PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

September 21-23, 2004
Belgrade, Serbia and Montenegro

PHYSICAL CHEMISTRY 2004

Proceedings

of the 7th International Conference on Fundamental and Applied Aspects of Physical Chemistry

Volume I and II

Editors
A. Antić-Jovanović and S. Anić

ISBN	86-82457-12-x
Title:	Physical Chemistry 2004. (Proceedings)
Editors	A. Antić-Jovanović and S. Anić
Published by:	The Society of Physical Chemists of Serbia, Studentski trg 12-16, P.O.Box 137, 11001 Belgrade, Serbia and Montenegro
Publisher:	Society of Physical Chemists of Serbia
Printed by:	"Jovan" Printing and Published Comp; 300 Copies; Number of Pages: x + 906; Format B5; Printing finished in September 2004.
Text and Layout:	Aleksandar Nikolić

300 – copy printing

GAMMA-RADIATION-INDUCED DAMAGE OF CHICKEN MYOSIN AND ACTIN

A. Nićiforović and M. B.Radojčić

Laboratory of Molecular Biology and Endocrinology
VINČA Institute of Nuclear Sciences, P.O.Box 522
11000 Belgrade, Serbia and Montenegro

Abstract

The single ^{60}Co gamma radiation-induced damage of purified chicken myosin and actin in N_2O saturated solution is dose dependent protein cross-linking. The differences in myosin and actin conformation and Mm do not influence the type of radiation-induced damage, but they influence the extent of radiation-induced damage, judged by the lower cross linking of fibrillar myosin compared to actin. The radiolytic behavior of myosin and actin in purified forms is different from their radiolysis in intact muscle, according to the absence of protein fragmentation in the former. The results confirm that industrially sterilized meat may contain significant amount (25-35%) of structurally altered proteins.

Introduction

Biological decontamination from pathogenic organisms in meat industry is commonly performed by irradiation with 4-7 kGy of x- or gamma-rays [1]. Although it is claimed that after irradiation organoleptic characteristics of meat are not significantly changed, marked structural changes of meat proteins were detected in irradiated frozen chicken muscle [2]. The dominant damage was protein fragmentation. Due to the complexity of muscle, the damage was difficult to ascribe to any individual muscle protein. In this paper purified chicken muscle proteins: fibrillar myosin and globular actin were irradiated with 1-3 kGy in N_2O saturated solutions. Radiation-induced products were resolved by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and quantified after densitometric scanning. The type and the extent of radiation damage of myosin and actin inducing changes in their molar mass (Mm) were determined. The results were compared with gamma-radiation-induced changes in Mm of another purified globular chicken protein, ovalbumin, which was previously thoroughly characterized [3] and herein used as a reference.

Experimental

Purified chicken muscle myosin (Sigma M-7266, Mm 520 000 g/mol) and actin (Mm 42 000 g/mol) were dialysed against 100 mM Na-phosphate buffer pH 7.4, saturated with N_2O, sealed in micro capillaries and irradiated with Co-60 gamma-rays for various time intervals. The dose rate was 47.8 Gy/min as determined by Fricke dosimetry. After irradiation samples were mixed with equal volume of 125 mM Tris-HCl pH 6.8 containing 2% SDS, 10% glycerol and 0.002% brom-phenol-blue, either

with or without 5% β-mercaptoethanol, boiled for 2 minutes and analyzed by SDS-PAGE. The gel consisted of 5% or 15% polyacrylamide resolving part, for analysis of myosin or actin, respectively. The protein bands were visualized by Coomassie Brilliant Blue and scanned using UltroScan XL scanning densitometer. Molar mass (Mm) of each component was determined after calibration with SeeBlue™ standards (Novex, San Diego). Densitometric scans were processed by PC UltroScan and Microcal Origin 4.0 software, and quantification of radiation products was performed by comparison of respective integral area of control and irradiated samples. The experimental error was estimated to be ≤ 8%.

Results and Discussion

In contrast with the results of gamma-radiation-induced effects in frozen (partially anoxic) chicken muscle proteins, where protein fragmentation was the dominant protein damage [2], the major damage of purified myosin and actin in N_2O saturated solution, was protein cross linking or agglomeration (*Figure 1 a,b*). The protein fragmentation was negligible. The Mm of cross linked myosin products exceeded 250 000 g/mol, so they mostly appeared in front of 5% resolving gel (mobility *cca* 0), and were partially unable to penetrate the stacking gel (not shown). The amount of cross linked myosin was proportional to the radiation dose (*Figure 1a*): *cca* 11% of cross linked myosin was observed after irradiation with 1Gy, while 20% and 27% of the protein was cross linked after 2- and 3 Gy, respectively (*Table 1*).

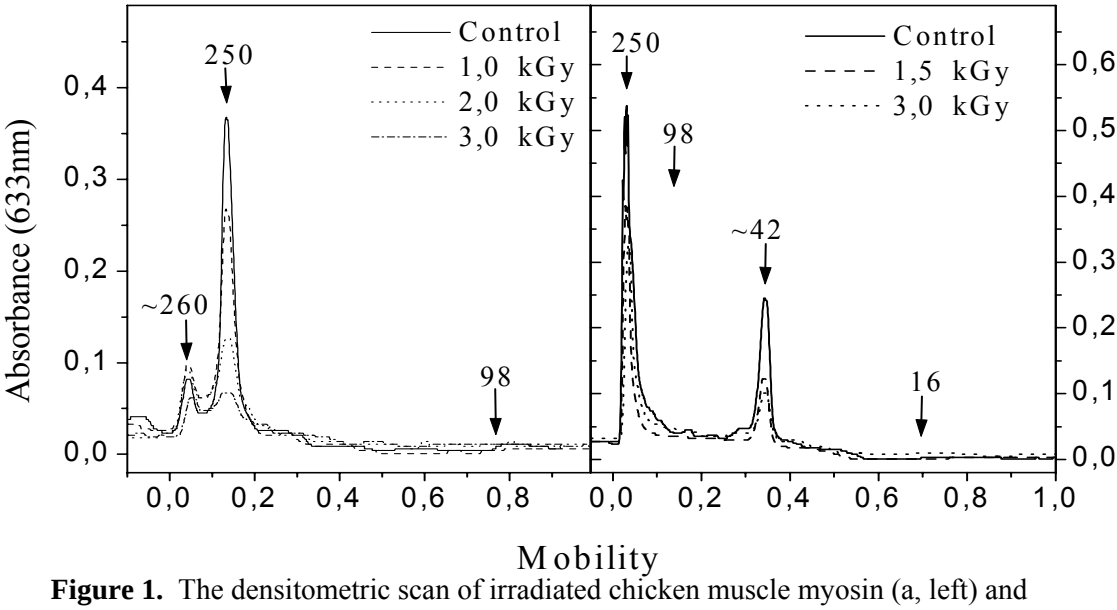

Figure 1. The densitometric scan of irradiated chicken muscle myosin (a, left) and actin (b, right). Mobility of Mm standards is indicated by arrows

Cross-linked actin products *(Figure 1b)* had Mm > 98 000g/mol and were unable to enter 15% resolving gel. They were found together with cross-linked myosin in the stacking gel (mobility 0.01-0.03). The actin cross linking was also dose dependent, yielding 24% and 36% of cross linked products after irradiation with 1.5- and 3 Gy, respectively (*Table 1*). The radiation damage of purified myosin and actin suggested that protein conformation (fibrillar *vs.* globular) and size (250 000 g/mol *vs.* 42 000 g/mol) did not influence significantly the type of radiation-induced damage. However, the extent of radiation-induced cross- linking (for protein concentration in the μM range) was significantly different (*Table 1*). Thus, for the radiation dose of 3 Gy, the cross linking was highest for ovalbumin (~77%), less pronounced for actin (~46%) and the least for myosin (~27%).

Table 1 The extent of the radiation-induced cross- linking of myosin, actin and ovalbumin (mean ± SEM, n=3) as a function of gamma-radiation dose

Radiation dose (kGy)	Protein agglomerates (%)		
	Myosin	**Actin**	**Ovalbumin**
1	11.4 ± 0.8	-	56.2 ± 0.6
1.5	-	23.8 ± 1.4	-
2	20.6 ± 0.5	-	67.3 ± 0.7
3	26.8 ± 1.1	36.1 ± 0.5	77.1 ± 0.6

Conclusions

The major damage of chicken muscle proteins myosin and acting, induced by Co-60 gamma-radiation under anaerobic conditions, is protein cross-linking. The protein conformation and/or size do not influence the type, but influences the extent of the radiation-induced protein damage. The extent of cross linking is dose dependent and is more pronounced in the case of actin, but is less pronounced for both muscle proteins compared with the globular protein ovabumin under the same experimental conditions. The radiation-induced crosslinking of proteins also occurs under aerobic conditions and may influence the digestibility of meat after the routine industrial radiation-sterilization.

References

[1] Irradiation in the Production, Processing and Handling of Food, 21 CFR Part 179, USA-Federal Register 1997, 62, 64107.
[2] A.Nićiforović, M.B.Radojčić, B.H.Milosavljević, Radiat.Phys.Chem. 1999, 55, 731.
[3] Z.Tuce, E.Janata, M.B.Radojčić, B.H.Milosavljević, Radiat.Phys.Chem. 2001, 62, 325.