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γ-RADIATION EFFECTS ON THE THERMAL STABILITY OF BOVINE γ-GLOBULIN

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Abstract

Low-dose γ-radiation produces subtle effects on bovine γ-globulin, as revealed by calorimetric investigations carried out on concentrated aqueous solutions. The irradiation rate used seems below the threshold that allows complete protein denaturation; the denaturation temperature, T_d, remained unaffected; nonetheless, modifications of the shape of the calorimetric signal reveal that other changes, such as the dissociation of protein oligomers, could take place.

Keywords: bovine γ-globulin, calorimetry, food irradiation, γ-radiation, protein denaturation

Introduction

Treatment of foods and/or food ingredients with γ-radiation is often used to protect them against degradation so as to achieve a longer shelf-life [1–5]. The state of the art can be summarized as follows: low-dose (up to 1 kGy, where 1 kGy≡1 J g^{-1}) treatment is used for the inhibition of sprouting, insect and parasite disinfectation, delay of physiological processes, etc.; medium-dose (1–10 kGy) treatment is used to delay mould growth, for spoilage decontamination, and to improve the technological properties of food (e.g. cooking time); high-dose (10–50 kGy) treatment is used for commercial sterilization, replacement of chemicals, etc. [6–13].

Food preservation by irradiation differs from other conventional physical preservation methods in its specific advantages: low-temperature treatment, good penetrating power (γ-rays), and less energy waste with respect to conventional methods (heating or freezing). On the other hand, this treatment can produce irreversible nutritional and sensorial damage. The absence of a reliable method to detect previous irradiation has so far been claimed by public opponents: this is indeed the case for irradiation processes up to 10 kGy, which pro-

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duce few detectable effects. The induced radiolytic changes cannot be definitively ascribed to irradiation, and are comparable with those of other preservation processes. Additionally, many of the radiolytic products are transient and/or present in trace quantities. For this reason, methods so far proposed to verify whether a given food has been previously treated with low-dose radiation cannot always be considered reliable [14–18].

One of the effects of food irradiation with γ-rays is the irreversible denaturation of the protein content. Although the denaturation of small globular proteins in aqueous dilute solutions and in the absence of radiolytic processes is often reversible [19, 20], for higher concentrations or in the case of large multi-domain or oligomeric proteins, dissociation and aggregation can result in irreversible unfolding [21, 22]. Furthermore, since the stability of the proteins is highly dependent on their environment [23–28], γ-ray-induced modifications of this can indeed compromise their thermal stability.

In this work, we present a further [29] DSC study of the influence of γ-rays on the thermal stability of bovine γ globulin protein in aqueous solution at concentrations comparable with those found in real foods (about 20%), to show that this experimental approach can reveal subtle changes produced by low-radiation treatments.

Materials and methods

Bovine γ-globulin (SIGMA product, from Cohn fraction II, III, purity approx. 90%) was used without further purification.

The irradiation was carried out at ambient temperature by using a ^{60}Co source at 5.5 kGy h^{-1} radiation rate (Issledovatiel cell, Institute of Nuclear Chemistry and Technology in Warsaw). 2.5, 5, 10 and 20 kGy doses were attained by exposing 50% protein aqueous suspensions for different times. After irradiation, the samples were lyophilized and frozen.

A Perkin Elmer DSC-6 with 60 μl cells was used to investigate the thermal denaturation of protein suspensions in buffered solutions (pH=6.0, 0.05 M phosphate and 0.10 M NaCl, with a protein concentration of about 18% w/w), in the temperature range 20–100°C at a scan rate of 2 K min^{-1}. The raw data were analysed with the software Theseus [30]. The excess heat capacity $<\Delta C_p(T)>$, i.e. the apparent heat capacity $C_p(T)$ of the sample (per gram of protein) with respect to the heat capacity of the 'native state', $C_{p,N}(T)$, can be evaluated [30, 31] from linear regression of the $C_p(T)$ predenaturation trend (namely, for $T<T_i$, T_i being the highest temperature at which only the native conformation is present). The total calorimetric denaturation enthalpy $\Delta_d H$ was determined by integration of $<\Delta C_p(T)>$ across the range T_i–T_f (where T_f is the end-temperature of the denaturation process), after definition of a sigmoidal base-line [30, 32] to reproduce the drop

$$[\Delta C_p(T_f) - \Delta C_p(T_i)] = \Delta_d C_p$$

across the same temperature range. The temperature of the peak maximum is referred to as the denaturation temperature T_d.

Results and discussion

The thermoanalytical curve of non-irradiated bovine γ-globulin is depicted in Fig. 1; T_d and $\Delta_d H$ values are reported in Table 1. The thermal denaturation (endothermic signal peak) is irreversible (as found in a second heating run) and partially overlapped with an exothermic contribution (possibly related to aggregation), which occurs in the temperature span 90–97°C. This finding is typical of large oligomeric proteins [21] such as bovine γ-globulin (a tetramer with MW 150 000). Because of the irreversibility of the denaturation, thermodynamic analysis cannot easily be applied [33]; nonetheless, this irreversibility is useful in this study since it implies permanent radiation effects.

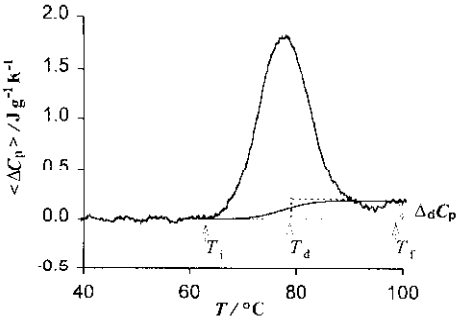

Fig. 1 DSC trace obtained for non-irradiated bovine γ-globulin at 18.4 w/w% concentration in buffered (pH 6) aqueous solution

Thermal denaturation peaks (scaled with respect to the sigmoidal $\Delta_d Cp$) for γ-globulin samples which underwent different radiation doses can be compared in Fig. 2. In all cases, thermal denaturation is irreversible. As shown in Table 1, where T_d and $\Delta_d H$ values are reported, γ-rays seem to have no effect on T_d, while they do affect $\Delta_d H$, which shows a decreasing trend towards a limit value.

It has been reported [33] that, when protein denaturation is irreversible at moderate concentration, this parameter plays an important role in the peak shape and position. This effect is not significant at high concentrations [34]; nonetheless, to rule it out in the present study, where slightly different concentrations were considered (Table 1), thermoanalytical curves were also obtained on samples with lower concentration (11%) previously irradiated with a 2.5 kGy γ-ray dose. When these were compared with thermoanalytical curves obtained on typical samples (18%, irradiated with equal doses), no significant difference was observed (Fig. 3).

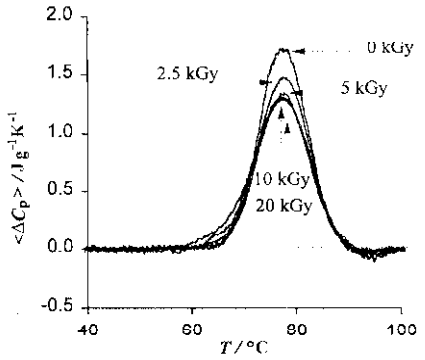

Fig. 2 DSC traces obtained for irradiation (0, 2.5, 5 10 and 20 kGy) bovine γ-globulin at about 18 w/w% concentration in buffered (pH 6) aqueous solution

Table 1 Experimental data (temperature and enthalpy of denaturation) from DSC traces of irradiated (0, 2.5, 5, 10 and 20 kGy) bovine γ-globulin at various percentage concentrations (w/w) in buffered (pH 6) aqueous solution

Dose/kGy	T_d/°C	$\Delta_d H$/J g^{-1}	Protein conc./w/w%
0	77.5±0.1	19.8±0.2	18.4
2.5	77.6±0.1	18.2±0.2	18.4
5	77.6±0.1	17.0±0.2	17.2
10	77.5±0.1	16.2±0.2	17.0
20	77.5±0.1	16.1±0.2	17.9

As there is no T_d variation on change of the radiation dose, the related entropic effects can be presumed to be independent of the radiation dose, which would therefore only affect the enthalpy. The denaturation of protein domains is a cooperative process [35, 36]: in the present case, this means that most of the γ-globulin molecules can undergo a conformation change only when the sample adsorbs an energy shot of at least 20 J g^{-1} (Table 1) in a time lapse that is short with respect to the relaxation of fluctuations between the native and denatured states. In other words, the irradiation rate should be large enough. Upon treatment at a lower irradiation rate, only a minor proportion of the protein molecules would undergo denaturation; this fraction would nonetheless increase with increasing exposure time (radiation dose), provided that the statistical radiation/reaction cross-section is not negligible. This fraction can accordingly be expressed as

$$f = \frac{\Delta_d H(0) - \Delta_d H(\text{dose})}{\Delta_d H(0)}$$

where $\Delta_d H(0)$ and $\Delta_d H(\text{dose})$ are the denaturation enthalpies of non-irradiated and irradiated samples, respectively. $\Delta_d H(\text{dose})$ decreases with increasing exposure time and eventually vanishes, i.e. f approaches unity.

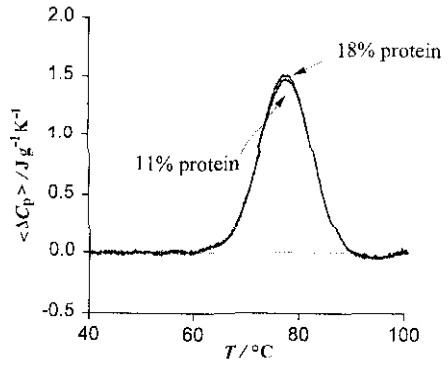

Fig. 3 Comparison between DSC traces obtained for bovine γ-globulin samples irradiated with a 2.5 kGy dose at two different concentrations (11 and 18 w/w%) in buffered (pH 6) aqueous solution

Figure 4 shows the f *vs.* time trend, which therefore accounts for the radiation dose. A saturation trend, at about f=0.18, is attained after 2 h. This indicates that the radiation power is not sufficient to produce complete denaturation.

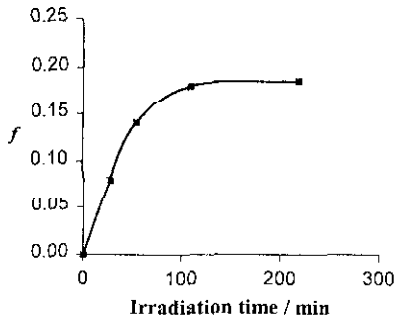

Fig. 4 Fraction of denatured bovine γ-globulin (mainly dissociated into oligomers, see text) in buffered (pH 6) aqueous solution as a function of the exposure time (irradiation rate 5.5 kGy h^{-1})

Although no specific scavengers were used, the effects of free radicals can be considered negligible under the conditions of the present study [37]. As a matter of fact, any indirect effect of irradiation (radicals and peroxides) can indeed be noteworthy at doses exceeding the range considered in this work [37]. On the other hand, radical-related damage would produce largely visible effects on the protein denaturation trend, which does not seem the case since the recorded DSC traces do not show any evidence of this.

Primary radiation effects on the proteins are certainly attenuated because of the low irradiation rates and doses acting on a liquid water environment [38]; according to the literature [39], the direct action γ-rays would not occur at the hit site, but would be spread through the transmission of excited electrons toward 'fragile' sites where bond breaking would take place. Due to this mechanism, the irradiation effect would mainly induce oligomer dissociation, which indeed seems the case in the present study. Furthermore, water ionization induced by γ-rays, and related pH changes, may occur [38] and also promote oligomer dissociation, since the solutions used for protein irridation in the present study were unbuffered. The small differences in peak shape on the left hand side (Fig. 2) are consistent with this interpretation, inasmuch as they often reveal dissociation steps [21], although such effects should not be large at the concentrations considered.

Conclusions

This investigation has shown that calorimetry allows the detection of subtle effects produced by a low γ-ray dose on protein stability. The results indicate that the irradiation must attain a threshold rate to affect the protein conformational state. The overall radiation dose up to a saturation effect is also important for the modified protein fraction. This is a consequence of the highly cooperative mechanism of denaturation. It is possible that γ-rays could somehow affect the mechanism of dissociation of protein oligomers.

To confirm these conclusions, the irradiation proteins should be investigated in dilute solutions where the dissociation and aggregation processes can be detected more precisely. Power tuning of the radiation source also seems necessary to determine the actual threshold of the energy rate that allows complete denaturation.

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