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Research on a wide-range biodosimeter based on the irradiation damage effect of proteins for γ radiation

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ABSTRACT

To realize the measurement of the biological dose in wide range, this paper dis discribes a biodosimeter based on the radiation damage effect of proteins (bovine serum albumin, bovine hemoglobin, and casein) and completed a dosimeter response study under γ radiation. Gafchromic film was used to measure the protein solution dose, and the solution dose was obtained by combining the film dose and the dose conversion factor calculated by Monte Carlo N Particle Transport Code (MCNP). The Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis technology (SDS-PAGE) was used to characterize the damage degree of proteins. By selecting the appropriate fitted range and weighted mean dose method, an accurate measurement of the film dose was realized. In the investigation of the irradiation damage effect of proteins, the damage degree of proteins gradually increased with increasing dose according to the exponential function. Different proteins have various response modes. For the three studied proteins, the protein with greater solution concentration were more difficult to degrade. The dose rate also influenced the damage effect of proteins. Damage degree was smaller at higher dose rates. A dose measurement from 2.3 to 2555.7 Gy was achieved by adjusting the concentration of the bovine serum albumin, and wide-range dose measurements is possible by changing protein types and the solution concentration. The novel dosimeter could realize wide-range biological dose measurement and prompt further research on the biological effect of radiation, given the great significance in the determination of the biological effects and in the health assessment of irradiated bodies.

1. Introduction

Biodosimeters (Ainsbury and Lloyd, 2010; de Lemos Pinto et al., 2010; Fenech, 2011) are dosimeters that measure radiation doses by analyzing human biological materials, such as DNA, cells, and proteins. A biological dosimeter reflects the actual damage degree of an exposed person and has advantages that physical dosimeters and chemical dosimeters cannot replace. At present, chromosomal aberration and micronucleus analyses (Agrawala et al., 2010; Bolt et al., 2011) are the most commonly used biological dosimeters. However, these dosimeters present disadvantages of small applicable range (usually about several Gy), large analytical workload, and complicated operation (Barquinero and Puig, 2017; Pujol et al., 2014; Vinnikov et al., 2010). Developing a new biological dosimeter with good dose–effect relationship, wide application range, and simple operation remains crucial for researchers.

Protein is an important component of the human body and is closely linked to various life activities. Protein accounts for 18% of the total mass and as high as 54% of the human body's dry weight. Studying protein behaviors after irradiation plays an important role in determining the damage degree caused by radiation and studying biological effects of radiations. After irradiation, disruption of the ordered structure of protein molecules occurs along with degradation, cross-linking, and aggregation of the polypeptide chains (Gaber, 2005; Kim et al., 2014; Lee et al., 2005; Lee and Song, 2002). The damage degree of proteins differs when the protein solution received different doses and has a response relationship with the dose. This paper proposes a novel biological dosimeter based on the damage effect of proteins after irradiation. As the damage effect of proteins after irradiation is related to the concentration of the protein solution (Lee et al., 2003), the adjustment of the measurement range of the novel dosimeter can be

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realized by changing the concentration of the solution. The dosimeter can achieve wide-range dose measurement, and can be applied in many fields, such as radiotherapy, biological research and so on. Moreover, given the wide variety of proteins, the dosimeter based on proteins can be used under different radiation conditions. The novel dosimeter has a wide range of applications. Therefore, research on a biodosimeter based on the protein damage effect is meaningful, and this work investigates the response of the novel dosimeter for γ irradiation.

To calibrate the novel dosimeter, the protein solution dose should be measured by a medium that is thin, size adjustable, and near-tissue equivalent. Radiochromic film (Aydarous and El Ghazaly, 2013; Devic et al., 2016; Neto et al., 2014) is an ideal choice, and the accuracy of the dose measurement of film determines the accuracy of the novel dosimeter. Accordingly, the response assessment of radiochromic film was studied. The influence of the fitted dose range and dose calculation method on film dose measurement was investigated and accurate evaluation of the film dose was attained. The protein solution dose was obtained by combining the film dose and the dose conversion factor calculated by MCNP (Monte Carlo N Particle Transport Code). Then, the response relationships between the damage degree of proteins and the doses, protein types, protein solution concentrations, and dose rates were established. Research on the response of the novel dosimeter under γ radiation was completed. Finally, the applicable dose range of the dosimeter was discussed by changing the concentration of the protein solution, and a wide-range dose measurement was realized.

2. Materials and methods

2.1. Film response evaluation and dose measurement of the protein solution

To preclude the influence of temperature on protein damage, an ice-box was designed (Fig. 1). The main material of the ice-box is polymethyl methacrylate, and the refrigerant is sodium polyacrylate gel with a density of 0.98 g/mL. A 15 mm space was designed in other directions to prevent the effects of scattering gamma rays. The temperature of the wall of the cuvette hole was measured using a FLUKE infrared thermometer. After the removal of the ice-box from the freezer compartment, the temperature of the wall was maintained at 2 °C–8 °C for about 300 min. This was sufficiently long for irradiation experiments. Thus, the effects of temperature were no longer considered in subsequent experiments.

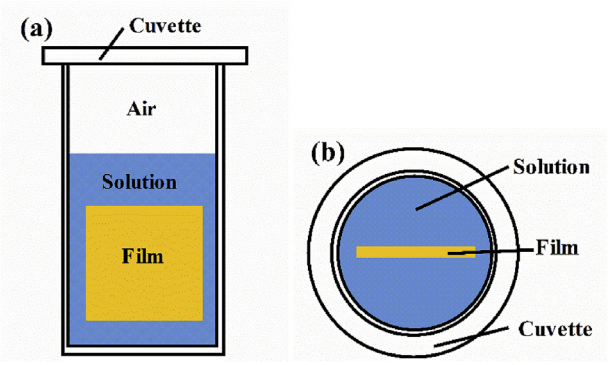

Fig. 2. Schematic diagram of the dose measurement of the protein solution.

The protein was irradiated inside the cuvette, and a radiochromic film was placed in the center of the cuvette to measure the dose of the protein solution (Fig. 2). Dose measurement can be achieved by evaluating the optical density (OD) change of the radiochromic film. This work used the latest model Gafchromic film HDV2 (Ashland Inc.), which is available for a dose range from 10 Gy to 1000 Gy. The film was cut into a 1 cm^2. To ensure that the film was not wet by the solution, the film was sealed in plastic package using a thermoplastic machine.

Using a flatbed scanner is the most common method to measure the

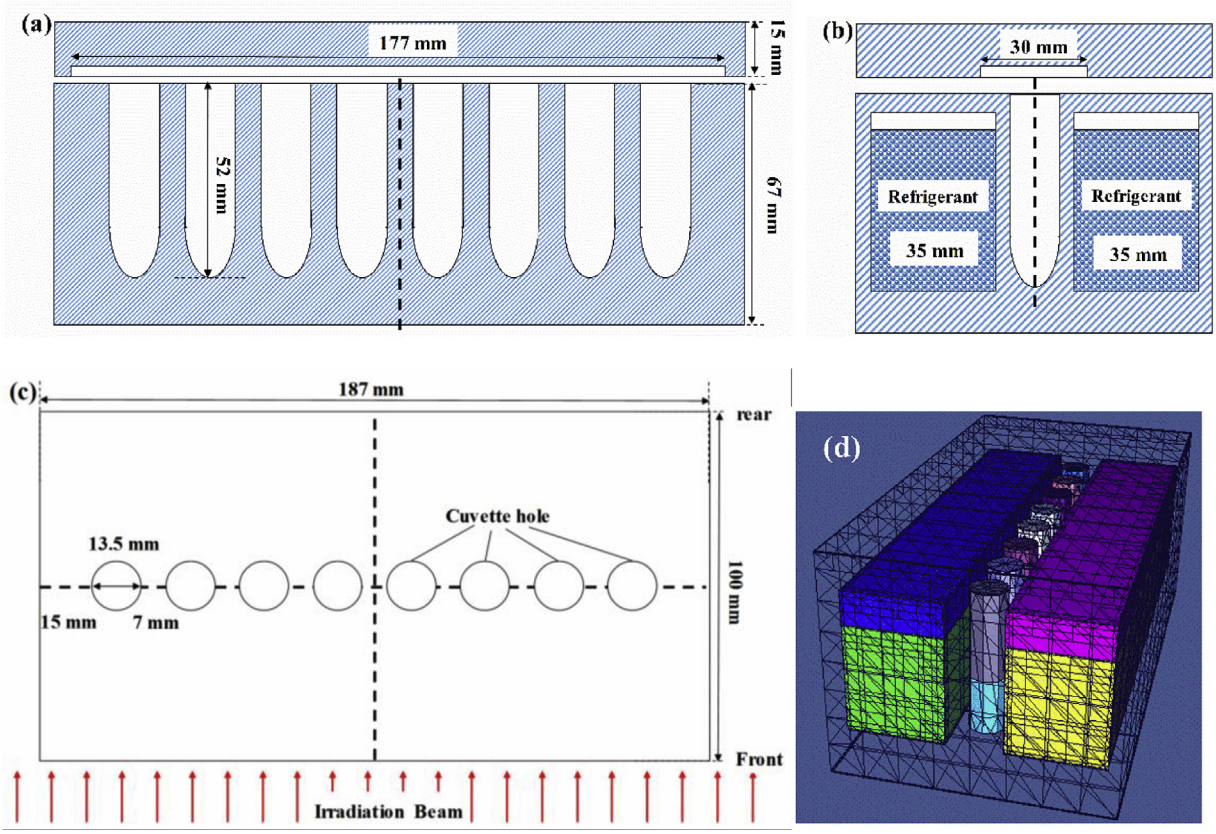

Fig. 1. Schematic diagram of the ice-box: (a) main view, (b) left view, (c) top view, and (d) the MCNP model.

response of radiochromic film (Devic et al., 2005). However, several scanning characteristics of radiochromic film when using a flatbed scanner may introduce large uncertainty when these factors are disregarded. These include post-exposure changes, lateral inhomogeneity, etc. (Devic et al., 2006). To solve this problem, a standard operating procedure proposed in our previous study was used, which is for the response evaluation of the radiochromic film using the flatbed scanner (Zhang Xudong et al. Under revision).

In this study, an Epson 12000 XL flatbed scanner was used to scan the film in the transmission mode. The Epson scan2 software was used to conduct the scanning, and all color correction of the software was turned off. RGB positive images were captured at a depth of 16 bits per color channel and saved as tagged image file format files. The scanner resolution was set as 2000 dpi. The operating temperature of the scanner was controlled at 15 °C–24 °C, at which the film response is unaffected by temperature.

The response of HDV2 film at 0–1600 Gy was calibrated at the National Institute of Metrology, China. The film was exposed using a ^{60}Co source and placed in a water phantom at 5 cm from the surface. The delivered dose was measured by an alanine dosimeter with an uncertainty of 4.0% (Confidence interval 95%). The film was scanned at 50 h after the irradiation. As a measure of film tint, the OD was obtained from the RGB color values according to the following equation: $OD = \lg (P_0/P_D)$. P_0 and P_D are the pixel values of the film before irradiation and after receiving dose D, respectively. The dose calibration curve of the film was established by the relationship between the OD change before and after irradiation and the dose: $D = a \times OD + b \times OD^c$.

The dose conversion factor F is defined as the ratio of the dose of the protein solution to the film dose. The dose of the protein solution can be obtained by multiplying the factor F by the film dose. Given the ice-box structure and materials, a Monte Carlo phantom was constructed in the MCNP6.1 (Pelowitz, 2013; Tang et al., 2011). The phantom was used to simulate the irradiation of the ^{60}Co source to calculate the dose conversion factor F.

2.2. Irradiation experiment and damage degree characterization of proteins

The protein solution was uniformly irradiated with an industrial ^{60}Co source (0.69 kGy/h). Proteins included bovine serum albumin (BSA), bovine hemoglobin (BHb), and casein, which are widely used in biological research. To study the response of the novel dosimeter to the dose, the concentration of the protein solution was fixed at 0.05% g/mL and the dose was changed from 0 Gy to 550 Gy. To determine the response of the dosimeter to the solution concentration, the dose of the protein was fixed and the concentration was changed from 0.01% g/mL to 0.1% g/mL. Moreover, irradiation experiments of BSA under 8.11 and 1.80 Gy/min were conducted to examine the effect of dose rate.

About one day after irradiation, the protein was characterized by the SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis technology) (Kurien and Scofield, 2012), and the damage degree of proteins was expressed as the remaining ratio of intact protein (RRIP). The protein sample was added to the 5 × loading buffer to the same protein concentration and mixed well. Then, the sample was boiled in water for 5 min and iced for 5 min. A 20 μL sample was introduced into a SurePAGE well, and electrophoresis was conducted at the voltage to 120 V until the bromophenol blue band ran to the bottom of the sheet. The gel was stained with 1 × Coomassie blue dye for 1 h, and was subsequently decolorized using a decolorizing solution (20 mL absolute ethanol, 20 mL glacial acetic acid, and 360 mL ultrapure water) until the background was clear.

After electrophoresis, the gel was scanned using an Epson scanner at 2000 dpi resolution. To avoid the occasional error that may be caused by a single scan, each gel was scanned five times. The gel was quantitatively analyzed using image processing software ImageJ. By comparing the gray values of the protein bands before and after irradiation, the RRIP was obtained.

2.3. Fitting formula for the irradiation damage effect of proteins

Given the long chain structures of protein and DNA (Cadet et al., 2005; Sutherland et al., 2000), protein is speculated to have some similarity to DNA after irradiation. Analogizing the survival score curve of cells after irradiation, RRIP after irradiation was fitted using the linear-quadratic formula (Brenner, 2008),

$$RRIP = e^{(a\times D+b\times D^2)} . \quad (1)$$

Here, D is the irradiation dose, and a/b are fitting parameters. Parameter a is the coefficient of the linear effect that indicates the part of the effect proportional to the dose and corresponds to click chain break events. Parameter b is the coefficient of the quadratic effect that indicates the part of the effect proportional to the quadratic dose and corresponds to multi-shot chain break events. Considering the simpler response of some proteins, the RRIP was fitted using a simplified first-order formula, as follows:

$$RRIP = e^{a\times D} . \quad (2)$$

3. Results

3.1. Dose measurement of the protein solution using Gafchromic film

To verify the role of the standard operating procedure, the 95% confidence intervals (CI) of the dose calibration curves of the radiochromic films using such procedure and an ordinary method are compared (Fig. 3). The 95% CI of the calibration curve established using the standard operating procedure is smaller than that using ordinary method.

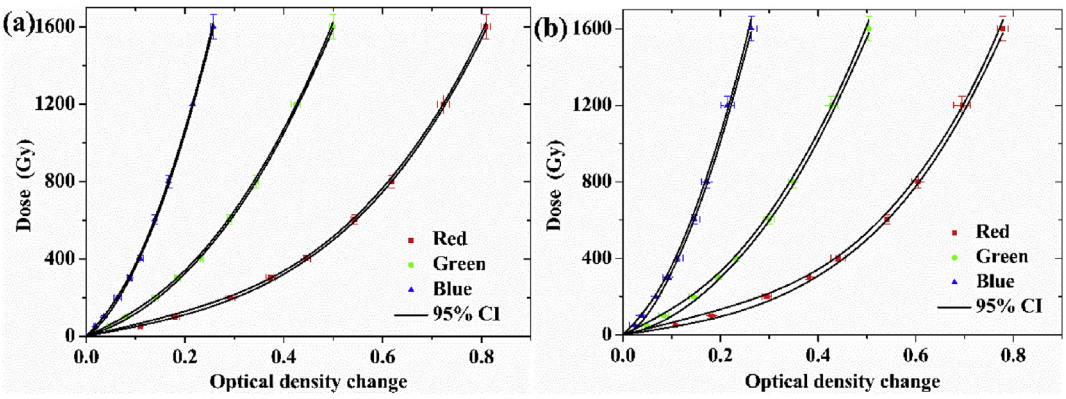

Fig. 3. The 95% confidence intervals of the dose calibration curves of radiochromic films using: (a) standard operating procedure and (b) ordinary method.

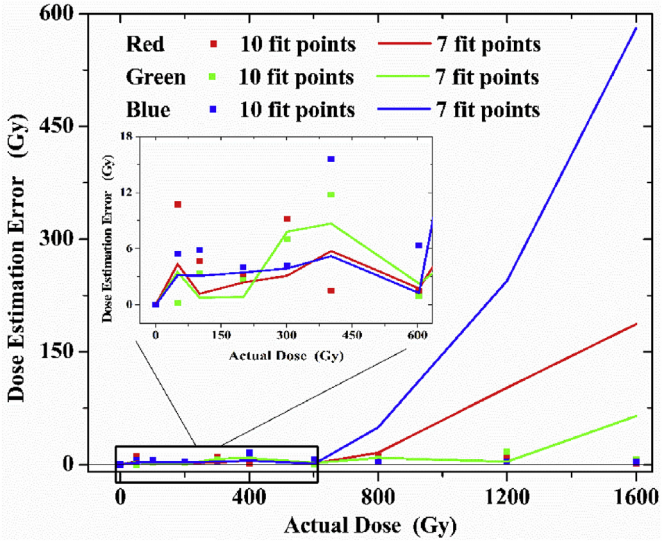

Fig. 4. Dose estimation error of film calibration curves using different fitted dose ranges (7 fit points: 0–600 Gy; 10 fit points: 0–1600 Gy).

Fig. 4 shows the dose estimation errors of two calibration curves fitted using 10 fit points (covering 0–1600 Gy) and 7 fit points (covering 0–600 Gy), respectively. The difference of two calibration curves is the fitted dose range. The dose estimation error is the absolute value of the deviation between the dose estimated by the calibration curve and the actual dose, which was calibrated at the National Institute of Metrology, China. The illustration in Fig. 4 is an enlargement figure from 0 Gy to 600 Gy. In the range 600–1600 Gy, the dose estimated by the 7-point fit curve significantly differs from the actual dose compared to the 10-point fit curve, especially in the blue channel. In the range 0–600 Gy, the dose estimated by the 7-point fit is better than that of the 10-point fit, especially in the blue channel.

The film dose can be obtained by the calibration curves of a single color channel or all three color channels. The three-channel methods (Micke et al., 2011) can reduce measurement noise and improve measurement accuracy. Among many three-channel methods, the mean dose and the weighted mean dose are commonly used. The inverse of the mean square error obtained during the film calibration for each channel was used as the weight to obtain the weighted mean dose (Méndez et al., 2014). To study the effect of the dose calculation method, the estimation errors and relative standard deviations of the film dose using the values for the single channel dose, the mean dose, and the weighted mean dose were compared. The results are shown in Fig. 5. The relative standard deviation is the ratio of the standard deviation of the dose estimation to the dose. In the dose range of 0–600 Gy, the estimation error of the mean dose and the weighted mean dose is better than that of the worst single channel and is inferior to that of the optimal single channel (Fig. 5(a)). Moreover, the relative standard deviation of the film dose calculated according to the weighted mean dose method is the smallest (Fig. 5(b)).

To calculate the dose conversion factor for converting the film dose to the dose of the protein solution, the irradiation of the ^{60}Co source was simulated with an ice-box phantom by using the Monte Carlo method. The dose conversion factor F was 0.997 ± 0.005.

3.2. Research on the response of the biodosimeter based on protein damage effect

3.2.1. Response of the dosimeter to different doses

Fig. 6 shows the remaining ratio of the intact proteins of BSA, BHb, and casein as a function of the dose, and Fig. 6(a) and (b) are fitted using the first-order formula and linear quadratic formula, respectively. Clearly, as the irradiation dose increases, the RRIP gradually decreases and eventually tends to be stable. BSA is most susceptible to degradation at all dose ranges. Casein is most difficult to degrade at low dose, but BHb is most difficult to degrade when the irradiation dose reaches 530 Gy or higher.

Table 1 presents the fitting parameters for the irradiation damage effect of proteins. The two fitting formulas for BSA are in good agreement. BHb is suitable for the first-order fitting formula, and its quadratic parameter is zero. Conversely, the linear quadratic fitting formula is more suitable for casein. For the two fitting formulas, the two linear correlation coefficients of BSA or BHb are basically the same, but quite different for casein.

3.2.2. Response of the dosimeter to different protein concentrations

Fig. 7(a) shows the actual exposure dose of 15 protein samples with different concentrations (3 proteins × 5 concentrations), with a mean dose of 78.72 ± 3.70 Gy. The doses of the 15 samples fluctuated within a standard deviation and were considered to be the same dose. Fig. 7(b) shows the RRIP of the three proteins after irradiation as a function of the concentration of the protein solution. With increasing solution concentration, the RRIP gradually increases and the degradation degree of the protein decreases. At the same concentration, BSA was most degraded after receiving the same dose, and casein was the least degraded.

3.2.3. Response of the dosimeter to different dose rates

Fig. 8 shows the RRIP as a function of the dose under two different

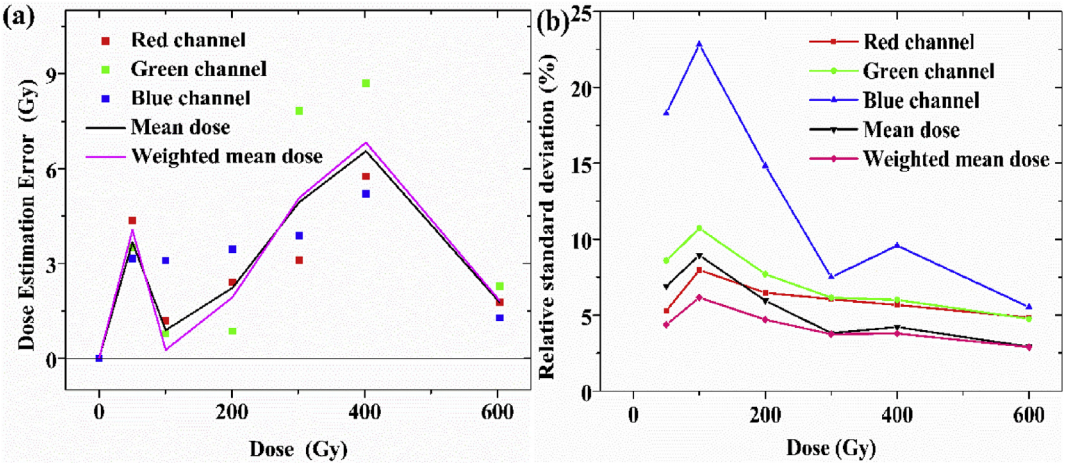

Fig. 5. (a) Dose estimation error and (b) relative standard deviation of film doses obtained using different calculation methods.

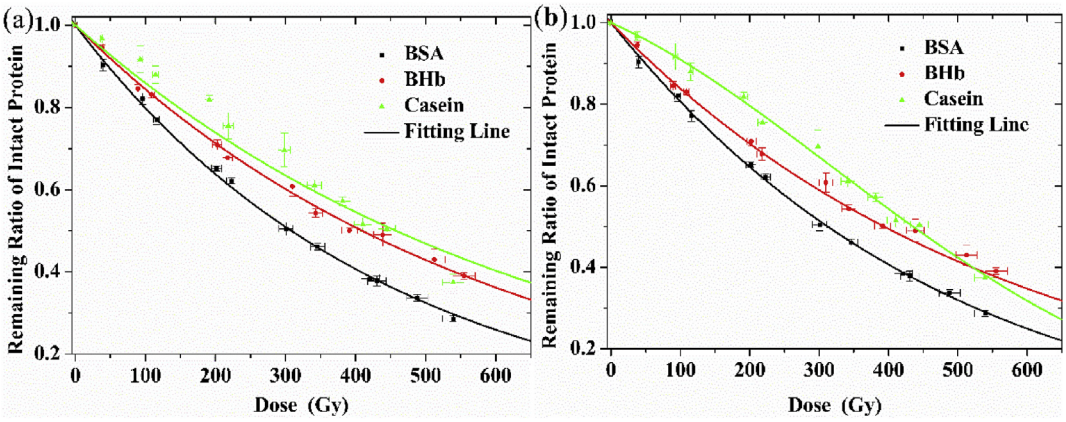

Fig. 6. Remaining ratio of intact protein as a function of irradiation dose (a) first-order fitting formula and (b) linear quadratic fitting formula.

Table 1
Fitting parameters of the radiation damage effect of proteins.

Protein type	Fitting Formula	a	b	R^2
BSA	$y = e^{a\times D}$	−0.00225		0.99832
	$y = e^{a\times D+b\times D\times D}$	−0.00212	−3.3E−7	0.99892
BHb	$y = e^{a\times D}$	−0.00169		0.99619
	$y = e^{a\times D+b\times D\times D}$	−0.00176	0	0.99135
Casein	$y = e^{a\times D}$	−0.00152		0.95551
	$y = e^{a\times D+b\times D\times D}$	−0.00075	−1.9E−6	0.99556

dose rates. Under different dose rates, the damage degree of proteins after receiving the same dose differs, and the damage degree of the protein is smaller under a higher dose rate.

3.3. Applicable dose range for the dosimeter

The dose interval corresponding to the remaining ratio of the intact protein from 0.05 to 0.95 is defined as the applicable dose range of the dosimeter. Table 2 presents the applicable dosage range for different proteins. By changing the concentration of the BSA solution, the minimum dose that can be measured is 2.3 Gy, and the maximum dose is 2555.7 Gy.

4. Discussion

This research developed a biological dosimeter based on the radiation damage effect of proteins and completed the dosimeter response study under γ radiation. To realize the calibration of the dosimeter, Gafchromic film was used to measure the dose of the protein solution,

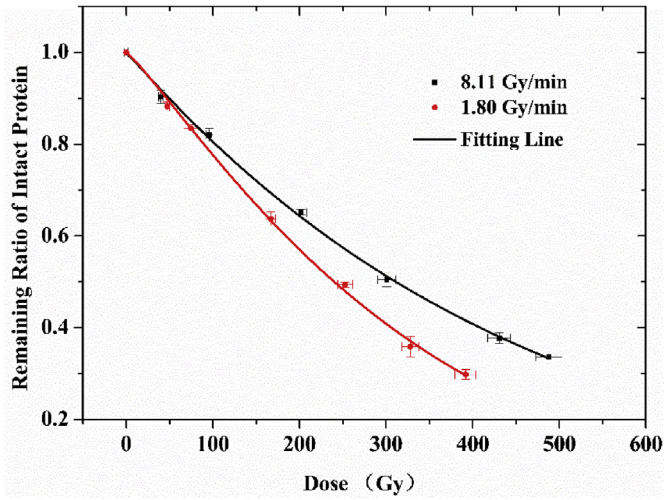

Fig. 8. Remaining ratio of intact protein as a function of the irradiation dose under two different dose rates.

and its response assessment was investigated. Then, the relationships between the damage degree of proteins after irradiation and the dose, protein type, protein concentration, and dose rate were established. Finally, a wide-range of dose measurement was achieved by changing the concentration of the protein solution.

In the response evaluation of Gafchromic film, the dose calibration curve established using the standard operating procedure helped achieve a more accurate dose measurement than the ordinary method.

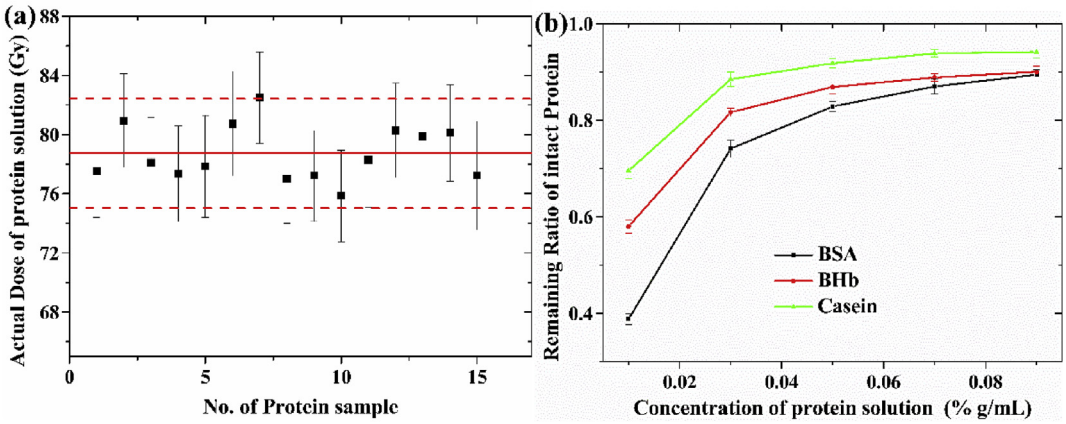

Fig. 7. (a) Actual dose of 15 protein samples and (b) remaining ratio of intact protein as a function of the concentration of the protein solution.

Table 2
Applicable dose ranges for different proteins.

Protein Type	Concentration (g/mL)	Available Dose Range (Gy)
BSA	0.01%	2.3–235.6
BSA	0.05%	23.7–1320.4
BSA	0.10%	29.9–2556.7
BHb	0.05%	27.9–1856.5
Casein	0.05%	70.9–1201.9

Moreover, the fitted dose range of the calibration curve affects the dose measurement, and better measurement can be achieved by selecting the appropriate range as needed. Considering that the dose used in this work is below 600 Gy, the 7-point fitting (covering 0–600 Gy) is more appropriate. In the full dose range, each color channel has its own dominant range, making it difficult to accurately evaluate the film dose through a single channel. The weighted mean dose method has good performance in all dose ranges and possesses the smallest relative standard deviation, so it is selected as the subsequent dose calculation approach for the film. Through the selection of the fitted dose range and the weighted mean dose method, the film response was accurately converted into the film dose. By combining the film dose and the dose conversion factor, the dose measurement of the protein solution was achieved.

The damage degree of the three proteins (BSA, BHb, and casein) differed for different dose ranges. This trend may result from the divergent amino acid compositions and spatial structures of the different proteins, and their responses to radiation and radiation-generated free radicals also vary. Thus, the responses of the proteins to radiation differ. A more accurate dose measurement can be realized at a special dose range by choosing the protein type. For the different protein species, the way the gamma ray causes damage was also distinctive. BSA and BHb were more suitable for the first-order response mode, while casein was more suitable for the linear quadratic response mode. This observation implied that the novel biodosimeter can be used under different conditions and applications.

The relationship between the damage degree of proteins and the dose is in accordance with the first-order formula for BSA and BHb. The protein damage curve can be determined by the damage degree of the protein under one irradiation dose. Through the study of the damage effect of proteins at different concentrations, establishing protein damage curves as a function of the dose with different concentrations was possible. For casein, however, the relationship between protein damage and irradiation dose is in line with the linear quadratic formula, and more data are needed when one concentration is used to establish the protein damage curve.

By using more radiation-tolerant proteins, such as BHb, the novel dosimeter can be used for larger doses and can achieve smaller dose measurements with more sensitive proteins. Therefore, the biological dosimeter based on protein irradiation damage effect can perform a wide range of dose measurements by adjusting the concentration of protein solutions and protein species.

At low dose, the difference in the fitting curves of protein damage caused by various dose rates was small and was disregarded. However, the effect of the dose rate on the damage degree of proteins cannot be ignored at high dose. Further research on the effect of the dose rate is needed. In addition, studies on the dosimeter response under greater radiation levels can extend the application range of the dosimeter. These concerns will be investigated in our future work.

5. Conclusion

In the response assessment of Gafchromic film, the accurate measurement of film dose can be achieved by selecting the appropriate fitted dose range and the weighted mean dose method. The Monte Carlo calculation revealed the dose conversion factor F to be 0.997 ± 0.005. The dose of the protein solution was obtained in combination with the film dose and the conversion factor. In investigating the damage response of proteins, the damage degree of proteins gradually increased according to the exponential function as the irradiation dose increased. Different kinds of proteins have different response modes and fitting coefficients. For the three proteins we studied, the greater the concentration of the protein solutions, the more difficult they were to degrade. In addition, the dose rate affected the radiation damage effect of proteins, and the damage degree was smaller at high dose rates. Finally, a wide-range dose measurement from 2.3 to 2555.7 Gy can be achieved by changing the concentration of the BSA from 0.01% to 0.1% g/mL. The biological dosimeter proposed in this paper can not only achieve the biological dose measurement of an irradiated body but also further strengthened the research on the biological effect of radiation.

Declaration of interest

The authors have no conflicts of interest.

Acknowledgments

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