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Physico-chemical changes taking place in gamma irradiated bovine globulins studied by thermal analysis

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Abstract Structural transformations induced in gamma and alpha globulins under influence of gamma irradiation using doses of 2.5 and 24 kGy were studied by differential scanning calorimetry (DSC) and thermogravimetry (TG, DTG). Thermal decomposition of the globulins irradiated in water suspensions occurs at higher temperatures, in comparison to the reference non-irradiated samples. This was related to formation of covalent linkages in the irradiated proteins, apart to chemical changes induced in amino-acids. Essential modification of thermal decomposition was detected already after irradiation with a dose of 2.5 kGy performed for water suspensions. Irradiation of solid native proteins induces decrease in decomposition temperature and gives evidence of proteins degradation.

Keywords Differential scanning calorimetry (DSC) · Gamma irradiation · Globulins · Proteins · Thermal analysis · Thermal decomposition · Thermogravimetry

Introduction

The present development of gamma irradiation techniques for modification of the proteins based biopolymers (i.e. hydrogels, packaging) [1–6] and for preservation, sterilisation and modification of medicines and food [7–12] induces necessity of better recognition of the physico-chemical changes induced in proteins by gamma radiation. It is the more important that recently relatively high doses till 10 kGy and in special cases even till 70 kGy are applied for protein food (meat, poultry, fish) [9–13]. Accordingly, necessity increases for further development of the methods appropriate for trade control of such foodstuffs. Good knowledge of physico-chemical changes occurring after irradiation in proteins based products and estimation of the applicability of the particular instrumental method for their detection are thus related to both biopolymer and food technology.

Chemical transformations of amino acids, breakdown of peptide bonds, and hydrogen and disulphide bridges, as well as crosslinking of the chains might occur under influence of ionising radiation and affect the tertiary structure of proteins and their physico-chemical properties [14]. Nature of damage that result from radiation processes taking place in the solid state might differ from those carried out in water environment.

During last years differential scanning calorimetry (DSC) became a useful method for the study of protein structure and foodstuffs properties. Although only a few attempts were made to apply DSC for the control of irradiated foodstuff [15] it seems possible to employ this method in future. It was also proved that thermoanalytical methods applied for thermal decomposition of proteins are helpful for characterisation of both proteins structure and the properties of tissues and products which contain proteins [16–25]. Last years thermogravimetry was also applied in the studies of pyrolysis of biomass as well as industrial or medical waste, including those containing proteins [26, 27].

Our previous DSC studies enable to discover the differences between denaturation of non-irradiated and

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gamma irradiated protein [28]. In particular, an essential irradiation effect was found for globulins. The preliminary studies [29, 30] result also in selection of the appropriate conditions enabling to detect the differences between decomposition of initial and gamma irradiated proteins using thermal analysis methods. In the present study the influence of gamma irradiation carried out for solid native proteins and for water suspensions on thermal decomposition of bovine gamma and alpha globulins were examined applying DSC and thermogravimetry (TG, DTG). Both types of measurements were carried out at oxidative conditions, in the range of temperature higher than the range of proteins dehydration, for the samples placed in open Al crucibles.

Experimental

Materials and irradiation

Two preparations of bovine globulins were G5009 and G8512 products of Sigma. These contain, respectively, Cohn fractions II, III (predominantly gamma globulins) and Cohn fraction IV-1 (predominantly alpha globulins). According to composition of these samples terms "gamma globulins" and "alpha globulins" are used in the paper for their specification.

Irradiation was carried out with ^{60}Co gamma rays in air in a gamma cell Mineyola installed in Department of Radiation Chemistry, Institute of Nuclear Chemistry and Technology. Irradiation of solid native proteins was performed at depressed temperature (in dry CO_2, the temperature of sublimation equal to −78.5 °C) with a dose of 24 kGy using a dose rate 0.47 Gy s^{-1}. Fifty percent water suspensions of both gamma and alpha globulins placed in closed polymer capsules were irradiated at ambient temperature with doses of 2.5 and 24 kGy applying a dose rate of 0.44 Gy s^{-1}. These samples were kept in water during 16 h before lyophilisation. Simultaneously, the non-irradiated reference samples were submitted to the same treatment with water.

Differential scanning calorimetry

DSC measurements were carried out in oxygen stream in the temperature range from 120 °C to 670 °C with a heating rate 3 °C min^{-1} for ca. 2 mg (the native samples, characterised by extremely high volume) or ca. 7 mg (the samples submitted to water treatment). A Perkin-Elmer heat flow DSC-7 calorimeter calibrated with thin and indium was used. Calculations of enthalpy (ΔH), peak (T_p) and onset (T_{on}) temperatures were done using DELTA PETOS programme basing three reproducible measurements.

Exact calculation of enthalpy connected to the particular decomposition stages was not possible because of superposition of thermal effects; in particular in the cases of the samples submitted to water treatment. In purpose to describe the differences between irradiated and non-irradiated samples, however, integration of the partial areas of thermal effects was done (I, II and III as well as IA and IB, IIA and IIB, IIIA and IIIB areas, selected as presented in Figs. 5 and 6). The increase and the decrease of the appropriate partial areas were attributed to the increase or decrease in the appropriate exothermal effects. The ratios between mean values of partial areas (determined as above) were calculated and presented in purpose of the better demonstration the differences between the samples. Additionally, the ratios between the partial areas were calculated for each separate DSC curve and the average values of such parameters (evaluated basing several DSC curves of the same individual sample) were shown.

In regard to the blurred profiles of thermal effects occurring in the wide range of temperature lower than 500 °C, T_p and T_{on} values were determined for the last fast decomposition stage and only T_p values were determined in the case of the samples treated previously with water.

Thermogravimetry

Thermogravimetry was carried out using two Mettler TA3000 thermobalances. The one (instrument A) was used for examination of irradiated dry native proteins and the other one (instrument B) was used for the samples irradiated in water suspensions. The measurements were performed in an oxygen stream applying heating rate 3 °C min^{-1}, within initial temperature of 120 °C and final temperature at 620 °C (alpha globulins) or 800 °C (gamma globulins). The samples of ca. 10 mg were used in the case of the both initial globulins. In the cases of proteins submitted to water treatment the applied weighted portions were equal to 7.8–10.6 mg (alpha globulins) or to 15.1–19.8 mg (gamma globulins).The trial was done to describe irradiation effects in terms of differences of mass loss connected to the particular stages of thermal decomposition. In this purpose the maxima of the successive effects on differential curves (DTG) were determined and treated as the borders between the subsequent pyrolysis steps (despite of the fact that exact separation of DTG effects was not possible ought to their superposition). The mass losses taking place within these selected temperature ranges were treated as connected to the particular decomposition steps. These were expressed in terms of the mass of dehydrated proteins (m_d = the sample mass corresponding to stabilisation of TGA and DTGA curves after dehydration). Calculations were done for each sample basing on two reproducible measurements.

Results

Oxygen flow was applied in regard to the necessity of the effective removal of the volatile decomposition products from DSC cell. Dehydration of proteins occurred in the range of low temperature, mainly during fast heating, preceding initiation of the measurements. The process was always totally accomplished at ca. 150 °C, with a mass loss of ca. 10 wt%. Several steps of oxidative thermal decomposition are observed at higher temperatures (Figs. 1, 2). Small amounts of non-volatile residues were still present after heating up to temperature as high as 800 °C.

Irradiation of dry native globulins

Two broad exothermal effects were recorded on DSC curves during heating of initial gamma and alpha globulins in the temperature range till ca. 514 °C, followed by a single sharp exothermal effects at higher temperature (Fig. 3). On the basis of these thermal effects three major temperature ranges of thermal decomposition were distinguished. Profiles of thermal effect recorded in the first temperature range (shoulders were observed at the higher temperature than peaks) indicates, however, that this effect correspond to two successive processes.

Occurrence of four decomposition steps in three basic temperature ranges is evident on the basis of thermoanalytical curves (TG, DTG) of both globulins (Figs. 1, 2). Temperature ranges of particular steps are given for each proteins in Tables 1 and 2. Two decomposition steps occur subsequently in the first temperature range (I). These are assigned as IA and IB stages. The processes taking place in the second temperature range are described as the second (II) step, while these occurring in the third temperature range are assigned as the third (III) step. Samples loose majority of their mass in a broad temperature range within the first (I; Δm_I) and the second (II; Δm_{II}) steps of pyrolysis. A relatively small mass loss (Δm_{III}) results also during the fast third (III) step taking place in the narrow range of the high temperature (Tables 1, 2).

The third decomposition stages of the both irradiated native dry gamma and alpha globulins took place at lower temperature as compared to the initial sample. This is shown as well by thermogravimetry as by DSC data (Table 1, 2 and Fig. 4a,b). Therefore, peak and onset temperature of DSC thermal effect were observed at 541.4 and 534.5 °C, respectively, for the initial gamma globulin bovine, while at 539.0 and 532.3 °C for the irradiated

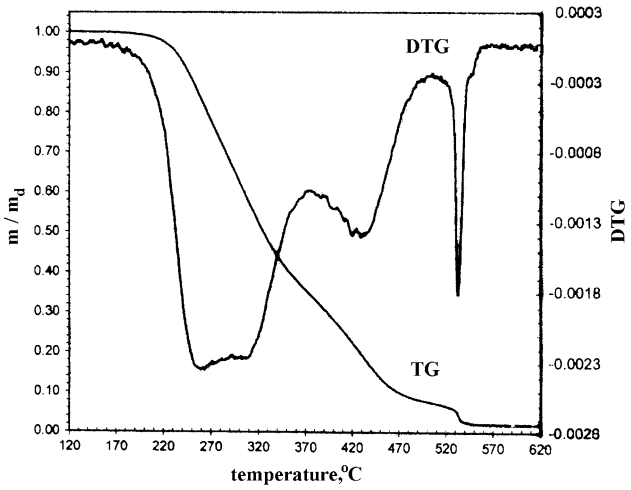

Fig. 1 Thermoanalytical curves recorded during heating of the initial gamma globulins (10.201 mg)

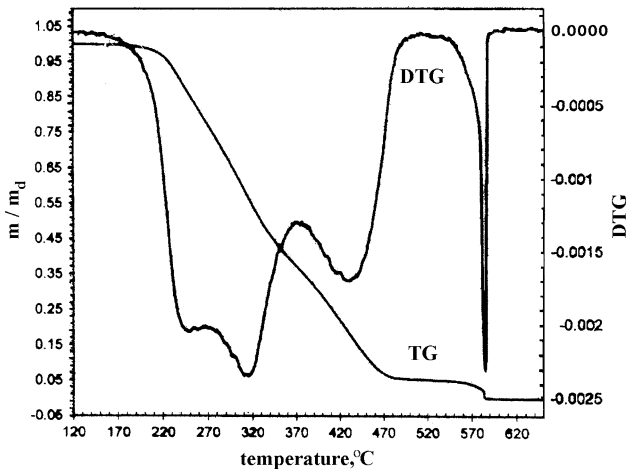

Fig. 2 Thermoanalytical curves recorded during heating of the initial alpha globulins (11.144 mg)

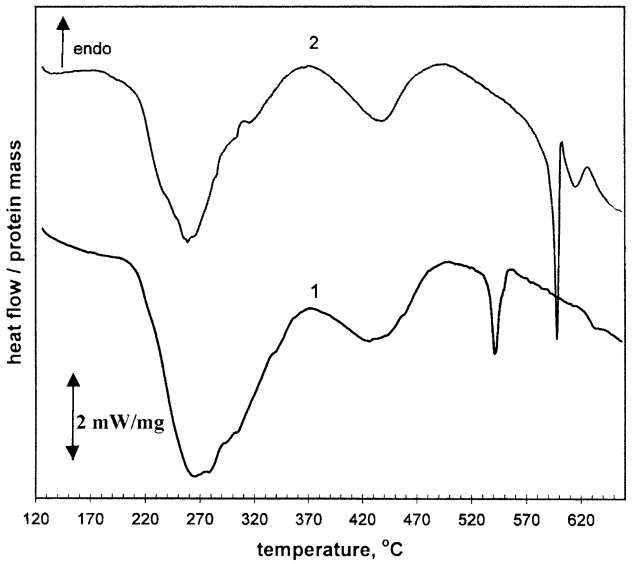

Fig. 3 DSC curves recorded during heating of the initial native gamma globulins (2.135 mg) (curve 1) and alpha globulins (2.034 mg) (curve 2)

Table 1 Thermoanalytical results obtained for the initial gamma globulin and the solid native sample irradiated (at instrument A)

Decomposition stage/ parameter determined	0 kGy		24 kGy	
	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%
I	II	III	IV	V
I + II stages	156.0–504.9	92.30 ± 0.30	156.3–498.1	92.89 ± 0.80
I stage	156.0–377.1	66.07 ± 0.10	156.3–383.8	68.55 ± 0.64
stage IA	156.0–286.3	31.47 ± 0.10	156.3–291.4	31.57 ± 0.30
stage IB	286.3–377.1	34.60 ± 0.30	291.4–383.8	31.51 ± 0.30
II stage	377.1–504.9	26.23 ± 0.30	383.8–498.1	24.34 ± 0.20
III stage	504.9–561.2	5.32 ± 0.20	498.1–556.9	5.36 ± 0.20
Residue at 800 °C	1.94 ± 0.20 wt%		1.72 ± 0.10 wt%	
T_p (III)	534 ± 2.0 °C		524 ± 2.0 °C	

T_0 and T_f means initial and final temperature of decomposition stage applied in calculations. Tp (III) means minimum of DTG effect attributed to the III decomposition stage

Table 2 Thermoanalytical results obtained for the initial alpha globulin and the solid native sample irradiated (at instrument A)

Decomposition stage/ parameter determined	0 kGy		24 kGy	
	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%
I	II	III	IV	V
I + II stages	138.5–514.2	93.86 ± 0.18	135.3–508.8	93.98 ± 0.20
I stage	138.5–375.6	63.38 ± 0.37	135.3–372.4	63.90 ± 0.50
stage IA	138.5–271.7	23.11 ± 0.94	135.3–273.0	23.70 ± 1.40
stage IB	271.7–375.6	40.24 ± 0.94	273.0–372.4	40.20 ± 1.40
II stage	375.6–514.2	30.50 ± 0.23	372.4–508.8	31.20 ± 0.35
III stage	514.2–600.2	5.26 ± 0.01	508.8 –595.7	5.23 ± 0.07
Residue at 620 °C	0.83 ± 0.13 wt%		0.59 ± 0.23 wt%	
T_p (III)	583.5 ± 1.0 °C		581.7 ± 1.0°°C	

T_0 and T_f means initial and final temperature of decomposition stage applied in calculations. Tp (III) means minimum of DTG effect attributed to the III decomposition stage

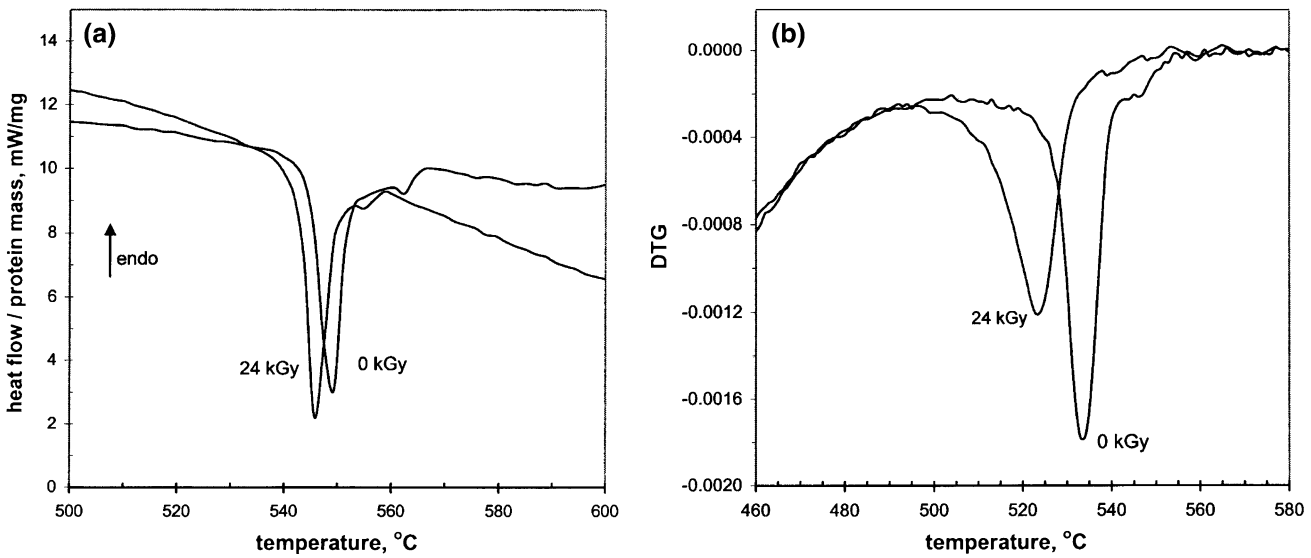

Fig. 4 Comparison of the effects corresponding to the fast last stage of decomposition of the initial gamma globulins and the irradiated native dry globulins (24 kGy): **a** DSC effects: the initial (1.990 mg) and the irradiated globulins (2.135 mg); **b** DTG effects: the initial (10.201 mg) and the irradiated globulins (9.059 mg)

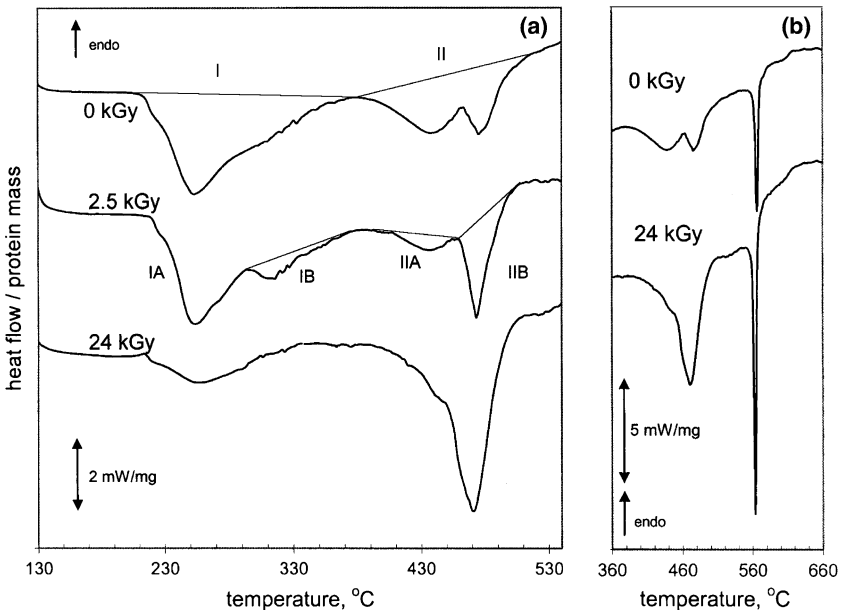

Fig. 5 DSC curves recorded for the reference gamma globulins (subjected to water treatment) (7.354 mg) and for the products irradiated with a 2.5 (7. 740 mg) and 24 kGy dose(7.249 mg): **a** within the range of the first and the second decomposition stages. Partial areas used for presentation of participation of IA, IB and IIA, IIB exothermal processes in the total decomposition heat is shown. **b** Within the range of second and the third decomposition stages (0 and 24 kGy)

product. Peak and onset of the exothermal effect attributed to that step were found at 598.0 and 593.1 °C, respectively, for the initial globulins but at 592.5 and 586.0 °C for the irradiated product.

The following observations can be done pointing out the decrease in temperature of the other decomposition stages of alpha and gamma globulins after irradiation performed in solid state.

Bovine gamma globulins

Degree of the irradiated gamma globulins decomposition achieved higher value in the first range (I) in the case of the irradiated sample as compared to the initial one. This concerns in particular the IB stage. Therefore, a larger total mass loss was detected in the first step (I) and a larger partial mass loss detected in the IB step, followed by the smaller mass loss recorded in the second decomposition range (II). Although this is accompanied by a small increase in the final temperature of the IB step (Table 1), the larger amount of material which decomposes in the IB step on the cost of the material decomposing at II step, shows that part of the material decomposes at somewhat lower temperature.

No particular differences can be noticed between exothermal effects attributed to the first (I) and the second (II) steps of the initial and the irradiated gamma globulins decomposition. ΔH determined jointly for these steps were equal to ca. 1235 ± 50 mJ mg^{-1} and for the third decomposition step to ca. 415 ± 15 mJ mg^{-1}, in the case of both initial and irradiated proteins.

Bovine alpha globulins

Particular steps of thermal decomposition were accomplished at slightly lower temperature (I—372.4 ± 0.04 °C, II—508.8 ± 2.7 °C and III—595 ± 2.3 °C) after irradiation as compared to the initial sample (I—375.6 ± 0.00 °C, II—514.2 ± 0.09 °C and III—600.2 ± 1.3 °C). Irradiation does not influences, however, the mass loss resulted in particular stages.

Irradiation of water suspensions

Likewise in the case of dry native proteins, three major temperature ranges of decomposition were distinguished for the reference gamma and alpha globulins subjected to water treatment on the basis of thermoanalytical and DSC curves, with a small participation of the additional process (IB) following the major decomposition process occurring in the first temperature range (IA) (Figs. 5–8). Occurrence of two subsequent processes is evident, however, in the second temperature range of decomposition, in contrary to the both native globulins. It is shown by two exothermal effects on DSC and two effects on DTG curves (IIA, IIB). IIA peaks correspond to the peaks (II) recorded for the native globulins while the additional IIB peaks appeared at higher temperature. Two exothermal effects are detected also in the third temperature range (IIIA, IIIB) in DSC curves of alpha globulins.

Decrease of the total heat evolved in the first decomposition range (I) was observed after irradiation of both globulins. This was accompanied by an increase in total

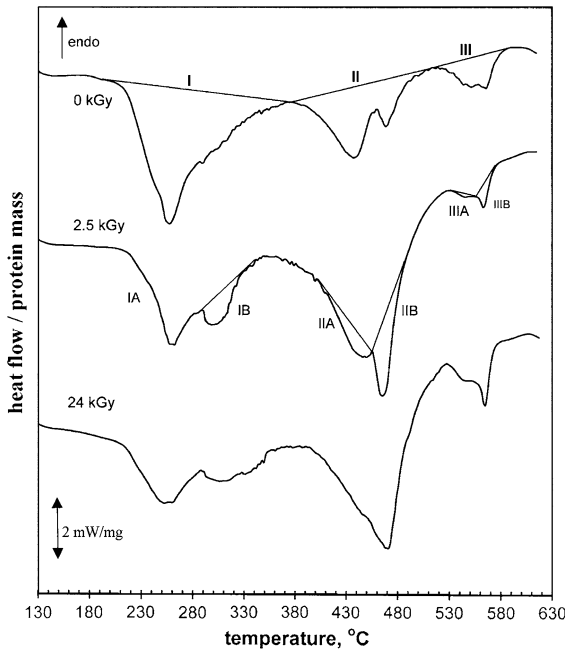

Fig. 6 DSC curves recorded for the reference alpha globulins (subjected to water treatment) (6.240 mg) and for the products irradiated with a 2.5 (6.715 mg) and 24 kGy dose (6.206 mg). Partial areas used for presentation of participation of IA, IB and IIA, IIB, IIA and IIIB exothermal processes in the total decomposition heat is shown

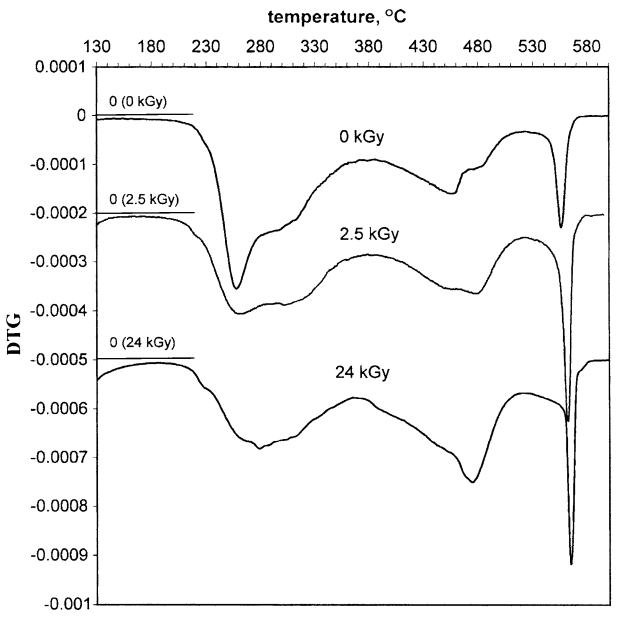

Fig. 7 Comparison of DTG curves recorded for the reference gamma globulins (subjected to water treatment) (19.759 mg) and for the products irradiated with a 2.5 (15.059 mg) and 24 kGy dose (19.313 mg). DTG values are expressed in terms of 1 mg of proteins

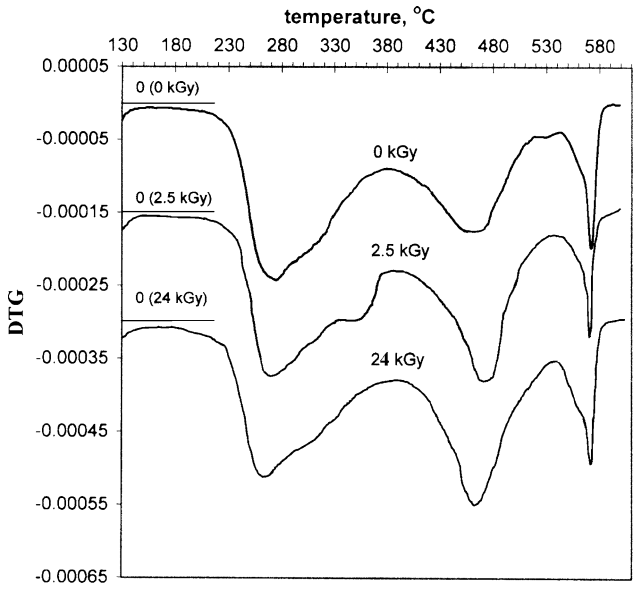

Fig. 8 Comparison of DTG curves recorded for the reference alpha globulins (subjected to water treatment) (10.548 mg) and for the products irradiated with 2.5 (8.868 mg) and 24 kGy dose (7.684 mg). DTG values are expressed in terms of 1 mg of proteins

heat evolved in the second decomposition range (Figs. 5 and 6 and Table 3). Simultaneously, exothermal effects corresponding to IB stage increased significantly already after irradiation with a 2.5 kGy dose. This IB effect became significant in the case of alpha globulin after irradiation with 24 kGy dose while a broad flat effect (I) was observed all over the first decomposition range in the case of gamma globulin due to essentially decreased participation of IA process. Moreover, the effects connected to the IIB stage became larger than these connected to IIA stage already after irradiation with a dose of 2.5 kGy. For both globulins irradiated with a dose of 24 kGy, the single peak was observed, at only slightly lower temperature than IIB peak observed for the reference sample. It is confirmed by the increased value of IB/I and IIB/IIA parameters with the increasing irradiation dose (Table 3).

A good correspondence occurs between DSC data and thermogravimetry. Increase of the mass loss in the particular decomposition stage induced by irradiation conforms to the increase in the appropriate enthalpy, while decrease in the mass loss conforms to the enthalpy decrease. Thus, the smaller mass loss Δm_I was found in the I decomposition stage and the larger mass loss Δm_{II} was stated in the II stage already after irradiation of both globulins with a 24 kGy dose (Tables 4, 5). That change in thermal decomposition was significant after irradiation with a dose of 24 kGy. The differences between the reciprocal magnitude and height of the particular DTG effects corresponding to the particular steps of thermal decomposition (for example, the first and the second ones) demonstrates

Table 3 Results of calculations done on the basis of DSC results obtained for the reference bovine gamma globulin and the products irradiated with doses of 3 kGy and 24 kGy in water suspensions

Parameter	Gamma globulins			Alpha globulins		
	0 kGy	3 kGy	24 kGy	0 kGy	3 kGy	24 kGy
I	II	III	IV	V	VI	VII
Results of integration of partial area (J/g)						
I	−4245 ± 400	−3985 ± 100	−1716 ± 600	−6183 ± 400	−4100 ± 200	−3700 ± 100
IB	−102 ± 50	−348 ± 80	−1716 ± 600	−95 ± 30	−517 ± 50	−746 ± 150
II	−1944 ± 600	−2226 ± 200	−3945 ± 200	−2829 ± 250	−5425 ± 600	−5683 ± 100
IIA	−441 ± 150	−200 ± 2	−33 ± 10	−1172 ± 40	−246 ± 130	−25 ± 20
IIB	−390 ± 18	−877 ± 60	−2390 ± 190	−311 ± 80	−942 ± 170	−1135 ± 20
III	−825 ± 120	−874 ± 100	−946 ± 120	−795 ± 70	−478 ± 40	−747 ± 20
IIIA	nd	nd	nd	−78 ± 10	−23 ± 3	−78 ± 10
IIIB	nd	nd	nd	−93 ± 4	−97 ± 9	−93 ± 4
Ratios of the mean values of partial areas (*shown above*)						
I_B/I	0.024	0.087	1.00	0.015	0.13	0.20
II_B/II_A	0.88	4.39	72.42	0.27	3.83	46.12
IIIB/IIIA	nd	nd	nd	0.83	4.22	4.75
II/I	0.45	0.55	2.30	0.46	1.32	1.54
Average ratios of the particular partial areas determined separately for individual curves						
I_B/I	0.05 ± 0.02	0.14 ± 0.03	1.00 ± 0.00	0.015 ± 0.004	0.13 ± 0.01	0.20 ± 0.02
II_B/II_A	0.60 ± 0.06	4.34 ± 0.30	71.43 ± 12	0.25 ± 0.05	3.01 ± 1.00	56 ± 20
IIIB/IIIA	nd	nd	nd	0.83 ± 0.10	4.43 ± 1.20	4.46 ± 0.25
II/I	0.50 ± 0.10	0.59 ± 0.02	3.19 ± 1.08	0.45 ± 0.01	1.55 ± 0,10	1.55 ± 0.15
T_{pIII} (°C)	566.4 ± 0.1	564.8 ± 0.3	563.1 ± 0.2	550.0 566.4	544.7 564.1	542.7 564.2

The ranges of the integration of partial areas IB, IIA, IIB are shown in Figs. 7 and 8

nd Not determined (single effect observed)

Table 4 Thermoanalytical results obtained for gamma globulins irradiated in water suspensions and the reference globulins subjected to water treatment (at instrument B)

Decomposition stage/ mass ratio	0 kGy		3 kGy		24 kGy	
	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%
I	II	III	IV	V	VI	VII
I stage	183.0–371.9	57.78 ± 0.80	189.3–382.8	52.09 ± 1.00	188.4–364.8	39.62 ± 1.00
stage IA	183.0–336.9	31.33 ± 2.00	189.3–300.0	26.90 ± 2.00	188.4–291.9	19.56 ± 2.00
stage IB	336.9–371.9	26.45 ± 2.00	300.0–382.8	25.19 ± 2.00	291.9–364.8	20.06 ± 2.00
II stage	371.9–520.5	31.95 ± 2.00	382.8–526.8	34.49 ± 0.20	364.8–516.9	44.63 ± 0.20
III stage	520.5–574.5	7.20 ± 0.10	526.8–586.5	12.00 ± 0.20	564.8–516.9	12.48 ± 0.20
$\Delta m_I/\Delta m_{II}$	1.81		1.51		0.89	
$\Delta m_{IA}/\Delta m_{IB}$	1.18		1.07		0.97	

Δm_I, Δm_{II}, Δm_{IA} and Δm_{IB} are the mass losses occurring during I and II as well as IA and IIA decomposition stages. T_0 and T_f means initial and final temperature of decomposition stage applied in calculations

well the differences between the reference and the particular irradiated samples (Figs. 7, 8).

These results showing that the same decomposition degree was achieved at higher temperature and that the connected heat was evolved at higher temperature after irradiation, demonstrates that irradiation induces increase in the temperature of process. Apart to the above observations common for both globulins, the following

Table 5 Thermoanalytical results obtained for alpha globulins irradiated in water suspensions and the reference globulins subjected to water treatment (at instrument B)

Decomposition stage/ mass ratio	0 kGy		3 kGy		24 kGy	
	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%	$T_0 - T_f$, °C	Δm, wt%
I	II	III	IV	V	VI	VII
I stage	190.5–384.6	55.20 ± 0.10	194.7–398.1	55.87 ± 1.00	185.7–372.9	44.35 ± 1.00
IA stage	190.5–384.6	55.20 ± 0.10	194.7–332.4	35.70 ± 2.00	ns	ns
IB stage	no	no	332.4–398.1	19.57 ± 2.00	ns	ns
II stage	384.6–543.0	37.30 ± 0.50	398.1–532.2	37.73 ± 1.00	372.9–534.9	45.91 ± 1.00
III stage	543.0–587.1	7.00 ± 0.50	532.2–588.0	5.14 ± 1.00	534.9–579.9	8.69 ± 0.50
$\Delta m_I/\Delta m_{II}$	1.48		1.44		0.98	

Δm_I and Δm_{II} are the mass losses occurring during I and II stages, respectively. T_0 and T_f means initial and final temperature of decomposition stage applied in calculations

ns Not seperated

particular observations were done, showing the increase in decomposition temperature.

Bovine gamma globulins

The mass losses connected to IA and IB stages were evaluated separately, despite of the strong superposition of the neighbouring DTG effects. Participation of mass loss in the IA stage in relation to the total mass loss occurring in the first decomposition stage ($\Delta m_{IA}/\Delta m_I$) decreases and participation of mass loss in the IB stage ($\Delta m_{IB}/\Delta m_I$) increases. This is shown by decreasing $\Delta m_{IA}/\Delta m_{IB}$ value after irradiation (Table 4).

Furthermore, after irradiation more of material decomposes in the third fast stage as compared to the non-irradiated proteins (shown by the increasing Δm_3 value) (Table 4). Accordingly, sharper exothermal effects accompanied by a larger heat correspond to that decomposition stage on DSC curves of the irradiated than of the reference samples (Fig. 5b, Table 3).

Bovine alpha globulins

Height of the exothermal peaks connected to the IIIB processes increases with irradiation dose in relation to IIIA peaks, while these peaks were almost equal in DSC curves of the reference samples (Fig. 6). This indicates the increase of enthalpy connected to IIIB stage, confirmed by the increase in IIIB/IIIA parameter (Table 4).

Discussion

It is known that during the first stage of thermal decomposition of proteins (following dehydration) the cleavage of peptide bonds occur, while in the second stage the resulting material decomposes [19–23]. Predominant process occurring during dynamic heating till ca. 450 °C consist thus on cleavage of disulfide bridges and peptide linkages while decomposition of the resulting polypeptide subunits takes place after splitting at the higher temperature [19–23]. It was also found that the structural changes caused by high accumulation of covalent linkages resulted in the shift of DTG peaks to the higher temperature [20]. Moreover, the last fast decomposition stage is attributed to decomposition of the crosslinked polypeptide subunits.

It is also known that a number of decomposition stages of free amino-acids and their temperature range depend on the chemical structure of individual species and result in the appearance of peaks observed on DTG, DTA and DSC curves [31–35].

Accordingly, chemical changes resulting in amino-acids as well as modification of protein tertiary structure affect DSC and DTG profiles recorded on dynamic heating. Chemical modification of amino-acids and consequently modification of their sequence in protein chain are probably the most important factors determining strong modification of thermal decomposition resulting due to irradiation of gamma and alpha globulins water suspensions [14, 36, 37]. Our results suggest, moreover, that crosslinking is more important than degradation in the case when irradiation was performed for such concentrated water suspensions (1:1). This is caused probably by the limited access of oxygen to material during irradiation, as proteins crosslinking is rather expected in absence of oxygen while degradation prevails in pure oxygen atmosphere under doses as high as 24 kGy [14, 38]. This occurs probably due to tight filling of the capsules and huge amount of protein in the highly concentrated suspensions. In result of the higher content of disulfide bridges between the polypeptide chains in the irradiated samples, both cleavage of polypeptide bonds and decomposition of fragmented

polypeptide chains occur at higher temperature, than in the non-irradiated reference sample. It results in the increased amount of material decomposed in the second range of thermal decomposition and increased areas of the appropriate DSC effects, on the cost of these corresponding to the first decomposition range. This is also the reason of the larger amount of material evacuated in the IB stage on the cost of material pyrolised within IA stage, and accompanying decrease in IA, IIA and IIIA partial areas of DSC effects followed with the increased IB, IIB and IIIB areas. It can be supposed, moreover, that the predominant process corresponding to the first DTG peak (IA) corresponds probably to the splitting of polypeptide bonds in α-helix structure. In fact, the loss of helical content was discovered after the very preliminary decomposition stage in keratin [18]. Moreover, increase of the material content, which decomposes at high temperature in the third decomposition stage, point out the increase in the content of strongly crosslinked polypeptide structure [21].

It is worth to mention, in relation to our previous results presenting denaturation of globulins induced by irradiation [28] that such disorganisation of the specific globulins' structure might occur without cleavage of peptide bonds, and that new disulphide bridges might be formed after disruption of the primary ones [39]. Simultaneous crosslinking is thus not excluded. Moreover, denaturation precedes probably as well proteins degradation as crosslinking, both induced by ionising radiation [40].

It seems worth to admit that the ratios between particular partial areas of exothermal effect in DSC curves (especially IB/I and IIB/IIA values) have appeared useful for description the differences between globulins irradiated in water environment and the reference globulins. For example, the IIB/IIA values were higher in an order of magnitude for both globulins irradiated with 2.5 kGy dose then for the references. Furthermore, it seems possible to adapt these parameters as the indicators of the performed irradiation.

In contrary to irradiation carried out for protein water suspensions, irradiation carried out for solid dry proteins induces degradation rather than crosslinking. It is shown, by decreased temperature of the particular decomposition steps, especially the last one (attributed to decomposition of crosslinked polypeptide network [21]). Moreover, the effect of irradiation on solid proteins is clearly smaller than that observed for irradiated water suspensions.

Conclusions

The differences in the route of thermal decomposition of the non-irradiated and the irradiated gamma and alpha globulins were observed using thermogravimetry and differential scanning calorimetry. The effect was larger when irradiation was carried out for water suspension, in comparison to the native solid samples irradiated. Thermal decomposition of the globulins irradiated in water suspensions occurs at higher temperatures, in comparison to the reference non-irradiated sample. This can be explained in terms of formation of covalent linkages in the irradiated proteins, apart to the chemical changes induced in amino-acids. Decrease in the temperature of the last decomposition stage gives evidence of degradation taking place under influence of gamma irradiation performed for solid dry proteins.

DSC and thermogravimetry detected the relatively large differences between decomposition of the irradiated and the non-irradiated samples already after irradiation of water suspensions with a dose of 2.5 kGy. The ratios between partial areas of exothermal effects drawn likewise in the present paper have appeared useful for description the differences between the irradiated and non-irradiated samples.

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