Effect of Thermal Protectants on the Stability of Bovine Milk Immunoglobulin G

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pH stability, thermal stability, and the effect of homogenization and ultrasonic treatment on the stability of bovine milk immunoglobulin G (IgG) in model systems was studied. Separated IgG (0.02 mg/mL) was found to be unstable and susceptible to denaturation when incubated at pH <4 or >10 or thermally treated at temperature >75 °C. IgG in the colostrum, on the other hand, was found to be much more stable than in whey or in PBS when thermally treated at temperatures in the range of 75−100 °C. The residual IgG content reduced more sharply with increasing heating times, and almost no IgG content was detected when IgG in PBS (0.15 M NaCl/0.01 M phosphate buffer, pH 7.0) was heated at 95 °C for 15 s, whereas the corresponding residual IgG contents in whey and colostrum were found to be 42 and 59%, respectively. For IgG in PBS heated at 95 °C for 15 s, addition of 5% fructose or maltose displayed most remarkable protection effects by raising the residual IgG content to 31%, followed by sucrose, lactose, glucose, and galactose. However, extravagant addition (>30%) to IgG in PBS led to a decline in residual IgG content. Addition of 0.4% glutamic acid and 2% glycine to IgG in PBS heated at 95 °C for 15 s also remarkably improved the residual IgG content by 13.5 and 26.7%, respectively. Glycerol and sugar alcohol, such as sorbitol, stabilized IgG during the thermal treatment.

Keywords: *Bovine milk immunoglobulin G (IgG); stability; thermal protectant; amino acid; sugar*

INTRODUCTION

Remarkable progress has been made recently in the area of active immunization, which allows testing of the efficiency and safety of several newly developed vaccines (Levine, 1991). Another possible protection of a host against a variety of infections by the use of passively derived antibodies has been considered. For example, oral administration of specific immunoglobulins from cow's milk have been found to be effective in experimental animal models as well as in clinical trials on humans against the specific enteropathogenic and enterotoxigenic *Escherichia coli* (Mietens et al., 1979; Tacket et al., 1988), rotavirus (Hilpert et al., 1987), and *Shigella flexneri* (Tacket et al., 1992). In addition, Musher et al. (1990) have reported that only 50 ng of specific antibody is sufficient to protect a mouse against a lethal dose of *Streptococcus pneumoniae*, whereas only 50 μg of that is estimated to be required to protect a child weighing 20 kg on the body weight basis against the same bacteria. Thus, cow's milk immunoglobulin is considered to possess a great potential for use in immunological supplementation of infant formulas and other foods (Goldman, 1989; Facon et al., 1993).

Colostral milk and normal milk contain approximately 50 and 0.6 mg/mL of immunoglobulins (Ig), respectively, of which ~80% has been reported to be of the IgG class (Jenness, 1988). Colostrum is limited in quantity, although surplus of colostral milk is usually treated as waste regardless of the high content of immunoglobulins therein. Normal milk or whey is a reliable source of immunoglobulins because they are available in large volumes. Recently, appropriate methods for the isolation of immunoglobulin by ultrafiltration and immobilized metal affinity chromatography (Fukumoto et al., 1994) and protein G affinity chromatography (Akerstrom et al., 1985) have been successfully developed, using cheese whey as the source of immunoglobulins. Thus, addition of IgG, separated from milk, appears to have potential for utilization in immunological supplementation of foods. However, thermal treatment is necessary during milk processing, and immunoglobulins have been reported (Goldsmith et al., 1983; Glover, 1985) to be thermally sensitive, especially when sterilization is conducted at high temperature. Thus, either modification of thermal treatment or addition of thermal protectants is considered to be important to preserve the immunoprophylactic or therapeutic potential of IgG during the thermal treatment.

In an attempt to understand the thermal stability of milk IgG before it could be used as an immunological supplement, studies on IgG in various media, such as in PBS (0.15 M NaCl/0.01 M phosphate buffer, pH 7.0), colostral whey, and colostrum, were conducted at temperatures ranging from 70 to 82 °C. The first phase of the study determined the effects of pH, buffer composition and concentration, and homogenization and ultrasonic treatment on the stability of bovine milk IgG in PBS. Subsequent studies were conducted to compare the thermal stability of IgG in PBS, colostral whey, and colostrum. Finally, the protective effects of sugars, amino acids, and sugar alcohols on the heat denaturation of IgG were evaluated.

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MATERIALS AND METHODS

Materials. Colostral whey was prepared according to the procedure described by Li-Chan et al. (1990). Bovine colostrum, collected immediately after milking, within 6 days of postpartum from the Taiwan University dairy herd, was kept frozen at −20 °C until use. Frozen colostrum was thawed in flowing tap water and subsequently centrifuged (10000*g*, 30 min, 4 °C) to remove the upper cream layer, and the pH of the colostrum was adjusted with 1 N HCl to pH 4.6. The cream-free colostrum was then kept at 40 °C for 30 min in a water bath for the ease of curd formation to prepare the colostral whey. Centrifugation was performed again to collect the clear whey, the pH of which was immediately raised to 7.0 by 1 N NaOH to protect the immunoglobulins from denaturation. Subsequently, centrifugation was repeated to remove the suspended precipitants formed during the pH neutralization. The finally collected supernatant (2.3 mg of IgG/mL) and the previously obtained cream-free colostrum (6.8 mg of IgG/mL) were used as starting materials.

Rabbit anti-bovine IgG whole serum, bovine serum IgG, *p*-nitrophenyl phosphate, agarose type IV, and alkaline phosphatase conjugated rabbit anti-bovine IgG were purchased from Sigma Chemical Co. (St. Louis, MO). Radial immunodiffusion plates were purchased from Fisher Scientific (Ottawa, ON, Canada). All other reagents were of analytical grade.

Preparation of IgG from Colostrum. IgG was prepared according to the method described by Akerstrom et al. (1985) and Fredriksson and Nilsson (1987). Protein G Sepharose 4 Fast Flow (Pharmacia, Uppsala, Sweden), packed in the column (10 × 1.0 cm i.d.) and equilibrated with binding buffer (0.02 M phosphate buffer, pH 7.0), was applied with 4 mL (∼10 mg of IgG/mL) of colostral whey and eluted with binding buffer until its absorbance at 280 nm was ∼0. Glycine buffer (0.1 M glycine, pH 2.8) was then applied to the column, and the collected effluent was neutralized with 1 M Tris-HCl (pH 9.0) to a pH of approximately 7.0 to protect IgG from denaturation. The IgG thus obtained was dialyzed against 100 volumes of PBS (0.15 M NaCl/0.01 M phosphate buffer, pH 7.0) at 4 °C for 24 h. The IgG content and total protein concentration in PBS was determined by ELISA (Kummer et al., 1992) and Bio-Rad protein assay dye reagent (Bradford, 1976), respectively. The IgG solution, thus obtained, with a recovery and purity of 97.1 and 86.6%, respectively, was freeze-dried and frozen (−20 °C). The separated IgG powder was dissolved to a final concentration of 0.02 mg/mL in PBS (pH 7.0), determined by ELISA, before being applied for the following stability studies.

pH Stability of IgG. The pH of the IgG in PBS was adjusted to 2.0−12.0 with 1 N HCl or NaOH, and then the samples were kept at 37 °C for 0−4 h. Sampling was conducted at the desired period of time, and the pH was adjusted immediately to ∼7.0 with 2 M Tris buffer (pH 7.0). The residual IgG content was determined according to an ELISA method.

Stability for Buffer Concentration. Various concentrations (0.005, 0.05, and 0.5 M) of Tris buffer and phosphate buffer were used to adjust the pH of samples to 4.2−11.0 and 4.2−9.0, respectively, and then the samples were kept at 37 °C for 1 h, before neutralization to pH 7.0 with 2 M Tris buffer (pH 7.0). The residual IgG content was then determined according to an ELISA method.

Homogenization and Ultrasonic Treatment. IgG in PBS (5 mL) was sampled in test tubes (10 × 1.1 cm i.d.) and homogenized (adapter PT-DA 1207/2, model Polytron Pt 1200, Kinematic AG, Switzerland) at 10000, 15000, or 20000 rpm for 0−30 min in an ice bath to avoid denaturation due to heat.

Ultrasonic treatment (Sonorex RK 100H, Bandelin Co., Berlin, Germany) (110 V, frequency = 35 kHz) was also administered to the same samples in microcentrifuge tubes (MT015, Kevin Science Technology Inc., Brea, CA) for 30 min in an ice bath. Sampling during both these treatments was conducted every 5 min, and the residual IgG content was determined according to an ELISA method.

Thermal Treatment of IgG Samples in Model Systems. Samples (200 μL) of IgG in PBS, colostral whey, and cream-free colostrum in disposable test tubes (40 × 3.5 mm i.d.), covered with Parafilm, were heated in temperature-controlled water baths at 63.5, 75, 85, 95, and 100 °C for 0−30 min, 0−10 min, 0−150 s, 0−60 s, and 0−60 s, respectively, to investigate the dependence of residual IgG content on temperature and time of thermal treatment. The temperature of IgG samples was measured using a thermometry system (Line Seiki, Tc-1100, Singapore). The time taken for the solutions to equilibrate to the temperature of the water bath ranged between 7 and 9 s. At specified time intervals, individual test tubes were removed from the water bath and immediately cooled in an ice−water bath. Each thermal treatment was conducted in triplicate.

Sugars, amino acids, and glycerol, usually used in food items, were added in IgG samples. In an attempt to understand the protective effect of concentrations on the IgG content, 0−50% (w/v) of glucose (G 8270), fructose (F 0127), galactose (G 0625), maltose (M 2250), or sucrose (S 8501) (Sigma Chemical Co., St. Louis, MO) was added to IgG in PBS (pH 7.0), and 0−30% (w/v) of the above sugars, except lactose (L 1768), was added to the colostrum and whey. Lactose in colostrum and whey was only 0−20% owing to its poor solubility. Amino acid, such as cystine, glutamic acid, or aspartic acid (Sigma Chemical Co.), was added to IgG in PBS to concentrations of 0−0.4%, whereas glycine was added up to 2%. Glycerol (G 6279), sorbitol (S 1876), or maltitol (M 8892) (Sigma Chemical Co.) was added to IgG in PBS to concentrations of 0−10% (w/v).

For the ease of evaluation of the thermal protection effect of these additives, IgG samples were treated at 95 °C for 15 s in a water bath.

Protein Determination. Protein content was determined according to the method described by the Bradford (1976) using Bio-Rad protein assay dye reagent. Various concentrations (100−500 μg/mL) of bovine IgG (B 8395) (Sigma Chemical Co.) were used to obtain the calibration curve.

Determination of IgG Concentration. Single radial immunodiffusion (SRID) was carried out according to the procedure described by Fukumoto et al. (1994). Rabbit anti-bovine IgG (1.0 mg/mL) was mixed with agarose type IV and PBS to make a gel. Bovine serum IgG was diluted with PBS to various concentrations (0.25, 0.5, 0.75,1.0, 1.5, and 2.0 mg/mL) to establish the standard curve for quantification of IgG in the samples. Triplicate samples were each analyzed twice for IgG by SRID.

Enzyme-linked immunosorbent assay (ELISA) (Kummer et al., 1992) was carried out to quantify the IgG contents in PBS, whey, and colostrum during the various treatments. For the analysis of thermal destruction of IgG in model systems, standard curves for ELISA were constructed using bovine serum IgG of known concentration, as determined by the Bio-Rad protein assay dye reagent, to prepare standard solutions of IgG in PBS. Fresh raw colostral whey and cream-free colostrum, the IgG concentrations of which were previously assayed by SRID, were used as reference milks for subsequent ELISA analysis of other milk samples. Triplicate samples of each temperature−time combination were analyzed for IgG by ELISA in duplicate.

RESULTS AND DISCUSSION

pH and Buffer Stability. Figure 1 presents the stability of IgG in PBS (0.02 mg/mL), the pH of which was adjusted to pH 2−12 with 0.1 N HCl or NaOH, kept at 37 °C for 0−4 h. IgG was found to be stable under neutral pH, but its content declined gradually with decreasing pH value, especially for pH <4. At pH 3, IgG content decreased by 30 and 73% in the first and second hours, respectively, and it decreased by 84% in the first hour at pH 2 (Figure 1a). This suggests that IgG is unstable at acidic pH values, which is consistent with the results reported by Kaneko et al. (1985) and Otani et al. (1991). At basic pH values, IgG was also

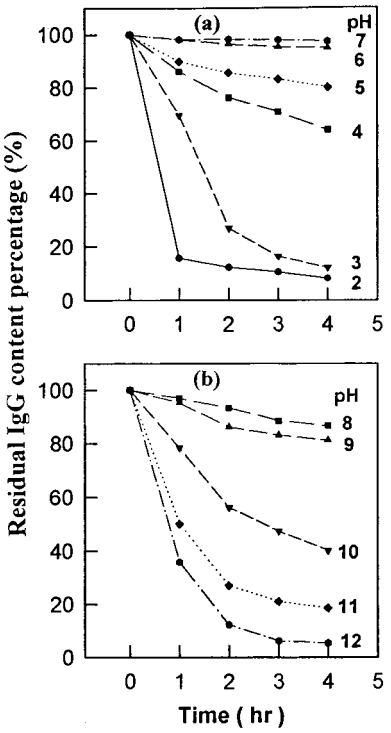

Figure 1. Stability of separated IgG (0.02 mg of IgG/mL of PBS) incubated at (a) pH 2.0−7.0 and (b) pH 8.0−12.0 at 37 °C for 0−4 h. pH of the solution was adjusted with 0.1 N HCl or NaOH. Each value is the average of three determinations.

unstable and the content declined gradually for pH >9. The IgG content was decreased to 50 and 36% in the first hour at pH 11 and 12, respectively. The effect of pH on IgG content has been reported to be due to the conformational change as a result of variation in charge on the protein surface (Shimizu, 1988), which finally results in the denaturation of IgG. Thus, strong acidity (pH 1−2) in the stomach is considered to be the major reason for causing instant denaturation of IgG upon ingestion. Adequate methods should therefore be applied to protect IgG to preserve its immunoprophylactic or therapeutic potential in the gastrointestinal tract.

Effects of various buffer systems with different compositions, concentrations, and pH values on IgG stability were also studied. As shown in Figure 2a, in the Tris buffer system, IgG stability declined remarkably when pH was far from neutral, regardless of the buffer concentration (0.005, 0.05, and 0.5 M) in the solution. Furthermore, IgG appeared to be more unstable under basic pH values than under acidic pH values, especially in high-concentration (0.5 M) Tris buffer systems with basic pH values. Increase in the ionic strength causes the decrease in IgG stability and is probably due to the rupture of solute−solute hydrogen bonds, ionic linkage, or water-mediated hydrophobic linkage (Lewin, 1974). However, the effects of pH (pH 5−9) and concentrations (0.005, 0.05, and 0.5M) of phosphate buffer system on IgG stability were not found to be remarkable (Figure 2b). The possible mechanism for the difference between Tris and phosphate buffers could be due to the higher ionic strength and higher binding ability toward metal ions of phosphate solutions (Good and Izawa, 1972).

Homogenization and Ultrasonication Treatment. Homogenization is commonly conducted during milk processing to stabilize the micelles in milk, and the stability of IgG after such treatment is of great concern. To obtain the fundamental results regarding

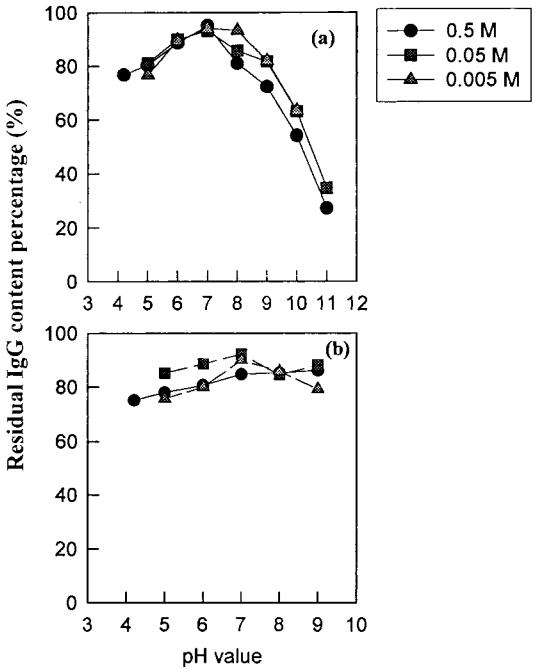

Figure 2. Stability of separated IgG (0.02 mg of IgG/mL of PBS) incubated in various concentrations of (a) Tris buffer and (b) phosphate buffer. Samples were kept at 37 °C for 1 h in the buffers before the pH was neutralized. Each value is the average of three determinations.

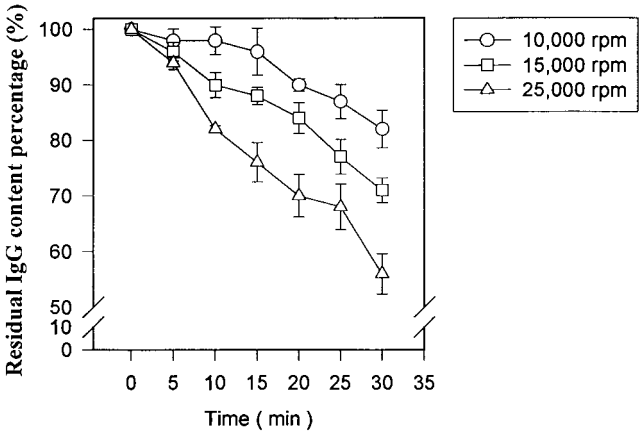

Figure 3. Stability of separated IgG (0.02 mg of IgG/mL of PBS) after homogenization for various periods of time. Each value is the average of three determinations.

IgG denaturation upon homogenization, IgG in PBS (0.02 mg/mL) (pH 7.0) was treated with a Polytron homogenizer at various speeds and the destruction of IgG was observed to be consistent with the severity of homogenizing operation. As shown in Figure 3, IgG content declined sharply with increase in the operating time, especially for homogenization at 25000 rpm. Residual contents of rabbit serum IgG and IgY (immunoglobulin in yolk) have been found to be 35 and 16%, respectively, when homogenization is conducted to prepare the multiple emulsion preparation (Shimizu and Nakane, 1995). Therefore, the shearing force is considered to be responsible for IgG denaturation, as for rennet, catalase, and carboxypeptidase (Ehrlich, 1978).

With regard to the ultrasonic treatment (Figure 4), the content of IgG was found to be decreased by an

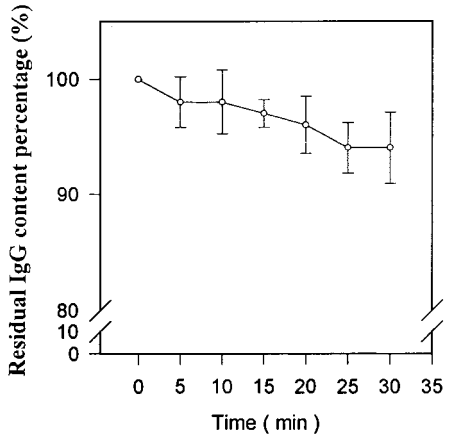

Figure 4. Stability of separated IgG (0.02 mg of IgG/mL of PBS) after ultrasonic treatment (frequency = 35 kHz) for various periods of time. Each value is the average of three determinations.

average of 7% after 30 min of treatment. Therefore, the ultrasonic vibration is unfavorable for the stability of IgG.

Thermal Stability. Thermal treatments including pasteurization, sterilization, evaporation concentration, and spray-drying are generally performed during milk processing. Thus, the effects of heating temperature and time on the content of IgG in PBS, whey, and colostrum are required to be properly understood. Cream-free colostrum, whey, and IgG were heated in a model system at 63.5, 75, 85, 95, and 100 °C in water baths for different periods of time, and the subsequent IgG content was determined according to an ELISA method. As shown in Figure 5, when the samples were pasteurized (63.5 °C) for 1800 s, the residual IgG content decreased in the order colostrum > whey > PBS. However, when the temperature was raised to 75 °C (HTST) and the treatment lasted for 600 s, the residual IgG content in PBS was decreased by 44%, compared to ~20% loss in colostrum and whey. The components other than IgG in whey are hence considered to be effective in preventing IgG from being denatured. At higher temperature (Figure 5c,d), the residual IgG content reduced more sharply with increasing heating times. Almost no IgG content was detected when the IgG in PBS was heated at 95 °C for only 15 s, whereas the corresponding residual IgG contents in whey and colostrum were found to be 42 and 59%, respectively. At 100 °C, the protective effect of the coexistent components on IgG heat denaturation became insignificant, and consequently the IgG content was reduced to nearly zero when any of these samples were heated for 15 s. IgG is thus highly susceptible to heat denaturation during thermal treatment, especially at temperatures ≥95 °C. Similar protective effects of milk proteins on serum IgG have been reported (Li-Chan et al., 1995) having *D* values that ranged from 90, 200, and 170 s at 80 °C to 25.5, 27.2, and 32.8 min at 72 °C for IgG in PBS, boiled milk, and UHT milk, respectively.

Sugars, which present a strong protection effect against freezing and thermal treatments in surumi processing, have also been reported (Shimizu et al., 1994) to be effective in protection of thermal denaturation of IgY. Hence, some monosaccharides and disaccharides were selected to investigate their influence on IgG stability during thermal treatment, and the results are shown in Figure 6. For IgG in PBS heated at 95 °C

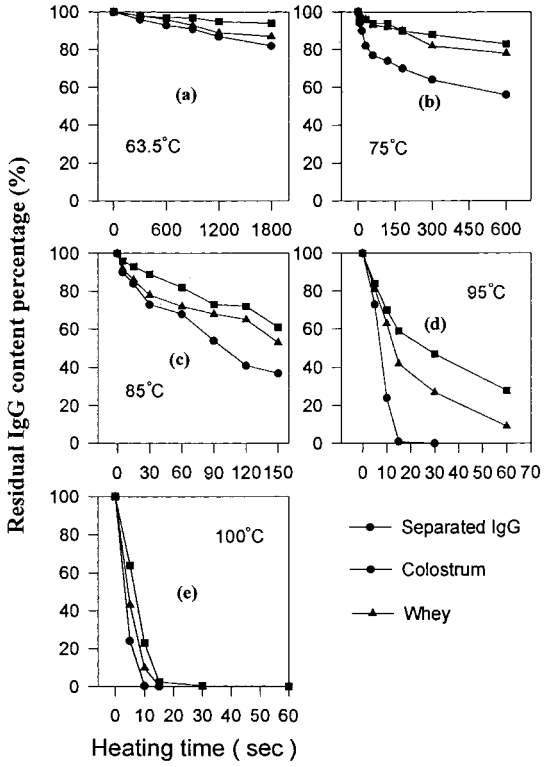

Figure 5. Stability of separated IgG (0.02 mg of IgG/mL of PBS), colostrum (6.8 mg of IgG/mL), and colostral whey (2.3 mg of IgG/mL) after thermal treatment at 63.5, 75, 85, 95, and 100 °C for various periods of time. Each value is the average of three determinations.

for 15 s (Figure 6a), a small addition (5%) of fructose or maltose displayed a most remarkable protection effect, by raising the residual IgG content to 31%, followed by sucrose, lactose, glucose, and galactose in that order, whereas only 4.2% residual content was observed in the absence of sugars. Higher content of sugars presented a more remarkable thermal protection effect. Among the sugars tested in this study, maltose was found to be the most effective. However, addition of up to 30% maltose did not show much significant improvement over that obtained by 5%. On the other hand, extravagant addition (>30%) leads to a decline in residual IgG content. From the results in Figure 6a, it can be seen that the addition of 5% maltose or fructose, which raises the residual content to ~30%, is very effective in protecting milk IgG from thermal denaturation. For the cream-free colostrum, the residual IgG content was observed to be ~60% in the absence of sugars when treated at 95 °C for 15 s; however, 5% addition of maltose or fructose stabilized the IgG by increasing its residual content to 73% (Figure 6b). Higher contents of sugars also increased the residual IgG content, similar to that shown in Figure 6a, and addition of 30% fructose or maltose exhibited a remarkable protective effect against thermal denaturation by raising the residual content to 77%. From Figure 6c, the protective effect of sugars can be seen to be between those of PBS and colostrum, and both maltose and fructose acted as the most powerful protectants against thermal denaturation of IgG in all of the IgG samples.

The protective effect of sugars on thermal denaturation of immunoglobulin have been pointed out to be due to the enhancement of hydrophobic interactions inside the protein molecules in the presence of sugar, which consequently stabilize the IgG structure (Shimizu

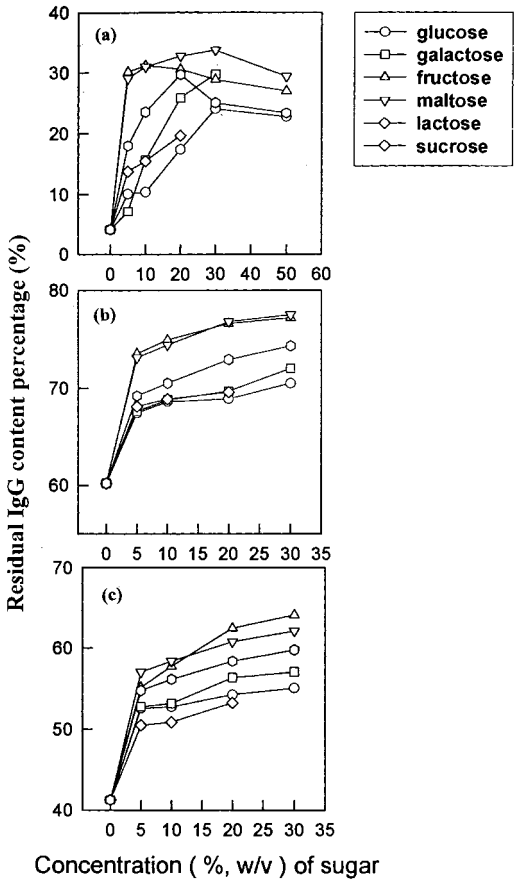

Figure 6. Influence of sugars on (a) separated IgG (0.02 mg of IgG/mL of PBS), (b) colostrum IgG (6.8 mg of IgG/mL), and colostral whey IgG (2.3 mg of IgG/mL) heated at 95 °C for 15 s. Each value is the average of three determinations.

et al., 1994). Increased hydrophobic interactions in the protein molecules (Back et al., 1979) and changes in preferential solvation of protein molecules (Timasheff, 1993) in sugar solution facilitate the stabilization of proteins during thermal treatment. The decreasing protective effect of sugar on the heat denaturation of Antarctic krill myofibrillar protein is in the order of lactitol, sucrose, sorbitol, and glucose, almost proportional to the number of hydroxy groups of sugar or sugar alcohol molecules (Ooizumi et al., 1983). The dissolution of sugar or sugar alcohol in the medium alters the properties of the medium and possibly results in the changes of properties of protein in the same medium (Ooizumi et al., 1981, 1983). However, the thermal protective effect of sugar or sugar alcohol on protein is related not only to the number of hydroxy groups but also to the structure, such as the orientation of the hydroxy groups, of the sugar or sugar alcohol molecule (Ooizumi et al., 1981).

Amino acids, such as cystine, glycine, glutamic acid, and aspartic acid, also displayed protective effect against thermal denaturation to some extent but not as remarkable as that of sugars. As shown in Table 1, residual IgG content was increased to 14.2 and 7.5% with a small addition of 0.2% glutamic acid and aspartic acid, respectively, for IgG in PBS heated at 95 °C for 15 s. Addition of higher amounts (0.4%) of these two amino acids did not exhibit proportional protective effect. Cystine almost did not show any influence on stabilizing IgG, whereas 2% glycine, which has been reported (Ou-Yang, 1997) to be an effective protectant for IgY against heat denaturation, was found to be effective in protecting IgG by raising the residual content to 26.7% during the thermal treatment of IgG in PBS at 95 °C for 15 s. The thermal protective effect of amino acids against the denaturation of mackerel myofibrils becomes lower in the order sodium glutamate, sodium aspartate, glycine = alanine = β-alanine, proline, glutamine, aspargine, whereas arginine, histidine, and glycylglycine are ineffective (Ooizumi et al., 1982). The effective functional group against the thermal denaturation of myofibrils is considered to be the extra carboxyl group of the acidic amino acid (Ooizumi et al., 1982). Furthermore, the number of the carboxyl group of carboxylic acids decides largely their protective effect on thermal denaturation of chub mackerel myofibrils (Ooizumi et al., 1984). However, in the present study, glycine was found to be more effective than acidic amino acids in protecting IgG from thermal denaturation. The mechanism of such a protective effect, although no reference is available, could be due to the strong and specific interaction between those two kinds of molecular structures.

Table 1. Effect of the Addition of Amino Aicd on the Residual Activity of Separated IgG (0.02 mg/mL PBS) Heated at 95 °C for 15 s

concn (%)	residual IgG content percentage[a]			
	cystine	glutamic acid	aspartic acid	glycine
0.0	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1
0.1	1.2 ± 0.3	8.2 ± 1.4	5.9 ± 0.6	7.8 ± 1.2
0.2	1.5 ± 0.2	14.2 ± 2.1	7.5 ± 0.4	13.4 ± 0.7
0.4	1.1 ± 0.2	13.5 ± 1.8	6.1 ± 1.5	19.3 ± 1.4
2.0	ND[b]	ND	ND	26.7 ± 1.3

[a] Average ± standard deviation of triplicate analyses. [b] ND, not determined.

Table 2. Effect of the Addition of Glycerol and Sugar Alcohol on the Residual Activity of Separated IgG (0.02 mg/mL PBS) Heated at 95 °C for 15 s

concn (%)	residual IgG content percentage[a]		
	glycerol	sorbitol	maltitol
0	4.1 ± 0.2	4.1 ± 0.2	4.1 ± 0.2
5	38.8 ± 1.7	12.8 ± 0.8	11.4 ± 0.2
10	42.7 ± 1.4	24.5 ± 1.6	18.3 ± 1.3

[a] Average ± standard deviation of triplicate analyses.

Oligosaccharides, gums, and sugar alcohols were all added to the IgG samples in the pretest to examine their effect on thermal denaturation of immunoglobulin. Among the samples tested, only glycerol and sugar alcohols, such as sorbitol and maltitol, were found to be effective. Addition of 5% glycerol was found to be capable of raising the residual IgG content to 38.8%, whereas only 4.1% content was noted in the absence of any additive (Table 2). Moreover, higher levels of glycerol stabilized IgG more remarkably. In the presence of 10% glycerol, residual IgG content was observed to be 42.7%, whereas only 24.5 and 18.3% residual contents were noted in the presence of the same amounts of sorbitol and maltitol, respectively (Table 2). Glycerol is thus found to be the most effective protectant for IgG against thermal denaturation and is worthy of further investigation.

Glycerol shows less protective effect on the heat denaturation of mackerel myofibrils (Ooizumi et al., 1981); however, it was observed to be a strong protectant against the thermal denaturation of IgG in the present study. The increased thermal stability of proteins and enzymes in the presence of glycerol has been considered to be due to the increased hydrophobic interactions inside the protein molecules (Back et al., 1979) and the

enhancement of the structure of the medium or of the solvation layer of the protein (Timasheff, 1993). Gekko and Timasheff (1981) have proposed that proteins in the glycerol−water mixture tend to minimize the surface of contact between proteins and glycerol and, thus, stabilize the native structure of proteins. The extent of thermal stabilization of proteins by glycerol could be related to the extent of molecular interactions between proteins and glycerol.

Conclusions. IgG was separated and purified from bovine colostrum with protein G Sepharose 4 Fast Flow affinity chromatography, and the change in its content during various thermal treatments with or without the addition of protectants was examined. IgG in the cream-free colostrum was observed to be more stable compared to that in PBS and whey, when heated at temperatures ranging from 63.5 to 95 °C. Certain kinds of sugars, amino acids, sugar alcohols, and glycerol were found to be effective in stabilizing IgG; however, the respective mechanisms are still incompletely understood. Glycine has been reported to exhibit only a little or no effect toward protecting fish myofibrillar protein against thermal and freezing operations (Noguchi and Matsumoto, 1971), but was found to be much more effective than sodium glutamate in stabilizing the bovine milk IgG, in the present study, during the heat treatment. Maltose, fructose, and glycerol also help to preserve the IgG content during thermal processing (HTST), although their influences on protein stabilization are different from those reported by Ooizumi et al. (1981). The addition of 5% fructose or maltose or 2.0% glycine stabilized IgG remarkably when heated at 95 °C for 15 s (Figure 6). This suggests that the use of low concentrations of those thermal protectants could be practical and would contribute greatly to the retention of therapeutic potential of immunoglobulins in cow milk.

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