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Gamma-radiation induced agglomeration of chicken muscle myosin and actin

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Abstract: Radiolytic behaviour of the major vertebrate muscle proteins: fibrillar myosin (molar mass, M_m = 520,000 g/mol) and filament forming actin (M_m = 42,050 g/mol) was studied using a SDS-polyacrylamide gel electrophoresis and quantified by high precision laser-densitometry. In order to study the OH radical contribution to the radiation damage, purified chicken myosin and actin (4 μM) were prepared in N_2O saturated solution and irradiated with 1–3 kGy at ^{60}Co gamma source. With respect to changes in the molecular mass, the only observed myosin and actin damage was dose dependent agglomeration of proteins. The corresponding radiation chemical yields of 5×10^{-8} mol J^{-1} and 6.3×10^{-8} mol J^{-1} were obtained for myosin and actin, respectively. This result confirmed that only the radiation-induced agglomeration is initiated with the reaction of the OH radical even in the situation where the OH radical concentration produced exceeds the protein concentration 500 times, thus enabling the multi-radical attack to occur.

Keywords: radiation, gamma rays, myosin, actin, protein agglomeration.

INTRODUCTION

The effect of ionizing radiation on the structure of the major vertebrate muscle proteins myosin (44 % of total muscle by mass) and actin (22 %) is of importance in applied as well as in basic science. The practical interest in the radiolysis of these proteins is based on the fact that biological decontamination from pathogenic organisms, in the meat industry is frequently performed by irradiation with 4–7 kGy of X or gamma-rays.[1,2] Although it is claimed that the organoleptic and nutritive characteristics of radiation-sterilized meat are not significantly changed,[3] structural differences, judged by M_m changes of the constituent proteins, were observed.[4] The interest of basic science in the radiolysis of muscle proteins is base on the fact that due their structural characteristics,[5–7] highly fibrillar myosin (fibril

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length, L = 160 nm; fibril diameter, D = 2.6 nm; M_m = 520,000 g/mol) and globular actin (M_m = 42,050 g/mol), which readily forms short filaments (L = 37 nm, D = 5 nm), represent a convenient model system for the evaluation of the effects of protein conformation and M_m on the type and extent of radiation-induced damage.[8,9]

In this study, purified chicken myosin and actin were prepared in N_2O saturated solution and irradiated with 1–3 kGy of gamma-rays from a ^{60}Co source. The ratiation-induced protein damage resulting in M_m changes were analysed by SDS-polyacrylamide gel electrophoresis and quantified by high precision laser-densitometry. The results of the radiolysis of purified muscle proteins were compared with their radiolytic behaviour in the intact muscle, as well as with the radiolytic behaviour of the highly globular chicken protein, ovalbumin (R_s = 3 nm) that was extensively characterized previously.[11]

EXPERIMENTAL

Chicken myosin (Sigma M-7266, M_m 520.000 g/mol, protein concentration, P_c = 10.6 μM or 5.4 mg/ml) containing purified actin was purchased from Sigma, St. Louis, USA. The preparation was dialyzed for 9 h at 25 °C followed by 15 h at 4 °C against 100 mM Na-phosphate buffer pH 7.4 in order to remove the high concentration of salt (0.6 M) and glycerol (50 %). The samples ($P_c \cong 4$ μM) were saturated with N_2O for 3 h to remove traces of oxygen, sealed in micro capillaries and irradiated with ^{60}Co gamma-rays for various time intervals. The radiation dose rate was 47.8 Gy/min as determined by Fricke dosimetry. After irradiation, the samples were denatured with 125 mM Tris-HCl pH 6.8 containing 2 % SDS, 10 % glycerol, 5 % β-mercaptoethanol and 0.002 % bromophenol blue, boiled for 2 min and 10 μl samples containing about 20 μg of proteins were analysed by discontinuous 5 % or 15 % SDS-polyacrylamide gel electrophoresis (SDS-PAGE).[10] The protein bands were visualized by Coomassie Brilliant Blue staining and scanned using an UltroScan XL scanning densitometer. The gel was calibrated using SeeBlue™ (Novex, San Diego) standards: rabbit myosin heavy chain $M_m \cong$ 250,000 g/mol, BSA M_m = 98,000 g/mol, glutamic dehydrogenese M_m = 64,000 g/mol, alcohol dehydrogenase M_m = 50,000 g/mol, carbonic anhydrase M_m = 36,000 g/mol, myoglobin M_m = 30 000 g/mol and lysozyme M_m = 16 000 g/mol. A linear relationship between log M_m and protein mobility was established, and used to estimate the M_m of the protein peaks. Radiation-induced damage of chicken ovalbumin ($P_c \cong 4$ μM) was determined as previously described.[11] Quantification of the radiation products was performed by comparison of the respective integral area of the control and irradiated samples. The experimental error was estimated to be ≤ 8 %.

RESULTS AND DISCUSSION

In our previous work, the ^{60}Co-gamma-ray radiation-induced damage of purified chicken egg white ovalbumin in solutions saturated with N_2O were studied.[11] Under these conditions, the single radiation-induced damage leading to molar mass (M_m) changes of ovalbumin was intra-molecular or inter-molecular agglomeration, which occured by the mechanism of grafting.[11] The same experimental conditions were applied in the present work for the irradiation of purified chicken muscle myosin and actin. The irradiated samples were analysed on 5 % SDS-PAG for quantification of the myosin damage (Fig. 1) and on 15 % SDS-PAG for quantification of the actin damage (Fig. 2). As can be seen, the dominant ^{60}Co gamma-ray radiation-induced damage of

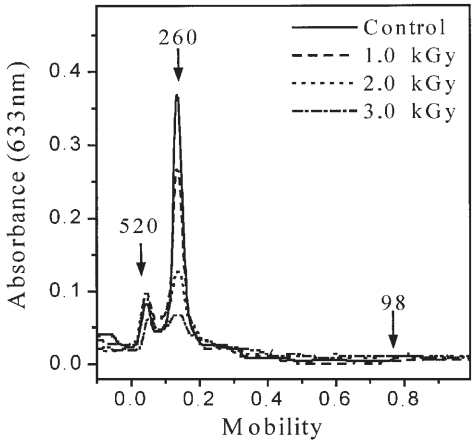

Fig. 1. Densitometric scan of control and irradiated chicken muscle myosin analysed on 5 % SDS-PAG. The M_m values of the standards are given in thousands and are indicated by arrows.

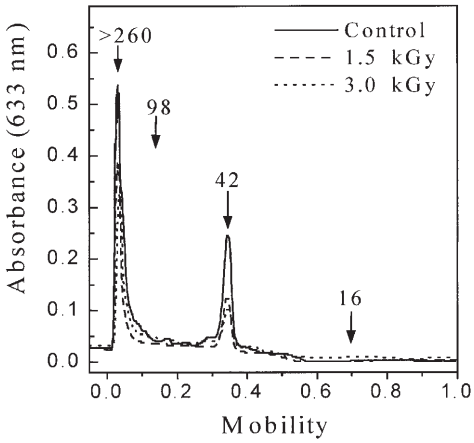

Fig. 2. Densitometric scan of intact and irradiated chicken muscle actin analysed on 15 % SDS-PAG. The M_m values of the standards are given in thousands and are indicated by arrows.

myosin was extensive protein agglomeration. The M_m of the myosin agglomerates exceeded 1,000,000 g/mol, preventing their penetration even through the 5% stacking gel, so they remained in the sample well during electrophoresis (not shown). Both myosin holoprotein (M_m 520,000 g/mol, mobility 0.05) and partially dissociated myosin, containing one heavy and one light chain (HC + LC, mobility 0.15) were agglomerated, as judged by the decrease in the magnitude of their respective peaks, and by the lack of protein fragments with lower M_m and so higher mobility (Fig. 1). Statistically relevant determinations were possible only in the case of the M_m 260 000 g/mol myosin peak. They showed that the extent of agglomeration was dose dependent. Thus after a dose of 1 kGy, *cca* 11 % of the myosin (HC+LC) had been agglomerated, while 20 % and 27 % of the protein had been agglomerated after 2 and 3 kGy, respectively (Table I).

Table I. The percent of ^{60}Co gamma-ray radiation-induced agglomeration of myosin, actin and ovalbumin (*mean ± SEM, n = 3-5)*; P_c-protein concentration; HC-heavy myosin chain; LC-light myosin chain; *L*-fibril length; *D*-fibril diameter; R_s-Stokes radius; *n.d.*-not determined

Protein (P_c = 4 μM)	M_m g/mol	Molecular Dimensions	Radiation-induced damage/%			
			1 kGy	1.5 kGy	2 kGy	3 kGy
Myosin *HC + LC*	260,000	*L* = 160 mm *D* = 1.3 nm	11.4 ± 0.8	*n.d.*	20.6 ± 0.5	26.8 ± 1.1
Actin	42,050	*L* = 37 nm *D* = 5 nm	*n.d.*	23.8 ± 1.4	*n.d.*	36.1 ± 0.5
Ovalbumin	42,650	S_r = 3 nm	56.2 ± 0.6	*n.d.*	67.3 ± 0.7	77.1 ± 0.6

The dominant form of ^{60}Co-gamma radiation-induced damage of actin was also protein agglomeration (Fig. 2). The actin agglomerates had M_m > 98,000 g/mol, which

prevented their penetration through the 15 % resolving gel. The actin agglomerates were stacked together with the two myosin bands at a mobility of 0.01– 0.03 (Fig. 2). The actin agglomeration was also dose dependent, thus 24 % and 36 % of the protein had been agglomerated after 1.5 and 3 Gy, respectively (Table I).

Taken together, the results indicated that ^{60}Co gamma-irradiation of both muscle proteins, in N_2O saturated solutions, led solely to the dose dependent agglomeration of the protein. This radiolytic behaviour was similar to that of ovalbumin,[11] and is also in accordance with the accepted postulates of the radiation chemistry of purified proteins.[13,14] As both myosin and actin were irradiated under non-denaturing conditions, the results also suggest that the differences in the degree of fibrillarity and the M_m of these proteins, had no influence on the type of radiation-induced damage. However, the extent of agglomertion of the highly fibrillar myosin (L = 160 nm, D = 2.6 nm) was notably lower than that of the actin filaments (L = 37 nm, D = 5 nm), at all the studied radiation doses (Table I). This finding suggested that the structural differences of myosin and actin influenced the degree of their damage. This observation was further strengthened by the finding that the agglomeration of the highly globular protein ovalbumin (R_s = 3 nm) irradiated under the same conditions (P_c = 4 μM) was significantly larger compared to both myosin and actin (Table I). This observation could be explained by the fact that there is less steric hindrance to the graffing process in the case of ovalbumin, which is a very soluble protein and does not form dimers of protein fibrils, compared to either actin or myosin.[8,9,11] Thus, the difference in the extent of agglomeration of actin and ovalbumin both of which have a monomeric M_m = 42,000 g/mol, could be the consequence of actin being able to form short filaments, shifting its radiolytic behaviour closer to that of myosin.

It should also be noted that the radiolytic behaviour of purified myosin and actin as well as purified ovalbumin were markedly different from their radiolysis in intact muscle or egg white, respectively,[4,12] judged by the absence of protein fragments in the purified proteins. This observation suggests that the radiolytic behaviour of proteins is vastly dependent on the higher order protein structures in which they are integrated *in vivo*.

Finally, the results confirm that industrial radiation-sterilization may generate significant amount (25–35 %) of structurally altered proteins. The effect of structurally changed proteins on the nutritive characteristics of meat should be further investigated, *e.g.*, by digestibility test using gastrointestinal enzymes, such as pepsin, trypsin and chymotrypsin.

CONCLUSIONS

The single ^{60}Co gamma-ray radiation-induced damage of purified chicken myosin and actin in N_2O saturated solution was dose dependent agglomeration. The differences in myosin and actin conformation and M_m did not influence the type of radiation-induced damage, but the extent of the damage was influenced, as

judged by the notably lower amount of agglomeration of the fibrilar myosin compared to the actin filaments, and the significantly lower amount of agglomeration of both muscle proteins compared to the highly globular protein ovalbumin. The radiolytic behaviour of myosin and actin in their purified forms was different from their radiolysis in intact muscle, as shown by the presence of protein fragments when muscle was irradiated. The results confirm that industrially sterilized meat may contain significant amounts (25–35 %) of structurally altered proteins.

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ИЗВОД

АГЛОМЕРАЦИЈА МИОЗИНА И АКТИНА ПИЛЕЋЕГ МИШИЋА ИЗАЗВАНА ГАМА-ЗРАЧЕЊЕМ

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Радиолиза главних протеина мишића: фибриларног миозина (моларне масе, M_m= 520.000 g/mol) и глобуларног актина који гради филаменте (M_m = 42,050 g/mol) има и практични и теоретски значај. Она је важна за индустријску стерилизацију меса, али такође може илустровати утицај конформације и молекулске масе протеина на врсту и степен њихових радијационих оштећења. У овом раду, пречишћени миозин и актин пилећег мишића припремљени су у раствору засићеном N_2O и озрачени дозама 1–3 kGy на ^{60}Co извору гама зрачења. Зрачењем индукована оштећења протеина која доводе до промене M_m анализирана су денатуришућом SDS-полиакриламид-гел електрофорезом и квантификована ласерском денситометријом. Једино оштећење миозина и актина изазвано зрачењем било је дозно зависно умрежавање протеинских ланаца. Резултати су показали да конформација и M_m протеина нису утицале на врсту оштећења. Међутим, миозин је био мање умрежен од актина, а оба протеина мишића су била знатно мање умрежена од глобуларног протеина овалбумина (M_m = 42.650 g/mol), озраченог под истим условима. Радиолиза миозина и актина у пречишћеном стању била је различита од њихове радиолизе у интактном мишићу, судећи по недостатку кидања протеина.

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