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Effect of γ-irradiation on the molecular properties of ovalbumin and ovomucoid and protection by ascorbic acid

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Abstract

To elucidate the effect of gamma-irradiation on the molecular properties of ovalbumin and ovomucoid, the secondary and tertiary structure and molecular weights of the proteins were examined after irradiation at various doses. Gamma-irradiation of protein solutions caused the disruption of the ordered structure of protein molecules. Circular dichroism showed that increase of radiation decreased the ordered structure of proteins. Fluorescence spectroscopy indicated that irradiation quenched the emission intensity excited at 280 nm. SDS-PAGE indicated that radiation caused initial fragmentation of polypeptide chains and, as a result, subsequent aggregation. Ascorbic acid protected against the aggregation and degradation of proteins by scavenging oxygen radicals produced by irradiation and the effect of irradiation on protein conformation was more significant at lower concentrations of proteins.

Keywords: γ-irradiation; Ovalbumin; Ovomucoid; Ascorbic acid

1. Introduction

Radiation treatments of biological materials have been applied in the field of food irradiation. The chemical changes that irradiation causes in proteins are fragmentation, cross-linking, aggregation, and oxidation by oxygen radicals generated in the radiolysis of water (Davies & Delsignore, 1987; Filali-Mouhim et al., 1997; Garrison, 1987; Schuessler & Schilling, 1984). These changes depend on the chemical nature of the protein, its physical state, and the irradiation condition (Woods & Pickaev, 1994). Especially, the effect of γ-irradiation on protein conformation appears to depend on several factors, such as protein concentration, the presence of oxygen, and the quaternary structure of proteins. Davies (1987) published a series of papers on protein damage and degradation by oxygen radicals. Hydroxy radical and superoxide anion radical generated by radiation can modify primary structure of proteins, which results in distortions of secondary and tertiary structure (Davies & Delsignore, 1987).

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 non-random and random fragmentation (Kempner, 1993). Fragmentation involves reaction of α-carbon radicals with oxygen to form peroxyl radicals which decompose to fragment the polypeptide chain at the α-carbon. The protein fragmentation in aqueous solutions is affected by the local conformation of an amino acid in the protein, its accessibility to the water radiolysis products, and the primary amino acid sequence (Filali-Mouhim et al., 1997). In Scuessler and Schilling's model (Schuessler & Schilling, 1984), bovine serum albumin is cleaved by oxidative destruction of proline residues, yielding specific protein fragments. Also, there have been reports on aggregation and cross-linking of proteins by irradiation (Filali-Mouhim et al., 1997; Garrison, 1987; Kume & Matsuda, 1995; Puchala & Schessler, 1993). Covalent cross-linkages are formed between free amino acids and proteins and between peptides and proteins in solution after irradiation (Garrison, 1987).

The oxygen effect in radiation biology is well known. Since oxygen enhances radiation-induced biological damage, antioxidants should be radioprotectors.

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Ascorbic acid is known to react with free radicals (Singh, Singh, & Henderson, 1990). The efficient radioprotection of ascorbic acid against radical formation has been interpreted in terms of the scavenging of protein radicals.

The radiation-induced alteration of the protein structure is observed in a number of protein systems by measuring changes in molecular properties of proteins. Therefore, to further elucidate the effect of oxygen radicals on protein structure, this study describes the radiation effect on the secondary and tertiary structure and molecular weight of the typical egg-white proteins, ovalbumin and ovomucoid, and protection by antioxidants such as ascorbic acid as an oxygen scavenger.

2. Materials and methods

2.1. Materials

Ovalbumin and ovomucoid were purchased from Sigma Chemical Co.(St Louis, MO, USA) and used without further purification. Protein concentrations were determined by measuring the absorbance at 280 nm. Standard marker proteins for SDS-PAGE were obtained from Bio-Rad, Inc.(Richmond, CA).

2.2. Sample irradiation

Ten millilitres solutions of proteins in 10 mM phosphate buffer (pH 7.0) were added in borosilicate glass vials (16×125 mm) and irradiated at room temperature using ^{60}Co gamma ray irradiation Type IR-79 (Nordion International Inc., Ontario, Canada) as source under air with 0, 0.5, 1, 5, and 10 kGy, respectively. ^{60}Co exposure was varied from 6 to 189 cm in order to achieve total doses of 0.5–10 kGy and the dose rates were 0.5, 1, 5, and 10 kGy/h.

2.3. Circular dichroism measurements

Circular dichroism (CD) spectroscopy was performed at 25°C with a JASCO J-720 spectropolarimeter according to the method reported previously (Cho & Song, 1997; Lee & Song, 1997). A 1-mm-pathlength cell was used. The protein solutions were diluted with 10 mM phosphate buffer (pH 7.0) to achieve the desired protein concentration. The reported CD spectra were averages of five scans, and were smoothed by a polynomial curve fitting program. CD data were expressed as molar ellipticity in deg·cm^2·dmol^{-1}.

2.4. Fluorescence spectroscopy

Fluorescence emission intensity of protein solutions irradiated was measured using spectrofluorometer (JASCO FP-750, Japan). Protein solutions irradiated were excited at 280 nm and the emission spectra were recorded from 300 to 450 nm.

2.5. Sodium dodecylsulfate gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed according to the method of Laemmli (1970). Protein samples 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 10% separation gel and stained with Coomassie Brilliant Blue. The following standard marker proteins were used. For ovalbumin and ovomucoid, bovine serum albumin (66,000), egg albumin (45,000), porcine stomach mucosa pepsin (34,700), bovine pancreas trypsinogen (24,000), bovine milk β-lactoglobulin (18,400), and chicken egg white lysozyme (14,300) were used and for ovotransferrin, rabbit muscle myosin (205,000), *E. coli* β-galactosidase (116,000), rabbit muscle phosphorylase b (97,400), bovine serum albumin (66,000), egg albumin (45,000), and bovine erythrocyte carbonic anhydrase (29,000) were used.

3. Results and discussion

Far-UV CD spectra show the conformational change in the secondary structure of proteins. Especially, in the case of change in the local environment of ordered structure of a polypeptide chain, it is easily differentiated from the native state. Far-UV CD spectra of ovalbumin solution irradiated at various doses were obtained (Fig. 1). The spectrum of native ovalbumin had a typical α-helical structure, which has negative minimum ellipticity values at 207 and 221 nm and a positive maximum at 193 nm. It has about 40% α-helical structure. However, γ-irradiation clearly affected the CD spectrum. Mostly, it increased the ellipticity values at 207 and 221 nm, indicating the decrease of ordered structure. Oxygen radicals, formed by the irradiation, subsequently destabilized the α-helical structure of proteins. The same trend was observed for ovomucoid (Fig. 1). Ovomucoid has mostly a β-sheet structure. Increase of γ-irradiation shifted the negative minimum ellipticity values into lower wavelength. This indicates the increase of random coil structure of the protein. The changes in CD spectra of these proteins by radiation were mainly due to the cleavage of covalent bonds of proteins and formation of aggregated products. CD results clearly indicate that γ-irradiation breaks covalent bonds easily and disrupts the ordered structure of proteins, resulting in unnatural products. This is also proved by the fact that some irradiated enzymes lose

their activity as well as immunogenicity (Kume & Matsuda, 1995; Yang, Kim, Matsuhashi, & Kume, 1996). Therefore even very weak γ-irradiation alters the native function of a protein.

To further examine the change in the molecular properties of the protein solutions by irradiation, fluorescence emission intensity was measured. When excited at 280 nm, which excites both trytophan and tyrosine residues of the protein, the tertiary structure of the protein is affected. Fig. 2 shows that γ-irradiation causes a decrease of emission intensity of ovalbumin due to the change of local environment around tryptophan and tyrosine residues. Increase of radiation quenches the emission intensity of proteins. Similar results were obtained for ovomucoid (data not shown).

Regarding the radiation damage to proteins, two types of damage are observed: fragmentation and aggregation (Filali-Mouhim et al., 1997). SDS-PAGE profiles of the proteins show that γ-irradiation, at low dose, causes breakdown of polypeptide chains and, as a result, formation of degraded small molecular weight molecules (Fig. 3). Similar results were observed in other studies (Le Maire, Thauvette, De foresta, Viel, Beauregard, & Potier, 1990; Schuessler & Schilling, 1984). Le Maire et al. (1990) suggested that the locations of the break points, caused by radiation on aspartate transcarbamylase, are fragile bonds in the polypeptide chain. Also, Schuessler and Schilling (1984) proposed that proline residues are the targets for chain scission

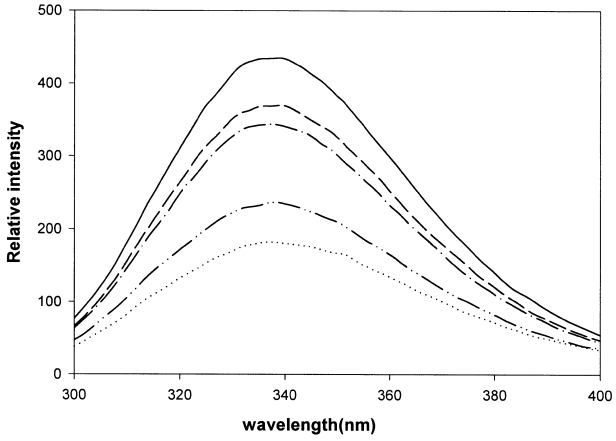

Fig. 2. Fluorescence emission spectra of ovalbumin irradiated. The protein concentration was 0.02%.—;, 0 kGy; —, 0.5 kGy; –●–, 1.0 kGy; –●●–, 5 kGy;—, 10 kGy.

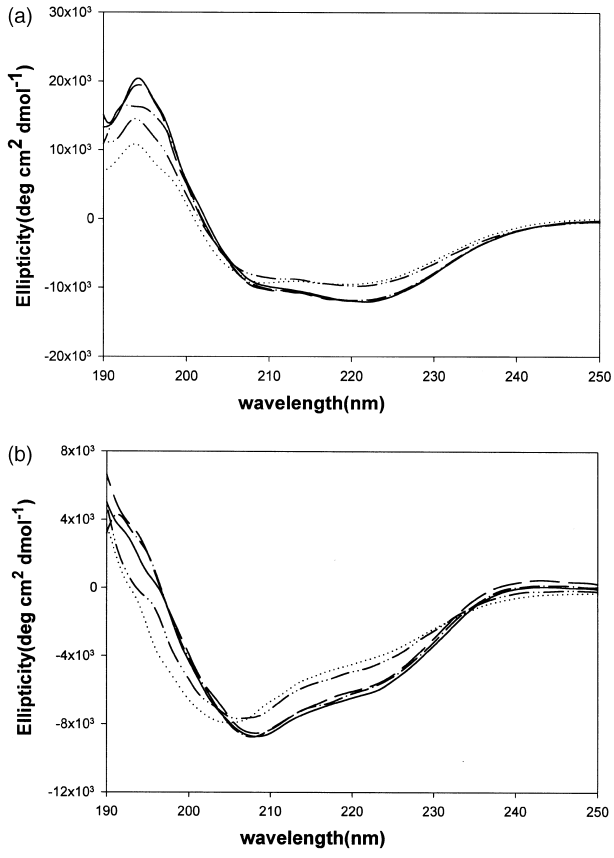

Fig. 1. CD Spectra of ovalbumin and ovomucoid irradiated. The protein concentrations were 0.02%. (a) Ovalbumin, (b) ovomucoid. —, 0 kGy; – – –, 0.5 kGy; –●–, 1.0 kGy; –●●–, 5 kGy;—, 10 kGy.

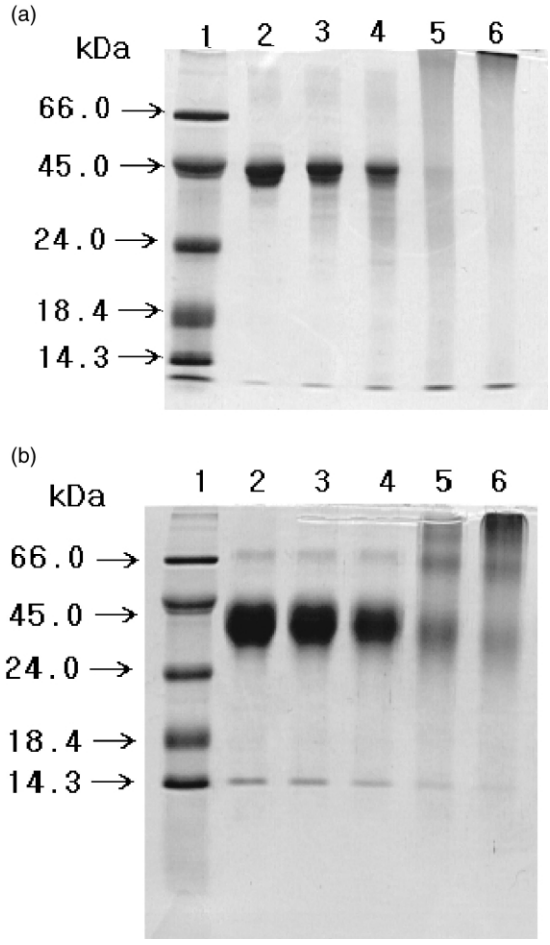

Fig. 3. SDS-PAGE profile of ovalbumin and ovomucoid irradiated. (a) Ovalbumin, (b) ovomucoid. (1) Molecular weight marker protein; (2) 0 kGy; (3) 0.5 kGy; (4) 1 kGy; (5) 5 kGy; (6) 10 kGy.

caused by radiation. Wolff, Garner and Dean (1986) reported that peptide bonds could be cleaved as a result of direct oxidation of proline residues. Usually, breakage of covalent bonds in irradiated proteins is shown as new bands below the major band. After doses above 5 kGy, on SDS-PAGE gel, there were only degraded pattern of protein molecules with some aggregated molecules which could not penetrate the separating gel of PAGE (Fig. 3). A similar result was observed in the case of hemoglobin (Puchala & Schuessler, 1993). When hemoglobin was irradiated in phosphate buffer at pH 7, under air, aggregation, as well as fragmentation, was observed.

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 (Davies & Delsignore, 1987; Le Maire et al., 1990). Any amino acid radical formed within a peptide chain could cross-link with an amino acid radical in another protein. At high dose range, proteins exposed to radiation underwent covalent cross-linking. The formation of the high molecular weight aggregates was negligible at the low doses, but increased significantly with higher doses (Fig. 3), partly caused by intermolecular S−S bonds. Similar interpretations were reported previously for egg-white lysozyme and BSA, where radiation damage was explained by the rupture of disulfide bonds and disulfide exchange (Schuessler & Schilling, 1984; Stevens, Sauberlich, & Bergstrom, 1967).

Ascorbic acid protected against the aggregation of ovalbumin and ovomucoid by scavenging oxygen radicals (Fig. 4). Especially, at 5 and 10 kGy, all the proteins studied showed distinct changes in the presence of ascorbic acid, resulting in maintaining of the intact bands. Ascorbic acid unambiguously showed a consistent pattern in which the native protein conformation was conserved by scavenging oxygen radicals. Similar results were observed in other studies where the formation of organic radicals was suppressed by the addition of ascorbic acid (Yoshimura, Matsuno, Miyazaki, Suzuki, & Watanabe, 1993).

It is known that protein concentration affects the degree of conformational change after irradating protein. Krumhar and Berry (1990) showed that radiation effect was more significant at low protein concentration. This was consistent with our observation where, at low concentrations of proteins, the effect of radiation was more significant (Fig. 5). In the range 0.1–1% of protein, SDS-PAGE shows only the aggregated pattern of protein bands. However, at 2 and 4% concentration, irradiation did not affect the molecular weight profile of ovalbumin significantly, compared with the native state. With increase of protein concentration, the SDS-PAGE profile became close to the control, which was not irradiated. This is strong evidence that protein molecules

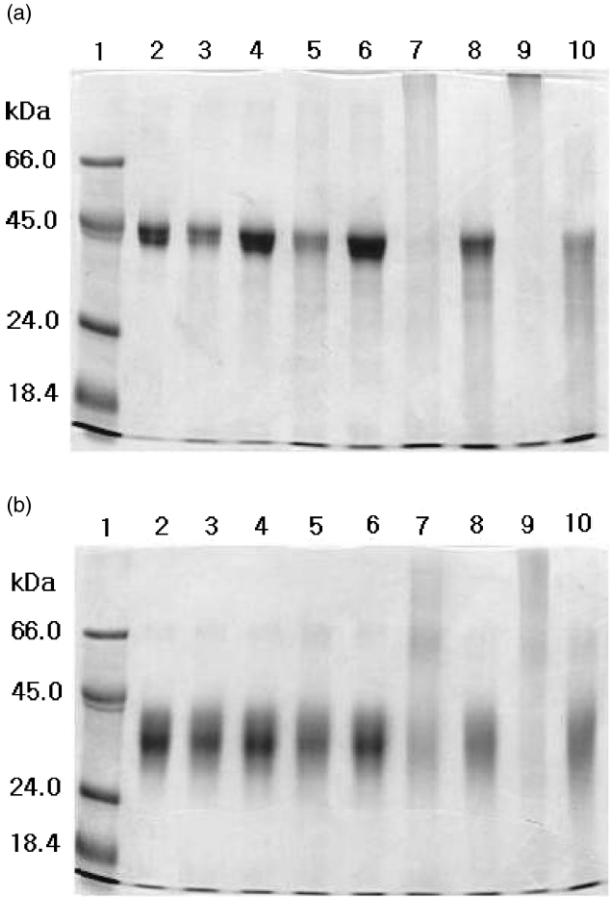

Fig. 4. SDS-PAGE profile of ovalbumin and ovomucoid irradiated in the presence of 0.02% ascorbic acid. The protein concentrations were 0.5%. (a) Ovalbumin (b) ovomucoid. (1) Molecular weight marker protein; (2) 0 kGy without ascorbic acid; (3) 0.5 kGy without ascorbic acid; (4) 0.5 kGy with ascorbic acid; (5) 1 kGy without ascorbic acid; (6) 1 kGy with ascorbic acid; (7) 5 kGy without ascorbic acid; (8) 5 kGy with ascorbic acid; (9) 10 kGy without ascorbic acid; (10) 10 kGy with ascorbic acid.

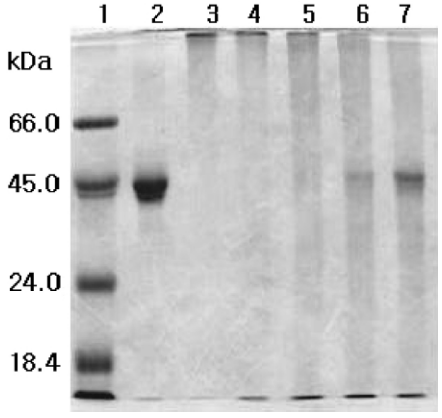

Fig. 5. Effect of protein concentration on SDS-PAGE profile of ovalbumin irradiated at 10 kGy. (1) Molecular weight marker protein; (2) native ovalbumin; (3) 0.1%; (4) 0.5%; (5) 1%; (6) 2%; (7) 4%.

are affected more significantly by oxygen radicals at low protein concentration.

In conclusion, γ-irradiation of protein solutions causes disruption of the ordered structure of protein molecules, as well as degradation and aggregation of the polypeptide chains, due to oxygen radicals generated by irradiation and alters the secondary structure, tertiary structure, and molecular weight profiles of ovalbumin and ovomucoid. Ascorbic acid protects against the aggregation and degradation of proteins by scavenging oxygen radicals produced by irradiation and the effect of irradiation on protein conformation is more significant at lower concentrations of proteins.

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