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THE INFLUENCE OF RADIO-FREQUENCY RADIATION ON THERMAL STABILITY OF BOVINE SERUM ALBUMIN IN AQUEOUS SOLUTION

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Abstract

Protein unfolding events were studied by differential scanning calorimetry (DSC) for bovine serum albumin (BSA) aqueous solutions exposed to radio-frequency radiation. No immediate effect of this radiation on thermal unfolding of BSA was observed. The differences between irradiated and control samples have appeared during the storage of BSA solution. The irradiated samples changed faster than non-irradiated. Our results indicated that the age-related changes were stronger for 3.5 and 5 MHz than for 247 MHz frequency and dependent on energy power of radiation. Deconvolution of DSC traces allowed to study the effect of radio-frequency radiation on each component transition.

Keywords: bovine serum albumin, DSC, radio-frequency radiation, thermal stability

Introduction

Interactions of radio frequency (*RF*) fields with biological bodies are complex function of numerous parameters. Radio waves in free space are characterized by the frequency, intensity of the electric (*E*) and magnetic (*H*) fields, their direction, and polarization. The distribution of electromagnetic fields in biological materials is governed by dielectric constant, conductivity, source configuration, and the geometrical factors that describe the material structure. *RF* energy absorption can be converted to thermal energy. The thermal interactions of electromagnetic fields with the exposed bodies are relatively well documented [1–3]. In recent years work has focused on possible effects at 'non-thermal' levels but such effects are not well established [2].

Differential scanning calorimetry (DSC) is widely used for the study of thermal protein denaturation [4–9]. DSC melting profiles of bovine serum albumin (BSA) depend on the experimental conditions, e.g. the kind of solvent, pH, ionic strength, protein concentration, fatty acid content, and other factors [10–17]. In aqueous solution at pH≈5.5, DSC curve of BSA containing endogenous fatty acids shows visually single, endothermic, partially reversible transition with the thermal midpoint T_m about 69°C [17].

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The current study concerns the influence of radio-frequency radiation (RFR) exposure on thermal stability of BSA in aqueous solutions.

Materials and methods

BSA as a crystallised and lyophilised powder (purity minimum 99%), essentially globulin free (BSG) (lot 79H7614) was obtained from Sigma. The protein characteristics were the same as previously reported [17].

DSC was carried out on a VP DSC ultrasensitive microcalorimeter (MicroCal Inc., Northampton, MA) with cell volumes 0.5 mL at heating rate 1 K min^{-1} in the temperature range 20–100°C. For all the measurements the protein concentration was 0.044 mM L^{-1}, pH was 5.5±0.3. All other DSC experimental details were the same as described previously [17].

The freshly prepared portion of BSA solution was divided into two parts and placed in two 10 mL glass flask. One of the sample was exposed to RF radiation, while the other (control) was kept in similar ambient conditions without irradiation. The source of electromagnetic wave was wave generator (Model RUFG-4) supplied by power generator (Model ZT-980-1M). The sample was situated directly in the front of the source of RF wave. Bovine serum albumin solutions were exposed to near field. In this region E and H fields vary rapidly with distance. Essential parameters (*f*-frequency, *E*-electric, *H*-magnetic fields and *S*-flux density (energy power) of exposure field are summarized in Table 1. These parameters were derivated from measurements with use of the E4403B Spectrum Analyser.

30 min expositions were performed at an air temperature 20°C. Temperature of solution did not change significantly during the period of exposure and the temperature differences before and after irradiation were ≤0.2°C.

Table 1 Essential parameters of the exposure field

f/MHz	$E/10^{-2}$ V m^{-1}	$H/10^{-2}$ A m^{-1}	$S/10^{-4}$ W m^{-2}
3.6	97÷10	1.9÷0.2	184÷2
5.0	163÷16	3.3÷0.3	532÷5
247	113÷13	2.3÷0.3	258÷3.6

DSC curves were analysed with MicroCal Origin software. The calorimetric data were corrected for the calorimetric baseline (by subtracting water–water scan) and for the difference in heat capacity between the initial and the final state by using a sigmoidal baseline.

Statistical analysis of the results was done with Statistica 5.1 using t-test, ANOVA or Kruskal-Wallis test.

Results

Time instability

DSC profile of BSA aqueous solution shows broad endothermic transition, with a positive value for the change in heat capacity Δc_p, connected with denaturation process (the melting of BSA). Figure 1 illustrates the averaged DSC curves (each from 2÷5 scans) for fresh (initial) and aged albumin solutions. The samples were stored in the refrigerator (in about 4°C) besides this time when they were thermostated in 20°C before DSC measurements. The BSA thermal characteristics are time dependent. The initial DSC curve and that after 3 days, are practically identical, thus BSA stability does not change significantly during a few days (or even longer if the sample is not bring out from the refrigerator). However, longer storage of protein solution decreases its stability. The intensity of denaturation peak becomes smaller, its width at half height ($T_{1/2}$) increases, the thermal midpoint (T_m) shifts to lower temperatures, the transition becomes bimodal. This bimodal character makes the melting DSC profile of the aged albumin similar to the melting profile of fatty acid free BSA [17]. The results of preliminary analysis based on integration of data in selected temperature range (area below the peak, ΔH, which represents the heat absorbed during the thermal unfolding transition), T_m and $T_{1/2}$, listed in Table 2, confirm above findings.

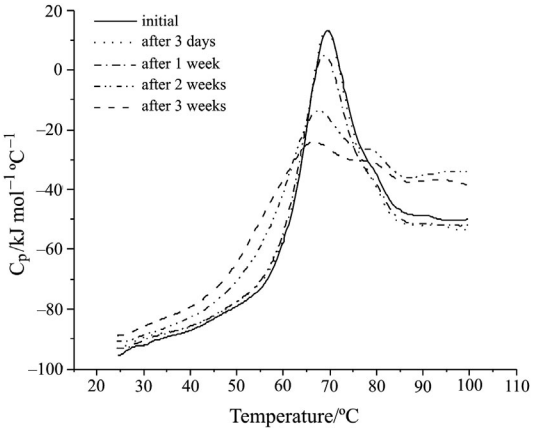

Fig. 1 The averaged DSC curves for fresh and aged BSA solutions

RF irradiation

Figure 2A–D shows original DSC scans (though after subtracting water-water scans) of the pair of samples: irradiated with 5 MHz and non-irradiated. One can see that the differences between irradiated and control samples have appeared during the storage of BSA solution. No immediate effect of this radiation on thermal unfolding of BSA was observed (DSC curves in Fig. 2A are almost identical) but the irradiated samples aged faster than non-irradiated. The differences between exposed and control sam-

Table 2 The averaged transition parameters (± SEM) for BSA endothermic peak

		T_m/°C	ΔH/kJ mol^{-1}	$T_{1/2}$/°C
Day '0'		69.0±0.1	920±20	10.7±0.1
After 1 week	Radiation frequency			
	none	67.6±0.5	910±30	12.0±0.5
	3.6 MHz	66.8±0.3	880±20	14.3±0.2
	5 MHz	66.6±0.4	900±15	12.8±0.4
	247 MHz	67.8±0.6	920±10	11.5±0.5

* SEM – standard error of the mean

ples are only just visible after 3 days (Fig. 2B) but well appreciable after 1 or 3 weeks (Figs. 2C D). Figure 3 illustrates similar effect and the differences for radiation frequency 3.6 MHz after 2 weeks.

The comparison of the averaged transition parameters listed in Table 2 for fresh control and irradiated samples (day '0') and after 1 week indicates decrease of BSA stability in time and suggests some differences between samples exposed to different radiation frequencies. The changes observed after 1 week seems to be the smallest for samples non-irradiated and irradiated with 247 MHz. The results of statistical analy-

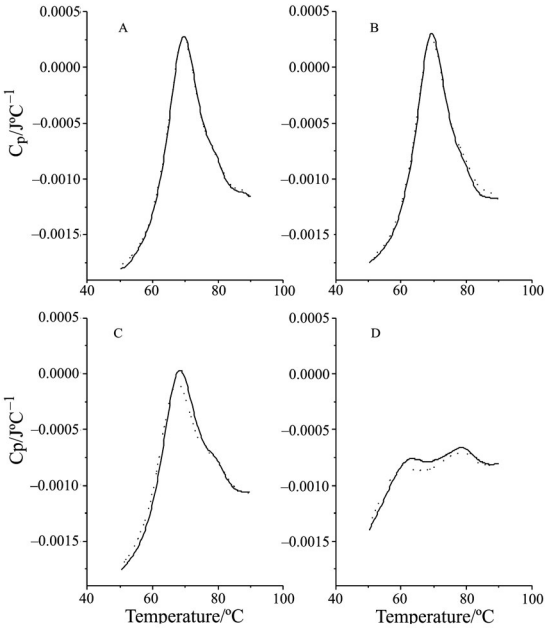

Fig. 2 DSC curves for the pair of BSA samples: —– non-irradiated and ····· – exposed to 5 MHz radiation; A – day '0', B – after 3 days, C – after 1 week, D – after 3 weeks

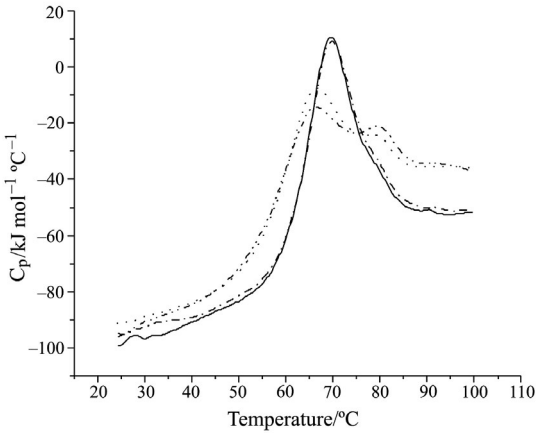

Fig. 3 DSC curves for BSA samples: — – control, fresh; -·-·- – exposed to 3.6 MHz radiation, fresh; ····· – control, after 2 weeks, -··-·· – exposed to 3.6 MHz radiation, after 2 weeks

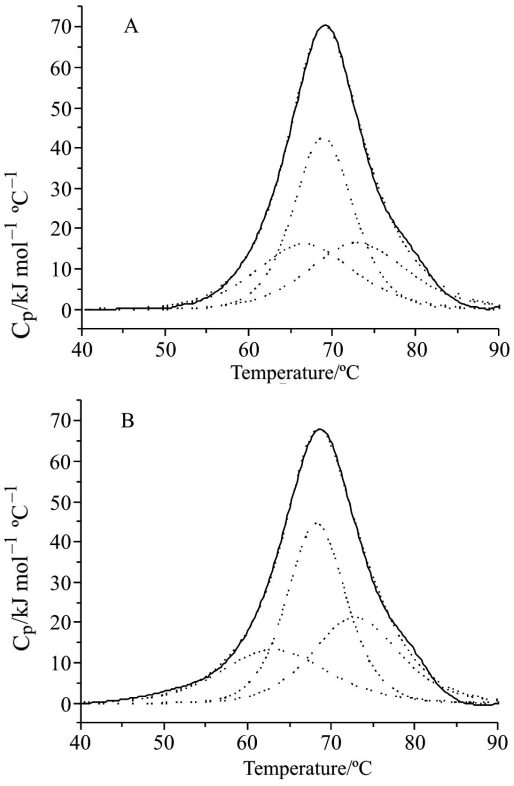

Fig. 4 The curve fitting of DSC profile for BSA solution: A – fresh, B – after 1 week, exposed to 247 MHz radiation; in 2-State model

sis show essential differences (p<0.05) between T_m and $T_{1/2}$ (but not ΔH) obtained for fresh and 1-week samples. No statistically essential differences in above transition parameters were found between different radiation frequencies.

Deconvolution analysis

A deconvolution of DSC traces were performed within the framework of 2-State and Non-2-State models (described earlier [11]). The exemplifying results of DSC curve fitting in 2-State model are shown in Fig. 4. The single endotherm peak is well approximated as the sum of three independent two-state transitions. Transition temperatures of the first and second transitions (T_1≈66°C, T_2≈69°C) decrease with time while that of the third (T_3≈74°C) practically does not changes. The detailed values of T_{mi} (i=1, 2, 3) for non-irradiated (n) and exposed to different radiation frequencies BSA are compared in Fig. 5 for fresh and 1 week samples. The statistically essential differences (p<0.05) were obtained: 1) in T_1 and T_2 – between fresh 'n' and all 1 week samples, 2) in T_1 and T_2 after 1 week – between 'n' and 5, 3.6 and 5 MHz, 3.6 and 247, 5 and 247 MHz, 3) in T_3 after 1 week between 3.6 MHz and other frequencies. No essential differences were found in ΔH_{mi} although the mean values shown in Table 3 suggest their slight decrease after 1 week.

Table 3 Comparison of enthalpy change ΔH_i of the three component transitions (obtained in 2-State model) for the denaturation peak of non-irradiated albumin solution: fresh (day '0') and after 1 week

	Enthalpy change ΔH_i/kJ mol^{-1}					
	ΔH_1		ΔH_2		ΔH_3	
	day '0'	1 week	day '0'	1 week	day '0'	1 week
Mean value	260	249	393	375	273	267
Confidence interval*	(243;276)	(234;264)	(358;429)	(346;405)	(256;290)	(248;287)

* 95% probability

The results of statistical analysis and Fig. 5 indicate that after 1 week T_1 decreases stronger than T_2. The biggest changes in thermodynamic parameters of component transitions occur for samples exposed to 5 MHz radiation. It should be noted however, that the energy power S was the biggest in this case (Table 1). The smallest difference between exposed and control samples after 1 week we observed for radiation frequency 247 MHz. Influence of exposure frequency on the differential '1 week' DSC curves is shown in Fig. 6.

Using of 2-State model gives poor results of fitting for DSC curves recorded for BSA solutions stored longer than 1 week. The best fitting of DSC curves corresponding to 2-week old BSA solution was obtained in Non-2-State model at assumption of 4 peaks. Figure 7 A and B shows such curve fitting for control and irradiated with 3.6 MHz samples respectively. Interpretation of the nature of the component transitions

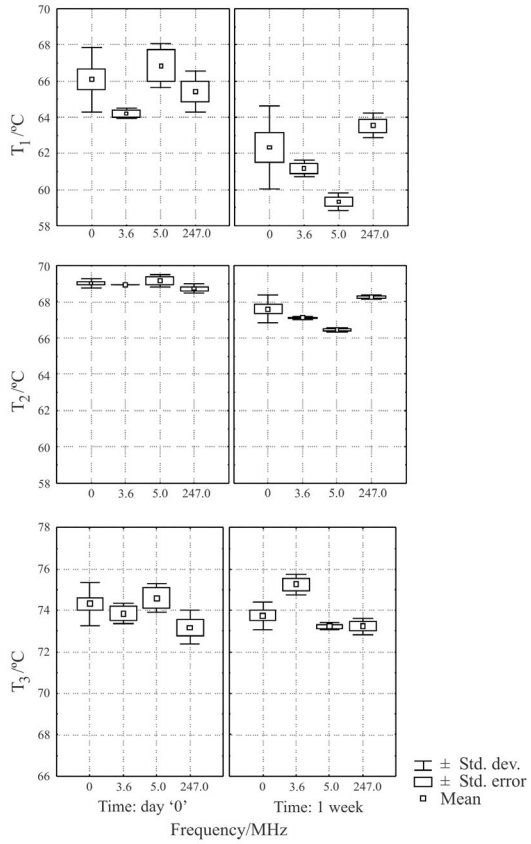

Fig. 5 The transition temperatures: T_1, T_2, T_3, of the component transitions obtained in 2-State model for the BSA denaturation peak

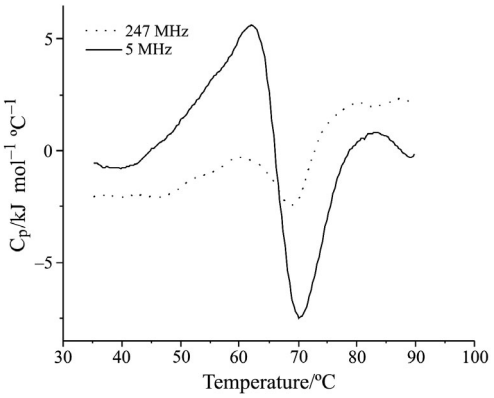

Fig. 6 Differential DSC curves for the pairs of BSA samples: irradiated with 5 or 247 MHz and non-irradiated; stored 1 week

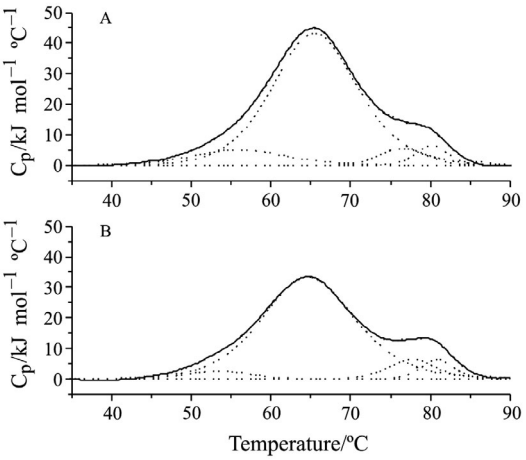

Fig. 7 The curve fitting of DSC profile for 2-week old BSA solution: A – control, B - exposed to 3.6 MHz radiation; in Non-2-State model

is not clear, thus the detailed results obtained within the framework of Non-2-State model will not be discussed in this paper.

Discussion

The energy per photon in RF range of the electromagnetic spectrum is feeble and molecules in living organisms can not be probably altered by RFR. However, some biochemical changes have been reported to result from exposure to RF energies. Non-thermal effects of RFR are suspect to occur, but it is very difficult to substantiate such effects [1–3].

In our experiment, we applied RFR that does not lead to significant temperature increases. The temperature differences before and after 30 min irradiation were negligible for 3.6 and 5 MHz and no bigger than 0.2°C for 247 MHz. It is interesting to note that all observed by us differences between irradiated and control samples were the smallest for this highest frequency used in experiment. It indicates that the effects described in this work are non-thermal.

We did not observe any immediate influence of RFR on the thermal unfolding of BSA in aqueous solution. However, the differences between DSC profiles for irradiated and control samples have appeared during the storage of BSA solution. The irradiated samples aged faster than non-irradiated. Our results suggested frequency dependence of the changes because the observed effect was distinctly stronger for lower radiation frequencies: 3.6 and 5 MHz than for 247 MHz. However, the energy power should also be taken into consideration.

Deconvolution analysis let us to see the effect of RFR on BSA molecule. It was shown [17] that in aqueous solutions BSG melts as a rather compact structure, al-

though three two-state transitions correlated to three domains in BSA molecule may be separated. DSC measurements presented in this work for stored BSA solution showed decrease of albumin stability with time. Transition temperatures respondent to two domains unfolding in lower temperatures decrease. Probably, the structure of BSA molecule becomes more loose, similar to that ascertained in a particular range of pH and ionic strength [14]. Radio-frequency radiation intensifies this process.

Generally, one can reaches the conclusion that radio-frequency radiation accelerates to some extent ageing of BSA aqueous solutions.

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