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GAMMA-RADIATION-INDUCED DAMAGE OF PROTEINS IN THE THICK FRACTION OF EGG WHITE

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Abstract

The ^{60}Co gamma-ray irradiation of ovomucin based protein network of the thick fraction of egg white in the absence of oxygen causes both protein cross linking and protein fragmentation. Protein fragmentation in the absence of oxygen is interpreted as a consequence of decreased diffusion of protein radicals within the protein network. Both protein cross linking and fragmentation, are dose dependent processes, with fragmentation prevailing below 10-15 kGy, and cross linking prevailing at the radiation doses >15 kGy. The radiolytic behaviour of the thick fraction of egg white, suggests that gamma irradiation of similar mucine containing structures might also result in accumulation of structurally altered and conceivably non-functional proteins *in vivo*.

Introduction

Due to its well-defined structure egg white represents a convenient model for investigation of radiation-induced damage of complex protein systems. Egg white is composed of the thin and the thick fraction. The thin fraction is a true solution of several proteins: ovalbumin, conalbumin, ovoglobulin and lysozyme. The thick fraction is composed of highly glycosilated, hydrated proteins ovomucine and ovomucoid which form a matrix encompassing other egg white proteins in a form of monomers, agglomerates or conglomerates [1]. Upon irradiation the proteins of the thin fraction, saturated with N_2O, undergo the reaction of cross-linking, but interestingly enough the scissoring reaction was also observed in the absence of oxygen [2]. This observation was in contrast with the results of the radiolytic behaviour of purified ovalbumin in N_2O saturated solution, when only cross-linking was observed [3,4]. The radiation-induced protein scissoring in the absence of oxygen was interpreted as a consequence of the thin fraction complexity, compared to the simple, one-component solution. In this paper we report the results of the study of radiation-chemical behaviour of the thick fraction matrix of egg white as an even more complex protein system.

Experimental

Fresh Brown Leghorn (*Gallus gallus)* farm hen's eggs were used as a source of thick fraction of egg white. It was separated by Buchner funnel filtration, saturated with either N_2O or Ar, sealed in ampoules and irradiated at a gamma source of ^{60}Co. The dose rate was 51.5 Gy/min is determined by Fricke dosimetry. The viscosity of egg white was measured by Ostwald viscometer at 20.0 °C (n=5). Protein analysis was performed by Sephadex G-200 gel filtration and SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The Sephadex G-200 column was calibrated by Blue Dextran

2000 (molar mass Mm=2.000,000 g/mol,) and cytochrom C (Mm=13,370 g/mol, R_s=1.79nm). For SDS-PAGE calibration was performed by chicken muscle myosin heavy chain (Mm=223,000g/mol) and ovalbumin (Mm=43,500 g/mol). The quantification of protein products was performed after gel scanning with Pharmacia-LKB UltraScan-XL densitometer. The experimental error of these measurements was less than 8%.

Results and Discussion

The Sephadex G-200 chromatography of the thick fraction proteins showed that at the radiation dose of 1.5 kGy most of egg white matrix was decomposed and major proteins: conalbumin (Mm=87,000 g/mol), ovoglobulins G_2 and G_3 and ovalbumin (Mm=43,500 g/mol) co eluted with ovalbumin (*Figure 1)*.

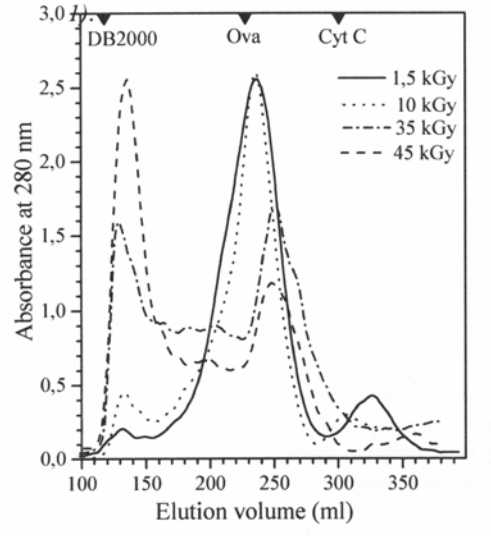

Figure 1. Sephadex G-200 chromatography of irradiated egg white proteins; R_s markers are indicated by arrows.

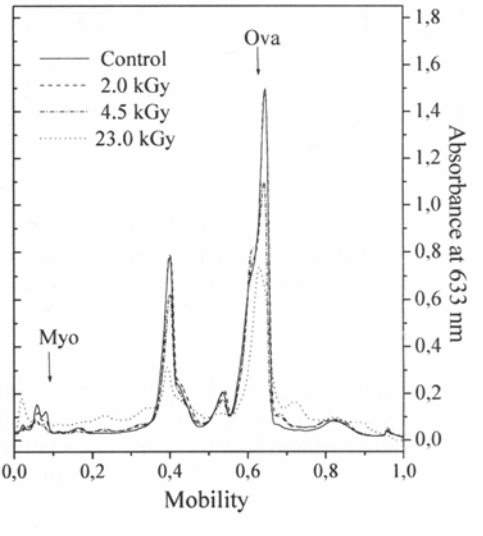

Figure 2. SDS-PAGE of gamma-irradiated egg white proteins. Mm markers are indicated by arrows.

Protein cross linking was also observed, thus that at 45 kGy the dominant protein peak contained products with Mm >250,000 g/mol (*Figure 1*). The SDS-PAGE analysis *(Figure 2)* confirmed that both protein fragmentation and cross linking of the thick fraction occurred upon irradiation. The former reaction was dominant at the lower radiation doses (10-15 kGy), while the later was prevailing above 15 kGy *(Figure 3).* The results suggested that while the egg white proteins were tightly bound inside the protein matrix, the diffusion of radiation generated protein radicals was slower and protein fragmentation was more likely to occur. With the increase in the radiation dose, S-S bridges and other matrix stabilizing bonds were disrupted, the diffusion of released protein radicals became faster and the cross linking reaction prevailed. These

results correlated well with viscosity measurements of the irradiated thick fraction, which showed more abrupt decrease when samples were saturated with Ar, *i.e.* when S-S bridges holding the matrix were broken, compared with of N_2O saturated samples (*Figure 4*).

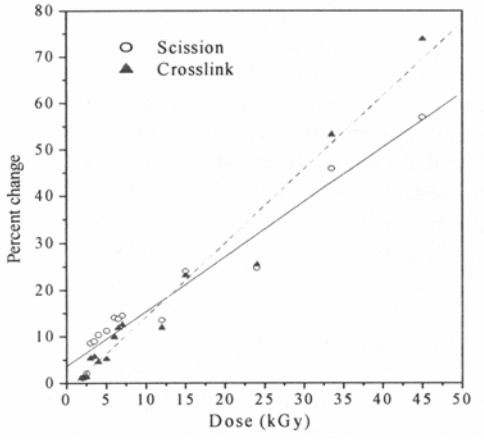

Figure 3. The percent of cross linked and fragmented thick fraction proteins

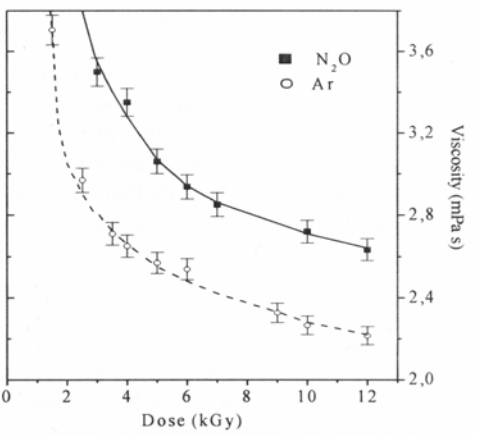

Figure 4. The viscosity of gamma-irradiated thick fraction proteins

Conclusions

The radiation-chemical behaviour of the thick fraction of egg white indicates that in the complex protein matrix both protein cross linking and fragmentation in the absence of oxygen take place. The later reaction is of potential importance for radiation-induced reactions *in vivo, i.e.* for radiation protection and radiotherapy.

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