylenediaminetetraacetic acid (EDTA, 2 g/L), to prevent coagulation, and centrifuged at 11,590×g for 30 min to remove blood cells. Plasma proteins were precipitated by adding 2% trichloroacetic acid (TCA) to the supernatant; and then freeze-dried.

Sample irradiation

Lyophilized plasma protein powder samples (50 g) were prepared for irradiation by wrapping in polyethylene film, and 0.5% protein solutions (20 mM phosphate buffer, pH 7.0) were poured into borosilicate glass vials (16×125 mm). All samples were irradiated at room temperature using a ^{60}Co gamma irradiatior Type IR-79 (Nordion International Inc., Ontario, Canada) to achieve exposure doses of 0, 1, 5, 7 and 10 kGy. ^{60}Co exposure was varied from 6 to 189 cm in order to achieve total doses of 1 ~ 10 kGy at dosage rates of 1, 5, 7 and 10 kGy/h.

Circular dichroism (CD) measurements

CD spectroscopy was performed at 25°C with a JASCO-720 spectropolarimeter as previously reported (11,12), at a pathlength of 1-mm and sensitivity of 10 mdeg. The reported CD spectra were averages of 5 scans, and were smoothed using a polynomial curve-fitting program. CD data were expressed as molar ellipticity in deg · cm^2 · dmol^{-1}.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (13). Protein samples (15 μg) for SDS-PAGE were prepared by mixing with sample buffer (60 mM Tris-HCl, 2% SDS, 14.4 mM β-mercaptoethanol, 25% glycerol, 0.1% bromophenol blue, pH 6.8). Proteins were resolved on a 12.5% or 15% separation gel and stained with Coomassie Brilliant Blue. The following standard marker proteins were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used: rabbit muscle myosin (205 kDa), *E. coli* β-galactosidase (116 kDa), rabbit muscle phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and bovine erythrocytes carbonic anhydrase (29 kDa).

RESULTS AND DISCUSSION

Effect of γ-irradiation on SDS-PAGE profile

SDS-PAGE shows the molecular weight profile of the proteins. The major component of bovine and porcine plasma proteins is albumin (66 kDa) on SDS-PAGE (Fig. 1). It is known that blood plasma proteins consist primarily of albumin and globulin fractions (14,15) and that their ratio is dependent on species and other conditions such as age and health of the animal. Bovine and porcine plasma proteins had very similar SDS-PAGE patterns (Fig. 1).

There were two different patterns of irradiation effects on plasma protein SDS-PAGE profiles, according to the physical state of the proteins. Plasma protein powders exhibited no significant change in molecular weight profiles, indicating that there was little degradation or aggregation of the protein molecules. We previously reported a similar lack of effect of irradiation in soy protein isolate and whey protein concentrate (16). However, there were significant changes in plasma protein solutions (Fig. 2). This is easily explained since hydroxy and superoxide anion radicals generated by radiolysis of water can modify the primary structure of proteins. Irradiated proteins sustain two types of observable damage: fragmentation and aggregation (6). SDS-PAGE profiles of plasma proteins showed that γ-irradiation at a low dose of 1 kGy causes a breakdown of the polypeptide chain resulting in the formation of degraded small-molecular-weight mol-

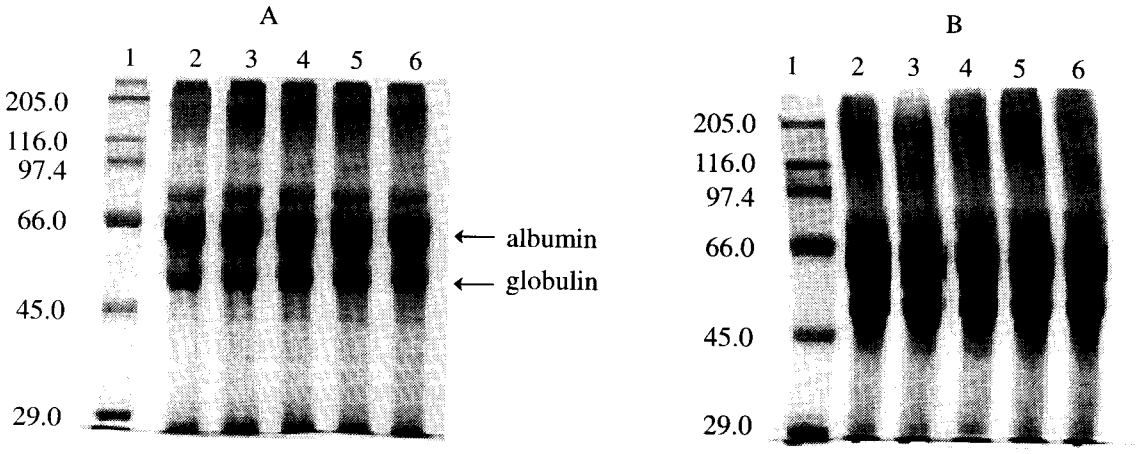

Fig. 1. SDS-PAGE profile of bovine (A) and porcine (B) irradiated plasma protein powders. About 15 μg of protein was loaded on each lane. Lane 1, marker proteins; 2, 0 kGy; 3, 1 kGy; 4, 5 kGy; 5, 7 kGy; 6, 10 kGy.

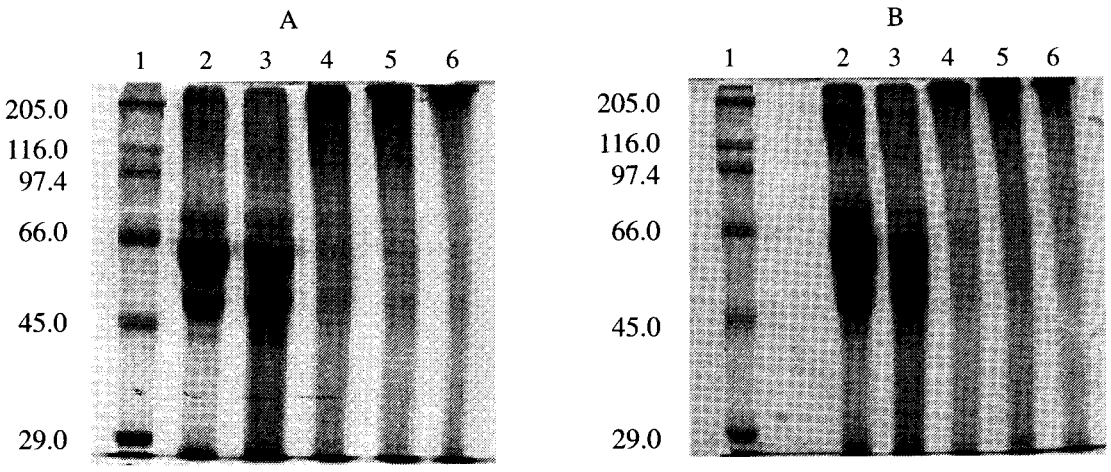

Fig. 2. SDS-PAGE profile of bovine (A) and porcine (B) irradiated plasma protein solutions. Lane 1, marker proteins; 2, 0 kGy; 3, 1 kGy; 4, 5 kGy; 5, 7 kGy; 6, 10 kGy.

ecules (Fig. 2), which are usually seen as a smeared band pattern, even in the presence of 10% gel. Similar results were observed in other studies (3,17,18). Schuessler and Schilling (3) proposed that proline residues are targets for chain scission caused by radiation. Wolff et al. (19) reported that peptide bonds could be cleaved as a result of direct oxidation of proline residues. Typically, breakage of covalent bonds in irradiated proteins is revealed by the appearance of new bands below the major band. When irradiation doses exceed 5 kGy, SDS-PAGE gel reveals only degraded patterns of protein molecules, with some aggregated molecules that could not penetrate the separating gel (Fig. 2).

Proteins may be converted to higher-molecular-weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions, and the formation of disulfide bonds (5,17,18). Any amino acid radical formed within a peptide chain could cross-link with an amino acid radical in another protein. At high doses, but not the lower doses, irradiated proteins underwent covalent cross-linking resulting in the formation of the high molecular weight aggregates (Fig. 2). A similar observation was previously reported for egg white lysozyme and BSA, in which radiation damage resulted in covalent cross-linking and the rupture of disulfide bonds or disulfide exchange (3,20).

Effect of γ-irradiation on the secondary structure

Far-UV CD spectra show the conformational changes in the secondary structure of proteins; and can easily distinguish between native proteins and those with conformational changes resulting from disturbances in the local environment of polypeptide chains. Far-UV CD spectra of plasma proteins irradiated at various doses were obtained (Fig. 3 and 4). Similar to the data of SDS-PAGE,

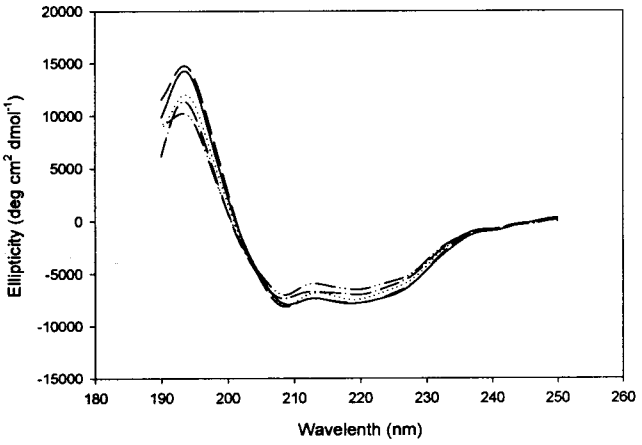

Fig. 3. Far-UV CD spectra of irradiated bovine plasma protein solution.
——, 0 kGy; – – – –, 1 kGy; ·····, 5 kGy; – · – ·, 7 kGy; – · · – · · –, 10 kGy.

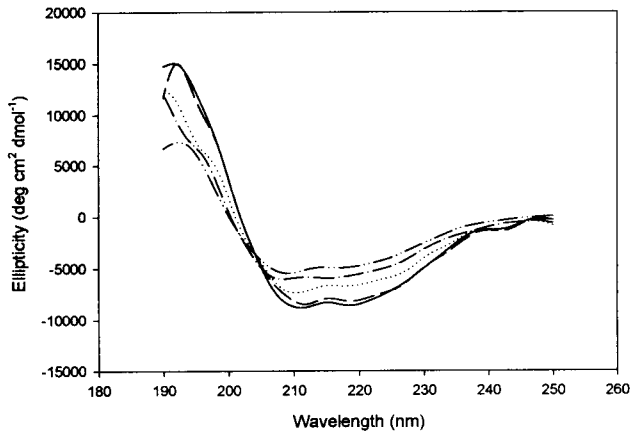

Fig. 4. Far-UV CD spectra of irradiated porcine plasma protein solution.
——, 0 kGy; – – – –, 1 kGy; ·····, 5 kGy; – · – ·, 7 kGy; – · · – · · –, 10 kGy.

the CD spectra of plasma protein powder samples showed little change, regardless of γ-irradiation dose (data not shown). However, CD spectra of plasma protein solutions were significantly affected. CD spectra of both bovine and porcine native plasma protein solutions indicate that they have mostly helical structure; since they have typical negative minimum ellipticity values at 207 and 221 nm. However, γ-irradiation decreased the negative ellipticity values significantly in the range of 210 and 225 nm, resulting in a decrease of ordered structure. This was also observed in other studies (21,22). The ellipticity value at 221 nm, which is a typical indicator of α-helix content, increased from -7680 for 0 kGy treated samples to -6410 for 10 kGy samples of bovine plasma protein. For porcine plasma protein, it increased from -8290 to -4620, indicating that γ-irradiation decreased the α-helix content with a concomitant increase of random coil structure. Oxygen radicals, produced by radiolysis of water during irradiation, subsequently destabilized the α-helical structure of the proteins in solution. The irradiation-induced changes in the CD spectra of the proteins were mostly attributable to the cleavage of covalent bonds and the formation of aggregated products. CD results clearly support that γ-irradiation easily breaks covalent bonds and disrupts the ordered structure of proteins, resulting in changes in the molecular properties of proteins in solution.

In conclusion, γ-irradiation of plasma proteins affected the molecular properties of the proteins in solution, but not in powder form. Gamma-irradiation below 10 kGy significantly affected the secondary structure and molecular weights of solubilized proteins by cleaving polypeptide chains that react with oxygen radicals produced by the radiolysis of water.

ACKNOWLEDGMENTS

This work was supported by a grant from the Korean Ministry of Health and Welfare.

REFERENCES

1. Mahrour A, Lacroix M, Nketsa-Tabiri J, Calderon N, Gagnon M. 1998. Antimicrobial properties of natural substances in irradiated fresh pourtry. *Radiat Phys Chem* 52: 81-84.
2. Foley DM, Dufour A, Rodriguez L, Caporaso F, Prakash A. 2002. Reduction of *Escherichia coli* 0157: H7 in shredded iceberg lettuce by chlorination and gamma-irradiation. *Radiat Phys Chem* 63: 391-396.
3. Schuessler H, Schilling K. 1984. Oxygen effect in the radiolysis of proteins. *Int J Radiat Biol* 45: 267-281.
4. Garrison WM. 1987. Reaction mechanisms in the radiolysis of peptides, polypeptides, and proteins. *Chem Rev* 87: 381-398.
5. Davies KJA, Delsignore ME. 1987. Protein damage and degradation by oxygen radicals III. Modification of secondary structure and tertiary structure. *J Biol Chem* 262: 9908-9913.
6. Filali-Mouhim A, Audette M, St-Louis M, Thauvette L, Denoroy L, Penin F, Chen X, Rouleau N, Le Caer JP, Rossier J, Potier M, Le Maire M. 1997. Lysozyme fragmentation induced by radiolysis. *Int J Radiat Biol* 72: 63-70.
7. Woods RJ, Pickaev AK. 1994. *Applied radiation chemistry*. John Wiley and Sons, New York.
8. Kempner ES. 1993. Damage to proteins due to the direct action of ionizing radiation. *Quart Rev Biophys* 26: 27-48.
9. Puchala M, Schuessler H. 1993. Oxygen effect in the radiolysis of proteins. *Int J Radiat Biol* 64: 149-156.
10. Kume T, Matsuda T. 1995. Changes in structural and antigenic properties of proteins by radiation. *Radiat Phys Chem* 46: 225-231.
11. Cho Y, Song KB. 1997. Effect of chaotropic salt on the secondary structure of pig skin gelatin. *Biosci Biotech Biochem* 61: 1194-1195.
12. Lee MJ, Song KB. 1997. Purification of streptodornase from *Streptococcus equisimilis* and its DNA-induced conformational change. *Biochem Biophys Res Comm* 230: 13-15.
13. Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.
14. Raeker MO, Johnson LA. 1995. Thermal and functional properties of bovine blood plasma and egg white proteins. *J Food Sci* 60: 685-690.
15. Park E, Lee H, Song KB. 1996. Characterization of plasma proteins from bloods of slaughtered cow and pig and utilization of the proteins as adhesives. *Agric Chem Biotech* 39(2): 123-126.
16. Cho Y, Yang J, Song KB. 1999. Effect of ascorbic acid and protein concentration on the molecular weight profile of BSA and β-lactoglobulin by γ-irradiation. *Food Research Int* 32(7): 515-519.
17. Le Maire M, Thauvette L, De Foresta B, Viel A, Beauregard G, Potier M. 1990. Effects of ionizing radiations on proteins. *Biochem J* 267: 431-439.
18. Cho Y, Song KB. 1999. Effect of γ-irradiation on the physicochemical properties of soy protein isolate and whey protein concentrate. *Korean J Food Sci Technol* 31: 1488-1494.
19. Wolff SP, Garner A, Dean RT. 1986. Free radicals, lipids and protein degradation. *Trends Biochem Sci* 11: 27-31.
20. Stevens CO, Sauberlich HE, Bergstrom GR. 1967. Radiation-produced aggregation and inactivation in egg white lysozyme. *J Biol Chem* 242: 1821-1826.
21. Cho Y, Song KB. 2000. Effect of γ-irradiation on the molecular properties of BSA and β-lactoglobulin. *J Biochem Mol Biol* 33: 133-137.
22. Moon S, Song KB. 2001. Effect of gamma-irradiation on the molecular properties of ovalbumin and ovomucoid and protection by ascorbic acid. *Food Chem* 74: 85-89.

(Received April 20, 2002; Accepted May 31, 2002)