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Effects of Pulsed Electric Fields and Heat Treatment on Stability and Secondary Structure of Bovine Immunoglobulin G

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Bovine immunoglobulin G (IgG) solutions were subjected to pulsed electric fields (PEF) or heat treatment to investigate the effect of processing on secondary structure monitored using circular dichroism spectrometry. Under heat treatment, the critical temperature for bovine IgG to change secondary structure at neutral pH in borate buffer is 72 °C. A conversion of the secondary structure from β-sheets into random coils along with the loss of immunoactivity of bovine IgG was observed when heated at 82 °C for 120 s. In contrast, PEF treatment at 41.1 kV/cm for 54 μs with bipolar pulses (outlet at 43.8 °C) caused no detectable changes in the secondary structure or the thermal stability of secondary structure. A shape factor, S_{217nm}^{200nm}, ratio of magnitude of the positive CD band at 200 nm to that of the negative CD band at 217 nm, was closely correlated to the immunoactivity of bovine IgG ($r^2 = 0.99$) and quantifies changes of secondary structure.

KEYWORDS: Bovine IgG, PEF, heat treatment, secondary structure, immunological activity, CD spectrometry.

INTRODUCTION

Oral administration of bovine colostrum has been reported to be effective in preventing diseases caused by microbial infections (*1*−*5*). Immunoglobulins in bovine colostrum are thought to provide the major antimicrobial protection and confer a passive immunity (*6*). Bovine serum and secretions contain three major classes of immunoglobulins: IgG, IgM, and IgA. The immunoglobulins are transported selectively from serum into the mammary glands. The first colostrum contains 40−200 mg/mL of immunoglobulins, in which IgG accounts for more than 75% (*7*). Through oral administration, bovine IgG in colostrum has demonstrated its ability to protect infants from intestinal infections caused by *Escherichia coli* (*2*, *3*), *Shigella flexneri* (*1*), and rotavirus (*4*, *5*). The antiinflammatory effect of milk protein concentrate, obtained from cows that were hyperimmunized with 26 human pathogens including *Salmonella enteritidis*, was evaluated by the significant relief ($p < 0.005$) of the symptoms of osteoarthritis of adult patients in a clinical study (*8*). The functionality of bovine immunoglobulins is dose dependent and relies on the bioavailability after ingestion (*1*). In addition, traditional food processing can result in loss of IgG immunoactivity (*9*). There is a need to investigate the stability of bovine IgG at different food processing conditions and the mechanism by which changes in bovine IgG activity take place during processing.

Bovine IgG concentration showed no significant change when thermally treated at 62.7 °C for 30 min or when held for 24 h at either ambient temperature or 4 °C (*10*). However, high-temperature processing ($T > 72$ °C) causes significant loss in bovine IgG stability. No IgG activity was detected in the ultrahigh-temperature pasteurized (UHT) or retorted sterilized milk products (*11*), particularly when pH is lower than 4.0 or higher than 10.0 (*12*). The *D* values of bovine serum IgG immunoactivity at 80 °C in phosphate buffered saline, boiled cow's milk (boiled for 5 min), and UHT cow's milk are 90, 200, and 170 s, respectively (*10*). In a survey of 254 raw milk samples collected in 1990 and 1991 in British Columbia, Canada, the concentration of bovine IgG in the raw milk was reported ranging from 0.28 to 0.71 mg/mL (*13*) and ranging from 0.04 to 0.24 mg/mL in commercially pasteurized milk using high-temperature short time process (*11*). SDS−PAGE results (*14*) suggest the possible formation of aggregates by bovine IgG upon being heated at 85 °C for 2 min, while cleavage of the peptide chains or any change in the primary structures of bovine IgG was not reported.

PEF is an emerging nonthermal technology alternative to thermal processing. Effectiveness of PEF in microbial inactivation was reported using *E. coli* (*15*, *16*), *Lactobacillus brevis* (*17*), *Listeria* (*18*), *E. coli* O157:H7 (*19*, *20*), *Bacillus cereus* (*21*), and *Mycobacterium paratuberculosis* (*22*) in foods. The inactivation of microorganisms by PEF is due to the high intensity of the electric fields, while thermal effects contribute minimally (*23*). PEF effectively inactivates microorganisms and may improve the quality of fruit and vegetable juices relative to thermal processing (*24*−*26*). PEF pasteurization retains heat-labile components in foods by reduced temperature during processing. Bovine IgG starts losing immunoactivity when the temperature is higher than 73 °C (*10*, *27*). PEF treatment at equivalent dosage with thermal treatment for 5-log reduction

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of natural flora of enriched soymilk does not result in loss of bovine IgG immunoactivity (*27*).

The mechanisms behind the loss of bovine IgG immunoactivity as a function of temperature are still not clear, and there is a lack of reports regarding the stability of secondary structure of bovine IgG under heat or PEF treatment. Changes in secondary or tertiary structures may be responsible for the changes in the immunological activity upon processing. The objectives of this study were to investigate the effects of PEF treatment or heat on the immunoactivity and secondary structure of bovine IgG and to identify the mechanism of the heat-induced changes. The relationship between bovine IgG immunoactivity and secondary structure was studied to quantify the contribution of changes in secondary structure to the loss of immunoactivity of bovine IgG.

MATERIALS AND METHODS

Materials. Freeze-dried bovine IgG was purchased from Sigma Chemicals (St. Louis, MO). Borate buffer (40 mM, pH 6.84), 1 mM EDTA, 30 mM sodium phosphate (pH 6.45), and 10 mM potassium phosphate (pH 4.85) were used for secondary structural measurements. The pH of the buffer was adjusted using boric acid/sodium borate or phosphoric acid/sodium phosphate. Hydrogen chloride and sodium hydroxide should not be used because of the strong absorption of circular polarized light by chloride ions and hydroxyl ions in the far-UV region. After the lyophilized IgG was mixed with buffer, the solution was stored in at 4 °C for 12 h to help distribute IgG uniformly through the solution before heat treatments.

Methods. *Heat Treatments of Bovine IgG Solutions.* The bovine IgG borate buffer solutions were treated using a continuous heat treatment apparatus (*27*). A seamless 316 stainless-steel holding tube (i.d., 0.18 cm; length, 609.6 cm) was immersed in a water bath maintained at the same temperature as the test temperature. The temperature was measured at the beginning and at the end of the holding tube. The difference between inlet and out temperature of the holding tube was in the range of 0.3−1.0 °C. The treatment temperature was calculated as the average of the temperature at the beginning and end of the holding tube. The treatment time was calculated On the basis of the flow rate and holding tube dimension. Three replicates were made for each treatment.

PEF Treatments of Bovine IgG Solutions. The OSU-4A PEF unit (The Ohio State University, Columbus, OH) was used for the PEF treatment, as illustrated in **Figure 1a**. An automatic syringe pump continuous fluid handling system was used. Four co-field flow PEF treatment chambers with a gap distance of 0.29 cm and an inner diameter of 0.23 cm were used. The PEF chambers were arranged electrically parallel. During a PEF treatment, the flow rate was 1.0 mL/s. The temperature was measured with type K thermocouples at the inlet and outlet of each pair of chambers. Bipolar square pulses were applied. The typical bipolar square waveform pulses are illustrated in **Figure 1b**. Samples after treatment were cooled to 4−6 °C immediately with an ice−water bath. Each treatment was repeated three times.

Determination of Protein Concentration in the Buffer Solutions. The protein concentration of the samples was determined using the Biuret method (*28*) with the following modifications. Biuret reagent, consisting of 2.25 g sodium potassium tartrate, 0.75 g of copper sulfate pentahydrate, and 1.25 g of potassium iodide, were dissolved in order in 100 mL of 0.2 M NaOH (0.8 g/100 mL) and brought to 250 mL with distilled water. One milliliter of each diluted sample and 9 mL of the Biuret reagent were added to a test tube, vortexed immediately, and incubated at room temperature for 20 min. The absorbance was measured at 550 nm with a Spectronic Genesys 5 spectophotometer (Milton Roy Company, Rochester, NY). Unlike the UV absorption method at 280 nm, which measures the UV absorption capacity of the side chains in aromatic amino acid residues, such as phenylalamine and tyrosine, the biuret method measures the amide peptide bonds in the protein backbone.

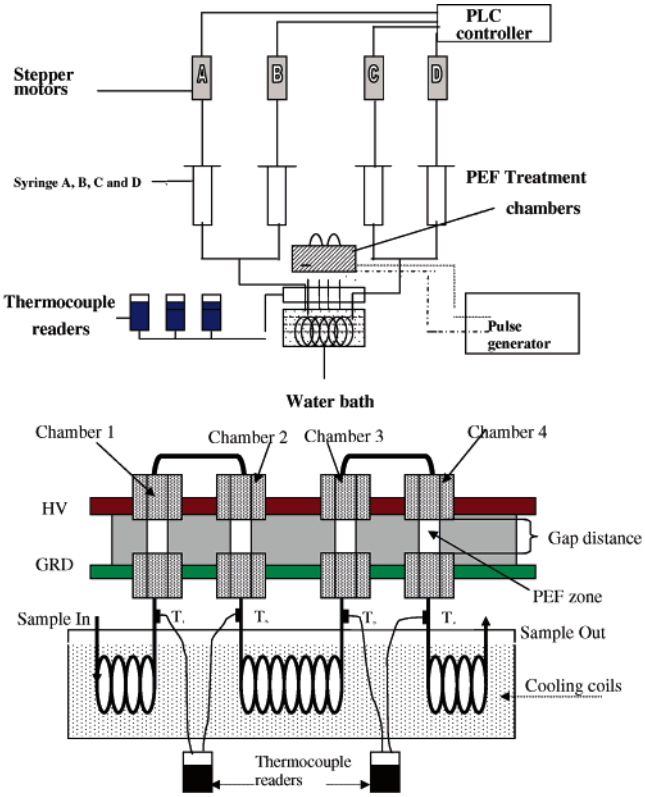

Figure 1. (a) Schematic diagram of the laboratory OSU-4 PEF treatment unit. (b) Temperature measurement points and schamatic diagram of structure and arrangement of the PEF treatment chambers.

Measurement of Secondary Structure of Bovine IgG. The secondary structure of bovine IgG was monitored using circular dichroism (CD) spectrometry. CD spectrometry is an analytical technique for measuring the secondary structure of protein in solution (*29*). Major optically active groups in proteins and polypeptides are the amide bonds and the aromatic side chains. Most commonly existing secondary structures of proteins and polypeptides, such as α-helices, *β*-pleated sheets (parallel or antiparallel), *β*-turns, and random coils, have characteristic CD spectra (*30*). CD bands in the region below 250 nm reflect the contributions only from the backbone of a peptide—from the amide groups. From the shapes and magnitudes of the spectra, the changes of secondary structure of proteins can be interpreted. In this study, we employed far-UV CD spectrometry to monitor the changes in secondary structure of bovine IgG contributed only from the changes in the backbone of IgG molecules under different processing conditions.

An AVIV 202 CD spectrometer (AVIV Instruments, Inc., Lakewood, NY) with 1 cm and 0.1 cm quartz cells (Hellma, Germany) was used to determine the secondary structure of bovine IgG. Secondary structure was detected using wavelength scanning from 300 to 180 nm with a wavelength step of 1 nm. The sample compartment temperature was maintained at 25 °C. The sample was scanned six times and the averages were recorded. During temperature ramping tests, the temperature started from 25 °C and increased to 80 °C with a temperature step of 2 °C. After being held at 80 °C for 2 min, the sample temperature was cooled to 58 °C with a temperature step of 2 °C. The ellipticity and CD dynode were measured at 217 nm during the temperature ramping tests with six scans for each treatment. The kinetic tests were conducted with an elapsed time starting at 0 and ended at 3600 s with a time step of 60 s, with ellipticity measurement at 217 nm. The ellipticity was measured and converted to molar ellipticity (*31*) of bovine IgG by using eq 1.

$$[\theta]_\lambda = \frac{\theta_{obs} M_w}{10LCN} \quad (1)$$

Where $[\theta]_\lambda$ refers to the mean residue ellipticity or molar ellipticity in (deg cm^2)/dmol, θ_{obs} the ellipticity of the samples, L the light path length

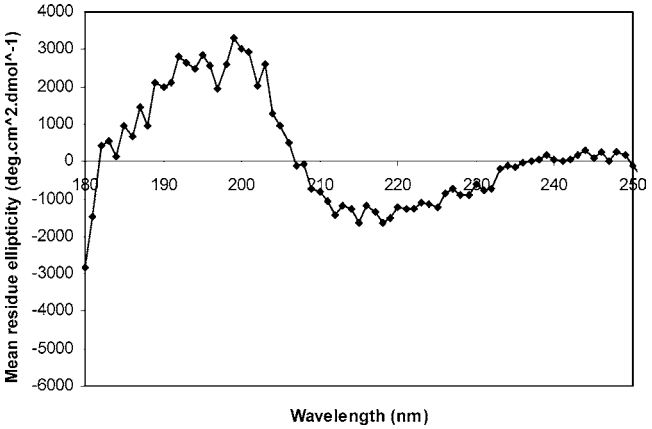

Figure 2. Bovine IgG dissolved in distilled water at room temperature with 1 cm cuvette.

in cm, M_w the molar mass of bovine IgG, which is 150 000 g, C the concentration of bovine IgG in the solution in g/mL, and N the number of amino acid residues in one IgG molecule, which is 1320 for IgG.

Measurements of Immunoactivity of Bovine IgG. Immunoactivity of an immunologically active compound was defined as its capacity to function as a pathogen or antibody so as to bind onto the surface of its counter peer. In this study, immunoactivity represents to the capacity of bovine IgG functioning as a pathogen to be bound by an anti-bovine IgG. The capacity of bovine IgG functioning as an antibody was assigned as antigen-binding activity. Bovine IgG immunoactivity was determined using a modified ELISA method, as previously described (*27*), with the following modifications. The 3,3′,5,5′-tetramethylbenzidine (TMB) substrate was a ready-to-use solution from Research Diagnostics, Inc. (Pleasant Hill Road, Flanders, NJ), and the incubation temperature was changed to room temperature from the previous 23 °C.

Statistical Analysis. Each treatment was replicated six times for CD measurement and three times for immunoactivity determination. The data presented are the means of the replicates for each treatment. Data were statistically analyzed with one-way analysis of variance (ANOVA) and Tukey's multiple comparisons method at 5% significance levels using Minitab 13.31 (Minitab, Inc., State College, PA). Correlation studies between the shape of the spectra and the immunoactivity of bovine IgG were conducted using regression analysis ($p < 0.05$).

RESULTS AND DISCUSSION

Bovine IgG Secondary Structure in Distilled Water and Different Buffers. Bovine IgG dissolved in distilled water has a typical β-sheet secondary structure in the far-UV region (**Figure 2**). A strong negative CD band was observed near 215 nm, while a strong positive band was observed close to 198 nm. A strong negative band in the region between 210 and 230 nm indicates the presence of an $n-\pi^*$ transition (electron transition from the n base level to the π^* stimulated level) in the amide bond in the IgG backbone. A positive band observed close to 198 nm illustrates the $\pi-\pi^*$ transition of the electrons in the valence shell of the oxygen atom in the amide bond. The CD spectrum of typical β-sheet structure of a protein has a negative band near 215 nm, a positive band near 198 nm, and a negative band near 175 nm (*28*, *32*). Theoretical calculations predicted 13 and 5 nm differences in the wavelength at which a maximum CD is observed for parallel and antiparallel β-sheets, respectively (*33*). Twisting causes increased CD band amplitude. Weakly twisted β-sheets have $n-\pi^*$ and $\pi-\pi^*$ bands of similar amplitudes, whereas the strongly twisted β-sheets have a stronger $\pi-\pi^*$ transition that leads to a stronger CD band near 198 nm than the CD band observed near 215 nm (*34*). **Figure 2** shows a stronger positive CD band near 198 nm than the

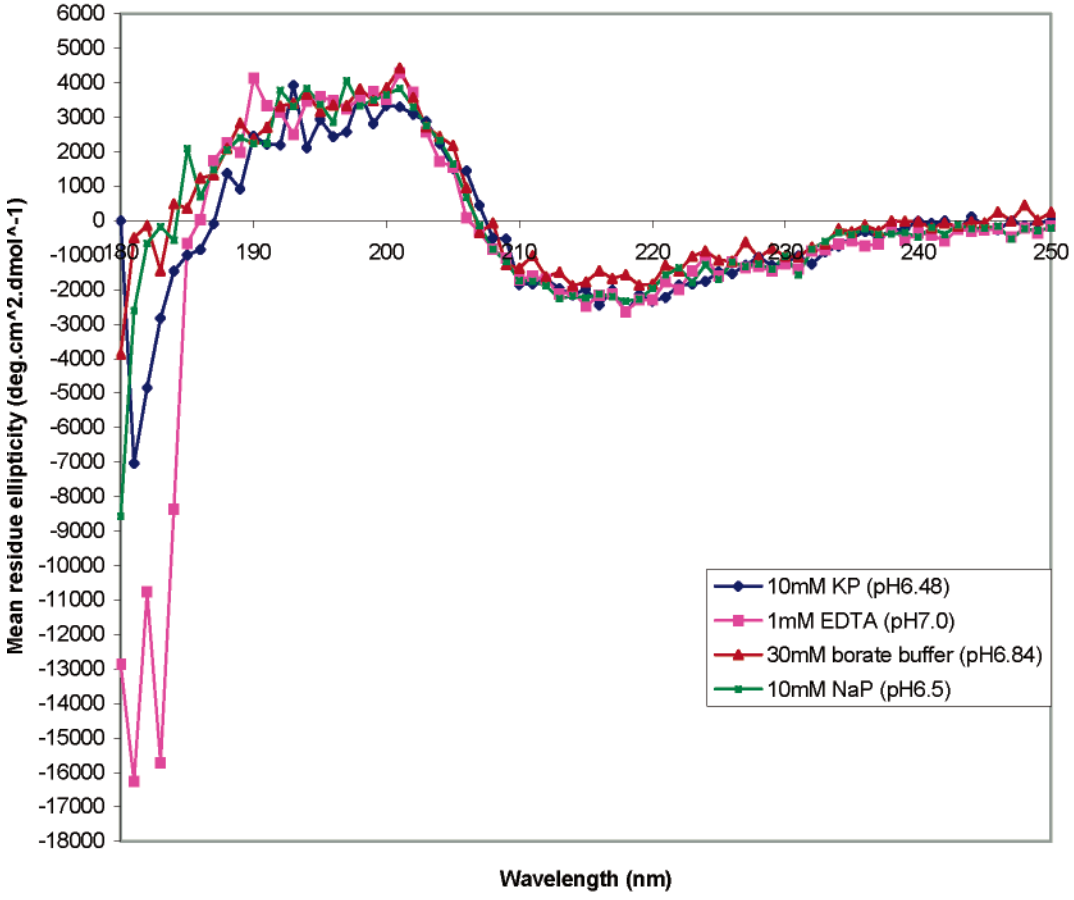

Figure 3. Secondary structure of bovine IgG in different buffers at neutral pH and room temperature (23 °C).

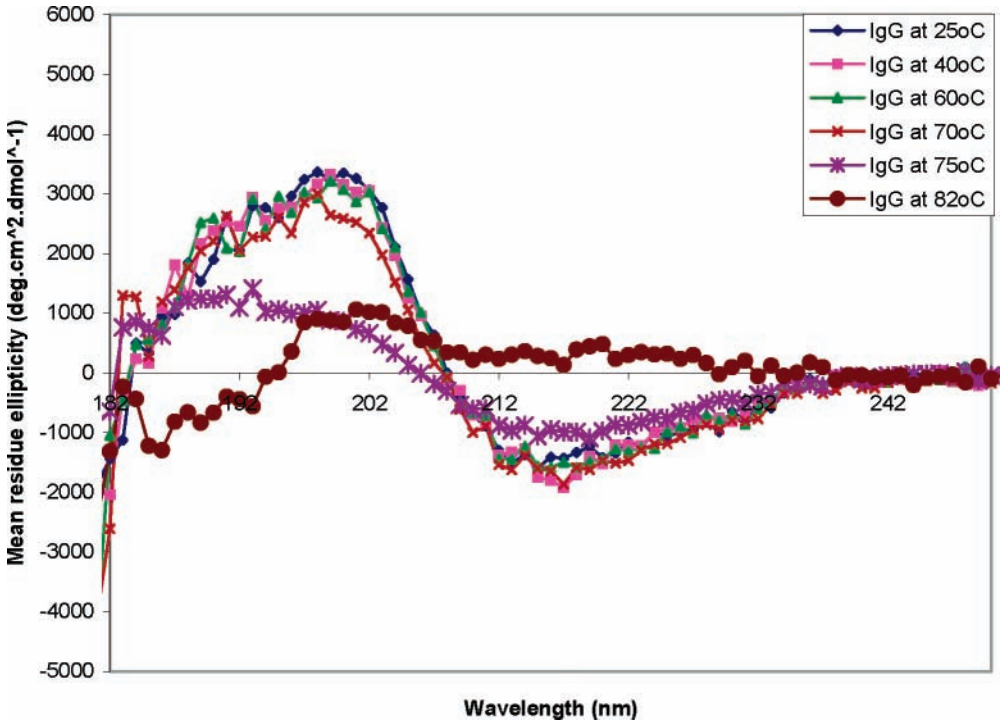

Figure 4. Secondary structure of bovine IgG under different heat treatment conditions. The holding time was 2 min for all treatments in this figure. The buffer used was 30mM borate (pH 6.84).

negative band near 215 nm. The CD spectrum illustrated in **Figure 2** suggests that, although bovine IgG does have typical β-sheets as the dominant secondary structure, the β-sheets in the bovine IgG are strongly twisted.

The influences on the structures of bovine IgG by the four selected buffers are illustrated in **Figure 3**. Both the shapes of the curves and the magnitude of the CD bands showed nondetectable differences with each other from 190 to 250 nm. However, the CD signal for the protein is masked and hard to explain when the wavelength is below 190 nm using 1 mM EDTA buffer. When absorbance of the solution is higher than 2.0 or the CD dynode is higher than 500 V, the recorded CD signal by the AVIV 202 spectrometer is not reliable and is affected strongly by the buffer background. One millimolar EDTA buffer should be avoided when information below 190 nm is needed.

Effects of Heat Treatment on Bovine IgG Secondary Structure. Heat treatment significantly alters the secondary structure of bovine IgG when the temperature is higher than 70 °C (**Figure 4**). Change in the IgG secondary structure takes place gradually, but a critical temperature exists (**Figure 5**). The structural changes developed with the increase of temperature (**Figure 5**). Simultaneously, we observed the corresponding decrease in bovine IgG immunoactivity (**Table 1**) with changes in its secondary structure when subjected to heat treatments at 70 °C or higher temperature. When treated at 82 °C for 120 s, bovine IgG secondary structure changed from its typical β-sheets to typical random coils, which implies the complete unfolding of the active sites along with the loss of the immunoactivity of bovine IgG (**Figure 4**). The decrease in the immunoactivity of bovine serum IgG measured with ELISA showed a parallel relationship with the changes of the secondary structure of bovine IgG from typical β-sheets to random coils. The more significant changes in the secondary structure are associated with the lower immunoactivity of bovine IgG. The relationship between the immunoactivity of bovine IgG and its secondary

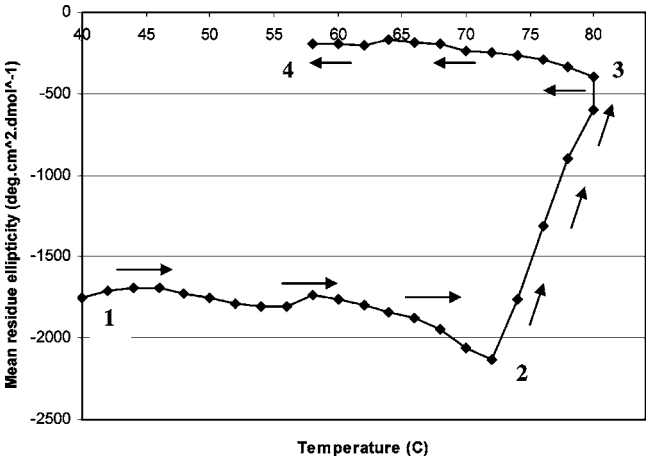

Figure 5. Temperature ramping tests of bovine IgG in borate buffer at pH 6.84 using an AVIV 202 CD spectrometer and 0.1 cm quartz cell with the control samples. **1**, **2**, **3**, and **4** in the figure refer to the sequence of temperature changes (the temperature was increased from 40 °C to 80 °C, held at 80 °C for 2 min, and then cooled to 58 °C). The buffer used for this set of data was 30 mM borate (pH 6.84). Arrows represent the sequence of temperature change during the ramping tests.

structure is illustrated in following equation, which was developed by linear regression using Minitab 13.31.

$$S_{217\text{nm}}^{200\text{nm}} = 0.0171 \times \text{immunoactivity } (\mu\text{g/mL}) + 0.1663 \quad (2)$$

where $S_{217\text{nm}}^{200\text{nm}}$ refers to the ratio of ellipticity measured at 200 nm versus that measured at 217 nm. Typical $S_{217\text{nm}}^{200\text{nm}}$ values were in the range from 1.5−2.0 for β-sheet structures. Another characteristic of β-sheet structures is the occurrence of a single band in the range from 205 to 230 nm. Random coils show typically positive ellipticity from 210 to 220 nm and negative ellipticity when the wavelength is shorter than 210 nm (**Figure 2**).

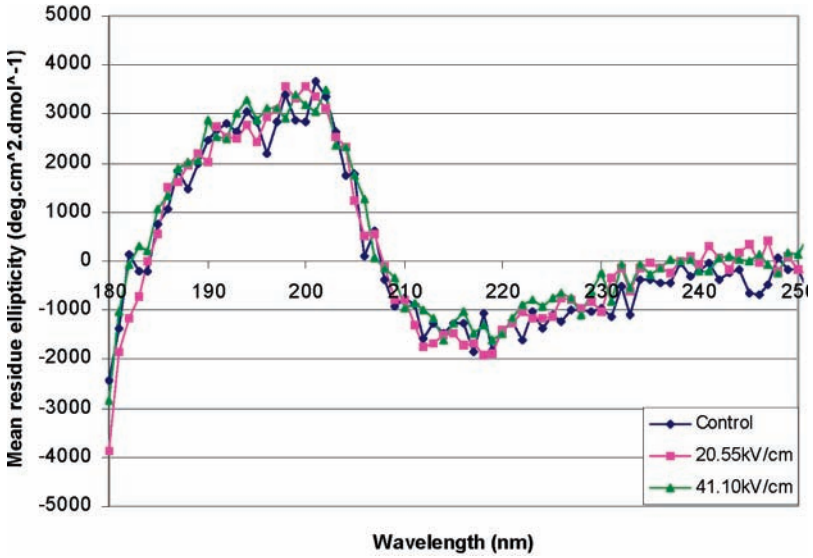

Figure 6. Stability of secondary structure of bovine IgG under different PEF electric field strength for 54 μs. ΔT for one pair of chambers was 23.2 °C at 41.1 kV/cm and 7 °C at 20.6 kV/cm. The outlet temperature of the sample after the fourth PEF chamber after treatment at 41.1 kV/cm was 43.8 °C.

Table 1. Changes in Bovine IgG Immunoactivity and Secondary Structure after Heat Treatments

T (°C) for 120 s	IgG immunoactivity (μg/mL)	S_{217nm}^{200nm} [a]
25	103.2 ± 1.8	1.94
40	101.4 ± 2.1	1.89
60	105.8 ± 1.4	1.92
70	72.2 ± 1.2	1.48
75	42.3 ± 2.5	0.91
80	1.2 ± 1.0	0.15
82	0	positive band at 217 nm

[a] S_{217nm}^{200nm} refers to the ratio of ellipticities measured at 200 nm (positive) and those measured at 217 nm (negative). Random coils show positive ellipticity at 217 nm wavelength.

Table 2. Changes in Bovine IgG Immunoactivity and Secondary Structure in Borate Buffer after PEF Treatments at pH 6.84[a]

PEF treatments	IgG immunoactivity (μg/mL)	S_{217nm}^{200nm}
pass through controls	102.8 ± 1.7	1.94
20.55 kV/cm–54 μs	101.6 ± 2.1	1.94
41.10 kV/cm–27 μs	101.9 ± 1.2	1.93
41.10 kV/cm–54 μs	102.2 ± 1.4	1.94
41.10 kV/cm–91 μs	100.1 ± 2.4	1.94

[a] ΔT per pair of chambers was 35.2, 23.2, and 10.8 °C at 41.1 kV/cm for treatment times of 91.4, 54, and 27 μs, respectively. ΔT per pair of chambers was 7 °C.

Transition of Bovine IgG Secondary Structure Upon Heat Treatment. Secondary structural change of bovine IgG under heat treatment covers a temperature range from 70 to 82 °C. However, a critical temperature point does exist (**Figure 5**). In borate buffer at pH 6.84, when the temperature increases from 40 to 80 °C, there is no significant change in IgG ellipticity at 217 nm when the temperature is below 72 °C. Seventy two degrees Celsius is a critical temperature point for IgG molecules to change their secondary structure (**Figure 5**). In the range from 72 to 80 °C bovine IgG secondary structure changes quickly, reflected in the CD spectrometry as a steep straight line with positive slope. This observation is consistent with a previous report by Li-Chan and his colleagues (*10*), who reported that 73 °C is the critical temperature for bovine IgG to lose its immunoactivity. When the temperature was cooled from 80 to 58 °C, the negative band at 217 nm in the CD spectrum did not return to its original magnitude (**Figure 5**). **Figure 5** suggests that the secondary structural changes caused by heat treatment at temperatures above 72 °C are irreversible.

Twistness of β-Sheets Due to Heat Treatment as Indicated in CD Shape Constant. Immunoactivity loss of bovine IgG associates closely with changes in its secondary structure. Compared with band magnitude, the shape of the CD spectra is more informative for interpretting the pathway in which the secondary structure change. The negative band close to 217 nm is the result of an $n-\pi^*$ transition of the nonvalanced electrons in the peptide bond (amide bond). The positive band close to 195 nm is caused by the $\pi-\pi^*$ transition of the nonvalanced electrons in the peptide bond. Typical 100% β-sheet proteins with twisted secondary structure have different magnitudes of the two bands (**Figure 2**). When the shape constant, S_{217nm}^{200nm}, is equal to 1, the β-sheets are weakly twisted. When S_{217nm}^{200nm} is larger than 1.0, the β-sheets are twisted. A positive S_{217nm}^{200nm} value indicates a stronger twist within the β-sheets. A negative S_{217nm}^{200nm} value implies loose packing of β-sheets. During heat treatment, when treatment temperatures are higher than the critical temperature, not only the magnitude of the CD ellipticity decreases but the shape constant also decreases until the β-sheet structures disappear (Table 1). This phenomenon suggests that the transition of secondary structure of bovine IgG during heat treatment undergoes a pathway in which the molecule first loses the packs of β-sheets. At a certain point, the second structural transition occurs and the structure collapses (**Figure 4** and **Table 1**).

Effects of PEF on Bovine IgG Secondary Structure. Effects of PEF treatments on the secondary structure of bovine IgG dissolved in borate buffer at pH 6.84 are illustrated in **Figures 6** and **7**. No detectable changes in the CD spectra among the controls and PEF-treated bovine IgG samples were observed. The shape of the CD spectra of PEF-treated samples is the same as that acquired from nontreated PEF control samples. This implies that the PEF-treated bovine IgG maintains the same secondary structure characteristics as that of the nontreated bovine IgG. Compared to heat treatments with a temperature higher than 72 °C, PEF treatments, ranging from 0 to 41.1 kV/cm and from 0 to 91.4 μs, demonstrate advantages in maintain-

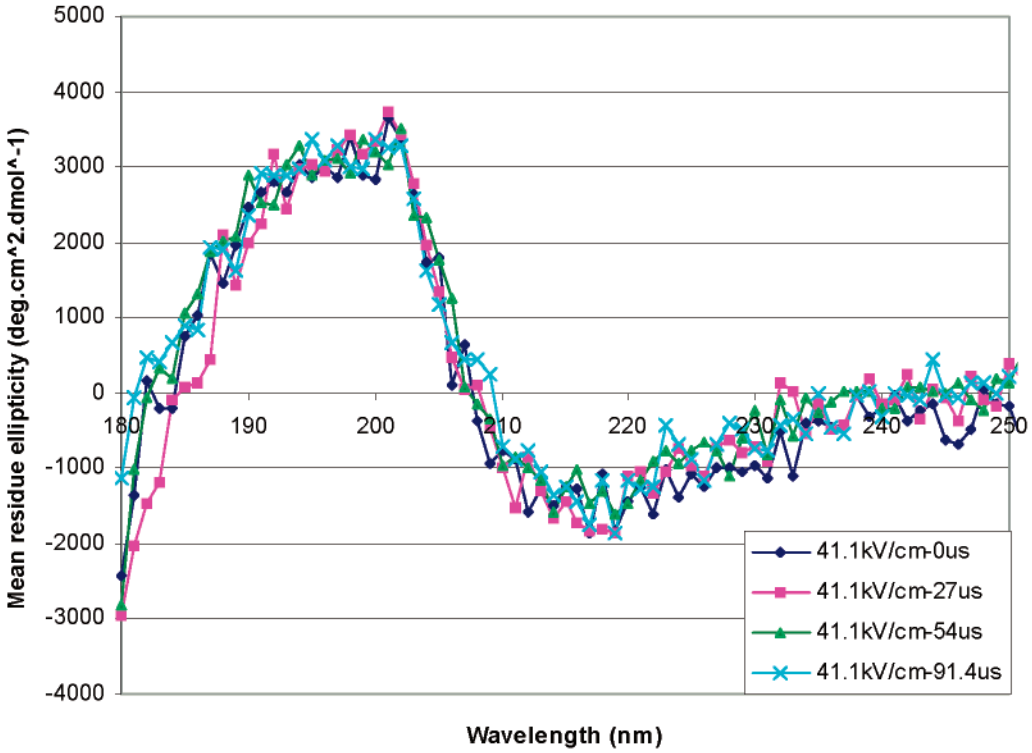

Figure 7. Effects of different total PEF treatment time on the secondary structure of bovine IgG in borate buffer. ΔT was 35.2, 23.2, and 10.8 °C at 41.1 kV/cm for treatment times of 91.4, 54, and 27 μs, respectively.

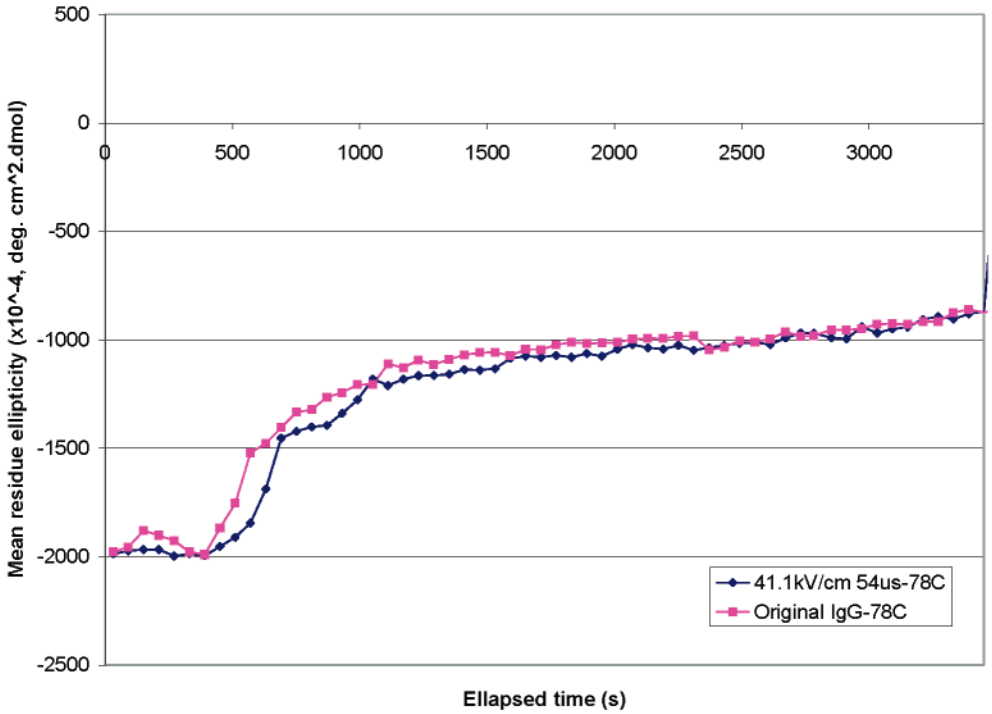

Figure 8. Kinetic tests of bovine IgG with different treatments using an AVIV 202 CD spectrometer at a cell compartment temperature of 78 °C.

ing bovine IgG intact and preventing loss of IgG immunoactivity. The immunoactivity of bovine IgG after different PEF treatments at same conditions shown in **Figures 6** and **7** was summarized in **Table 2**. There was no significant change among the IgG samples before and after PEF treatment ($P > 0.05$). The shape factor, S_{217nm}^{200nm}, also showed no significant change after PEF treatment at a level up to 41.1 kV/cm for 91.4 μs.

There is no significant difference between the thermal stability at 78 °C (cell compartment temperature) of secondary structure of bovine IgG samples before and after PEF treatments (**Figure 8**). Kinetic studies measuring the negative CD band at 217 nm revealed that the changes in secondary structure are also a function of incubation time (**Figure 8**). As the time increases, the magnitude of the negative band at 217 nm decreases until the negative ellipticity disappears. Exposure to 78 °C (cell compartment temperature) causes complete secondary structure transition only when the elapsed time reaches 3500 s for both PEF-treated samples and the controls, while at 72 °C, this time

period is too long for this study. No significant change in the thermal stability of secondary structure of bovine IgG was observed before or after PEF treatments. PEF treatment at 41.1 kV/cm for 54 μs did not cause significant change in either the secondary structure (**Figures 6** and **7**) or thermal stability (**Figure 8**) of bovine IgG. This finding is consistent with a previous report regarding the effect of PEF on the immunoactivity of bovine IgG (*27*).

Conclusions. Heat treatment at a temperature higher than 72 °C causes a loss in immunoactivity and a change in secondary structure of bovine IgG at neutral pH. Loss of immunoactivity of bovine IgG caused by heat treatment is strongly correlated to changes in secondary structure. Deviation of pH from neutral leads to less stability of bovine IgG secondary structure. Heat-induced changes in the secondary structure of bovine IgG takes place in multiple steps through which the molecule first loses its β-sheet pack and, gradually, the β-sheets transit into random coils. Heat-induced change in secondary structure of bovine IgG is irreversible. The shape factor, S_{217nm}^{200nm}, quantifies the change in the secondary structure of bovine IgG during heat treatments, indicating the intensity of twisting and the intactness of the β-sheets. PEF treatment ranging from 0 to 41.1 kV/cm and 0 to 91.4 us does not cause detectable changes in either bovine IgG secondary structure or immunoactivity.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Tacket, C. O.; Binion, S. B.; Bostwick, E.; Losonsky, G.; Roy, M. J.; Edelman, R. Efficacy of bovine immunoglobulins concentrate in preventing illness after *Shigella flexneri* challenge. *Am. J Trop. Med. Hyg.* **1992**, *47*(3), 276−283.

(2) Tacket, C. O.; Losonsky, G.; Link, H.; Hoang, Y.; Guesry, P.; Hilpert, H.; Levine, M. M. Protection by milk immunoglobulin concentrate against oral challenge with enterotoxigenic *Escherichia coli*. *N. Engl. J. Med.* **1988**, *1988*(May), 1240−1243.

(3) Mietens, C.; Kenhorst, H.; Hilpert, H.; Gerber, H.; Amster, H.; Pahud, J. J. Treatment of infantile *E. coli* gastroenterititis with specific bovine anti-*E. coli* milk immunoglobulins. *Eur. J. Pediatr*. **1979**, *132*, 239−252.

(4) Davidson, G. P.; Whyte, P. B. D.; Daniel, E.; Franklin, K.; Nunan, H.; McCloud, P. I.; Moore, A. G.; Moore, D. J. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. *Lancet* **1989**, *1989*(9), 709−712.

(5) Brussow, H.; Hilpert, H.; Walther, I.; Sidoti, J.; Mietens, C.; Bachmann, P. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. *J. Clin. Microbiol.* **1987**, *25*(6), 982−986.

(6) Korhonen, H.; Marnila, P.; Gill, H. S. Bovine milk antibodies for health. *Br. J. Nutr*. **2000**, *84*(Suppl. 1), S135−146.

(7) Korhonen, H.; Marnila, P.; Gill, H. S. Milk immunoglobulins and complement factors. *Br. J. Nutr.* **2000**, *84*(Suppl. 1), S75−S80.

(8) Zenk, J. K. The effects of milk protein concentrate on the symptoms of osteoarthritis in adults: and exploratory, randomized, double-blind, placebo-controlled trial. *Curr. Ther. Res.* **2002**, *63*(7), 430−442.

(9) Chen, C. C.; Tu, Y. Y.; Chang, H. M. Thermal stability of bovine milk immunoglobulin G (IgG) and the effect of added thermal protectants on the stability. *J. Food Sci.* **2000**, *65*(2), 188−193.

(10) Li-Chan, E.; Kummer, A.; Losso, J. N.; Kitts, D. D.; Nakai, S. Stability of bovine immunoglobulins to heat treatment and processing. *Food Res. Int.* **1995**, *28*(1), 9−16.

(11) Kummer, A.; Kitts, D. D.; Li-Chan, E.; Losso, J. N.; Skura, B. J.; Nakai, S. Quantification of bovine IgG in milk using enzyme-linked immunosorbent assay. *Food Agric. Immunol.* **1992**, *4*, 93−102.

(12) Dominguez, E.; Perez, M. D.; Calvo, M. Effect of heat treatment on the antigen-binding activity of anti-peroxidase immunoglobulins in bovine colostrum. *J. Dairy Sci.* **1997**, *80*, 3182−3187.

(13) Li-Chan, E.; Kummer, A.; Losso, J. N.; Nakai, S. Survey of immunoglobulin G content and antibody specificity in cow's milk from British Columbia. *Food Agric. Immunol.* **1994**, *6*(4), 443−51

(14) Dominguez, E.; Dolores, P. M.; Puyol, P.; Sanchez, L.; Calvo, M. Effect of pH on antigen-binding activity of IgG from bovine colostrum upon heating. *J. Dairy Res*. **2001**, *68*(3), 511−518.

(15) Zhang, Q.; Qin, B. L.; Barbosa-Canovas, G. V. Swanson, B. G. Inactivation of *E. coli* for food pasteurization by high-intensity short-duration pulsed electric fields. *J. Food Proc. Preserv.* **1994**, *19*, 103−118.

(16) Li, S. Q.; Zhang, Q. H. Inactivation of *E. coli* 8739 in enriched soymilk by pulsed electric fields. *J. Food Sci.* **2004**, *69*(7), M169−174.

(17) Jayaram, S.; Castle, G. S. P.; Pargaritis, A. Kinetics of sterilization of *Lactobacillus brevis* cells by the application of high voltage pulses. *Biotech. Bioeng.* **1992**, *40*, 1412−1420.

(18) Reina, L. D.; Jin, Z. T.; Zhang, Q. H.; Yousef, A. E. Inactivation of *Listeria monocytogenes* in milk by pulsed electric fields. *J. Food Prot*. **1998**, *61*(9), 1203−1206.

(19) Iu, J.; Mittal, G. S.; Griffiths, M. W. Reduction in levels of *Escherichia coli* O157:H7 in apple cider by pulsed electric fields. *J. Food Prot*. **2001**, *64*(7), 964−969.

(20) Evrendilek, G. A.; Zhang, Q. H.; Richter, E. R. Inactivation of *Escherichia coli* O157:H7 and *Escherichia coli* 8739 in apple juice by pulsed electric fields. *J Food Prot.* **1999**, *62*(7), 793−796.

(21) Rowan, N. J.; MacGregor, S. J.; Anderson, J. G.; Fouracre, R. A. Farish, O. Pulsed electric filed inactivation of diarrhoeahenic *Bacillus cereus* through irreversible electroporation. *Lett. Appl. Microbiol*. **2000**, *31*, 110−114.

(22) Rowan, N. J.; MacGregor, S. J.; Anderson, J. G.; Cameron, D.; Farish, O. Inactivation of Mycobacterium paratuberculosis by pulsed electric fields. *Appl. Environ. Microbiol*. **2001**, *67*(6), 2833−2836.

(23) Zhang, Q.; Molsalve-Gonzalez, A.; Barbosa-Canovas, G. B.; Swanson, B. G. Inactivation of E. coil and S. cerevisiae by pulsed electric fields under controlled temperature conditions. *Trans. ASAE* **1994**, *37*, 581−587.

(24) Jia, M. Y.; Zhang, Q. H.; Min, D. B. Pulsed electric field processing effects on flavor compounds and microorganisms of orange juice. *Food Chem*. **1999**, *65*, 445−451.

(25) Min, S.; Jin, Z. T.; Min, S. K.; Yeom, H.; Zhang, Q. H. Commercial Scale Pulsed Electric Field Processing of Orange Juice. *J. Food Sci.* **2003**, *68*(4), 1265−1271.

(26) Min, S.; Jin, Z. T.; Min, S. K.; Yeom, H.; Zhang, Q. H. Commercial Scale Pulsed Electric Field Processing of Tomato Juice. *J Agric. Food Chem*. **2003**, *51*(11), 3338−3344.

(27) Li, S. Q.; Zhang, Q. H.; Lee, Y. Z.; Pham, T. V. Effects of pulsed electric fields and heat treatment on the stability of Bovine Immunoglobulin G (IgG) in enriched soymilk. *J. Food Sci.* **2003**, *68*(4), 1201−1207.

(28) Gornall, A. G.; Bardawill, C. J.; David, M. M. Determination of Serum proteins by means of the Biuret reaction. *J. Biol. Chem.* **1949**, *177*, 751.

(29) Adler, A. J.; Greenfield, N. J.; Fasman, G. D. Circular dichroism and optical rotatory dispersion of proteins and polypeptides. In *Methods in Enzymology*, Volume 27, Enzyme structure, Part D; Hirs, C. H. W., Timasheff, S. N., Eds.; Academic Press: New York, 1973; pp 675−735.

(30) Greenfield, N. J. Methods to estimate the conformation of proteins and polypeptides from circular dichroism data. *Anal. Biochem*. **1996**, *235*, 1−10.

(31) Snatzke, G. Circular Dichroism: An Introduction. In *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, K., Woody, R. W., Eds.; Wiley-VCH: New York, 2000; p 29.

(32) Koslowski, A.; Sreerama, N.; Woody, R. W. Theoretical approach to electronic optical activity. In *Circular Dichroism: Principles and Applications*, 2nd ed.; Berova, N., Nakanishi, K., Woody, R. W., Eds.; Wiley-VCH: New York, 2000; Chapter 3, p 77.

(33) Woody, R. W. Optical properties of polypeptides in the β-conformation. *Biopolymers* **1969**, *8*, 669−683.

(34) Manning, M. C.; Illangasekare, M.; Woody, R. W. *Biophys. Chem.* **1988**, *31*, 77−86.

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