9146　食品蛋白質加熱ゲル形成における食塩の効果

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【目的】ホエータンパク質は重要な加工特性の一つである加熱ゲル形成性を有する。ゲル形成の主因子はβーラクトグロブリン（βーＬｇ）であることが知られているが、その他のタンパク質の寄与についてはあまりはっきりしていない。そこで、αーラクトアルブミン（αーＬａ）のゲル形成における寄与を明らかにするためにαーＬａとβーＬｇの混合比を変えて種々の食塩濃度とグルタチオン（ＧＳＨ）濃度において加熱することにより形成するゲルについて調べた。

【方法】αーＬａとβーＬｇはホエータンパク質単離物より調製した。それぞれの調製物の純度をゲルロ過法により調べ、αーＬａとβーＬｇの混合比が２：８、５：５、８：２となるようにそれぞれ混合し、ゲル形成用試料とした。これに食塩濃度25-150mMの６レベル、還元型グルタチオン濃度0-75mMの４レベルにおいてタンパク質濃度８％、pH6.3 とし、80℃、15分間加熱してゲルを形成させた。レオメーターによりゲル強度を測定し、得られたデータをＳＡＳプログラムを用いて解析した。

【結果】αーＬａとβーＬｇ混合比２：８の試料ではいずれの食塩濃度においてもＧＳＨ無添加のものがゲル強度が高く、25mMの添加で若干の減少がみられ、50mM以上で著しく低下した。一方、混合比５：５ではＧＳＨ無添加においてゲル形成が見られず、25mMＧＳＨの添加で強固なゲルを形成した。しかしながら、より多量のＧＳＨを添加すると逆にゲル強度が低下した。混合比８：２においても混合比５：５と同様の傾向がみられた。食塩はいずれの混合比においても100mM 前後の添加によりゲル強度を増加させた。ゲル強度の対数に対して食塩濃度とＧＳＨ濃度を関数とした重回帰分析により次のような最適条件が得られた。すなわち、混合比２：８では食塩86mM、ＧＳＨ０mM、混合比５：５では食塩116mM、ＧＳＨ43mM、混合比８：２では食塩83mM、ＧＳＨ45mMであった。　以上の結果よりαーＬａの割合が多くなると適量の食塩とＧＳＨの存在により強固なゲルが形成されることが明かになった。

Effect of Salt on Heat-induced Gelation of Food Porteins

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SUMMARY

The optimum heat-induced gelation of the mixed α lactalbumin and β-lactoglobulin has been investigated at different concentrations of glutathione and NaCl by the gel strength determination. The gel strength was determined under the gelling conditions of pH 6.3, protein concentration 8% and heating at 80°C for 15 min. The optimum gel strength of the mixed α-lactalbumin and β-lactoglobulin in ratio 2:8, 5:5, and 8:2 was obtained at glutathione 0 mM and NaCl 86 mM, glutathione 43 mM and NaCl 116 mM, and glutathione 45 mM and NaCl 83 mM respectively. A multiple regression analysis showed the significant contribution of NaCl and glutathione ($p<0.01$) to form the gel. In case of the gel formation influenced by glutathione, it was supposed that the glutathione reacted with intramolecular disulfide bonds in the α-lactalbumin and β-lactoglobulin, and resulted in their conformational changes. Moreover, the gel-forming ability of α-lactalbumin and β-lactoglobulin assisting each other in the mixed proteins gelation.

INTRODUCTION

Interest in the use of whey protein and theirs protein components as constituents in food product has grown steadily in recent years. The major protein components of whey protein are β-lactoglobulin and α-lactalbumin, which comprises 50% and 12% respectively, of the total whey protein (Evans and Gordon, 1980), and are probably important in the physico-chemical properties of whey protein.

An important function of whey proteins in food system is gelation. This phenomenon involves the formation of a three-dimensional matrix mainly through interprotein hydrogen bonding and allows the immobilization of water within the gel structure

(Gosset et al, 1984). Several structural properties, electrostatic interaction, hydrophobic interaction and disulfide bond formation are also considered responsible for the gelation of proteins. Protein content, pH, ionic strength, solvent composition, and reducing agents are some of the factors influence on the gelation of whey proteins (Schmidt et al, 1979; Mulvihill and Kinsella, 1987; Wang and Damodaran, 1990). Few studies have been published on heat-induced gelation of individual whey proteins. Nevertheless, quantitative information regarding the interrelationships between the factors and their influence on gel structure, gel strength, and other gel textural properties is very limited and much needed.

The process of proteins gelation involves the structural changes, which is characterized by the progression from native protein to denatured protein to gel (Ziegler and Foegeding,1990). Kim et al (1989) showed that β-lactoglobulin was highly correlated with gel strength. However, it is still not clear the importance of the structural changes in α-lactalbumin in the course of the gel formation of whey protein. The presence of disulfide bonds in α-lactalbumin, and both of disulfide bond and sulfhydryl groups in β-lactoglobulin (Brunner, 1977; Walstra and Jennes, 1984), would undergo a variety of reaction which are of importance in proteins structural changes during the process of heat-induced gelation.

The purpose of the present study was to determined the effect of NaCl and glutathione on the gel strength of heat-induced gelation of the mixed α-lactalbumin and β-lactoglobulin.

MATERIALS AND METHODS

Materials.

Glutathione (GSH)-reduced form (G-4251, Lot 31H0197) and Bovine Serum Albumin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ethylenediaminetetraacetic Acid (EDTA) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Fractionation of α-lactalbumin and β-lactoglobulin.

The obtained WPI was fractionated to be α-lactalbumin (α-La) and β-lactoglobulin (β-Lg) according to the method of Armstrong

et al (1967) with some modifications. α-La was dialyzed against distilled water thoroughly and was concentrated to 10% by an ultrafiltration with a Advantec UK10 membrane. β-Lg solution was freeze dried, after dialyzing against distilled water. Then it was dissolved to 10% solution in 0.9% NaCl and dialyzed against distilled water thoroughly. The precipitate was collected by vacuum filtration, and dissolved in 0.9% NaCl solution. The obtained β-Lg finally was concentrated to 10% by ultrafiltration, after dialyzed against distilled water.

Determination of protein content was performed by the method of Lowry et al (1951) using a bovine serum albumin as standard proteins.

The purity of α-La and β-Lg was checked using polyacrylamide gel electrophoresis (PAGE) and gel filtration Sephacryl S-100 column (20 x 60 cm) with HCl-imidazole at pH 6.3 and ionic strength 0.043 as eluent buffer.

Gel preparation.

The α-La and β-Lg solutions at pH 6.3 were mixed to be the ratio of 2:8, 5:5, and 8:2. NaCl concentration were adjusted to 25, 50, 75, 100, 125 and 150 mM; and GSH concentration were also adjusted to 0, 25, 50 and 75 mM. The final protein content of the mixed solution was 8 %. Each 5 ml of the mixed solution was filled in the small petridish (diameter 2.2 cm) covered with a silicone plate and a glass plate , then were heated in the waterbath at 80^{o}C for 15 min.

Gel strength determination.

The procedure of gel strength determination was carried out according to the method of Hayakawa and Nakamura (1986). Gel strength was measured by using a Rheometer (Yamaden RE-3305 Rheoner) with a 0-50 g load cell. A gel sample prepared in the small petridish was place on a stage moving upward at a speed of 0.5 mm/sec. The samples were tested with a special probe with 0.36 x 0.03 cm at the edge. Gel strength was expressed as the force in dyne/cm^{2} applied to probe edge when the surface yield point was reached.

Data analysis.

Data analysis were performed using the statistical analysis

system (SAS) program with a FACOM M-780/20 computer. Multiple regression and response surface plots have been employed to investigate the factors affecting heat induced gelation of the mixed proteins. To estimate regression coefficients for the combined effects of NaCl and GSH on gel strength according to the model: $y = b_o + b_1X_1 + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$. The average of two replicate trials were fitted to the model.

RESULTS AND DISCUSSION

Effect of GSH

Table 1 showed the gel strength at different levels of NaCl and GSH. The gel strength of the mixed α-La and β-Lg in ratio 2:8 was high at GSH 0 mM, and slightly decreased at GSH 25 mM, then drastically decreased with increasing concentration of GSH. In contrast, a different pattern was obtained from the gel of the mixed α-La and β-Lg in ratio 5:5 and 8:2. They did not form the gel in the absence of GSH, but formed a hard gel at GSH 25 mM, and they decreased at excess amount of GSH.

In case of the absence of GSH, β-Lg seemed play a more important role in the gel formation of the mixed proteins than α-La. The gelation of β-Lg and other globular protein, were the result of major conformational changes induced by the thermal protein denaturation. On heating, β-Lg undergoes denaturation and aggregation (Bottomley et al 1990). Moreover, Paulsson et al (1986) investigated that gelation of β-Lg started in the range 75-80^{o}C. This condition corresponded with the present study.

α-La did not form a heat-induced gel of concentration up to 20% (Paulsson et al, 1986). The addition of GSH was required to form the α-La gel, but an excess of GSH weakened the gel. It is supposed that the concentration of GSH corresponded to the reactive groups of α-La and β-Lg. The GSH will reduce the intramolecular disulfide bonds to sulfhydryl groups (Jocelyn, 1972; Haschemeyer and Haschemeyer, 1973). Reduction of disulfide bonds involves the conformational changes and exposure of hydrophobic regions which promotes the interaction of proteins and led to the gel formation (Hayakawa and Nakamura, 1986; Li-Chan and Nakai, 1991). The reacted GSH is limited with the

equivalent amounts of disulfide bonds. Hayakawa and Nakamura (1986) suggested that the partial cleavage of the disulfide bonds in lysozyme was presumably better for the formation of a hard gel than full cleavage. This suggestion corresponded to the present study. An excess amounts of GSH resulted a weak gel of α-La, and also β-Lg.

The structure of α-La is stabilized by the presence of four disulfide bonds. The GSH reduced the disulfide bonds to sulfhydryl groups that may responsible for destabilised of α-La structure. This process of reduction happened slowly during heating and more rapidly after denaturation (Jocelyn, 1972). It was supposed that the gel formation of α-La is similar to the gel formation of lysozyme which has been investigated by Hayakawa and Nakamura (1986) and Li-Chan and Nakai (1991). This suggestion based on the fact that the lysozyme and α-La are considered to have the similar structure, and the four disulfide bonds of them are located identically (Walstra and Jennes, 1984).

Figure 1 showed the three dimensional surface plots for the gel strength of the mixed proteins as functions of NaCl and GSH concentrations. These plots indicated an increasing trend in the maximum gel strength as increasing the proportion of α-La of the mixed proteins. The maximum gel strength of the mixed α-La and β-Lg in ratio 2:8, 5:5, and 8:2 was 120, 294, and 342 x 10^4dyne/cm^2 respectively (Table 1). It was supposed that the gel-forming ability of β-Lg not so strong as that of α-La. Therefore, the β-La present in large amounts of the mixed proteins would form the best hard gel.

Combination effects of GSH and NaCl

The gel strength for all of the mixed proteins ratio increased at NaCl 75, 100, and 125 mM (Table 1). These results reflected the fact that gel strength of the mixed proteins was influenced by salt concentration. Mulvihill and Kinsella (1988) suggested that salt concentration is one of the factors influencing the heat-induced gel formation of whey proteins. Moreover, Khun and Foegeding (1991) found that increasing levels of NaCl caused a sharp increased in the shear stress of the WPI gels to a maximum 50-75 mM, but it caused a decreased in shear strain to a maximum

with 150 mM. Table 1 showed that low gel strength was obtained at both low and high NaCl concentrations. This results in accordance with the argumentation of Ziegler and Foegeding (1990) that too little salt inhibits protein-protein interactions and an excess of salt produces protein precipitate. The gel formation may be influenced by the critical balance between attractive and repulsive forces of proteins which in turn depend on salt concentration (Mulvihill and Kinsella, 1988), and the effect of ion-specific on hydrophobic interactions in the protein structure (Wang and Damodaran, 1991).

Under the conditions of no GSH and at any concentrations of NaCl, the high proportion of α-La of the mixed proteins did not formed the gel, but the high proportion of β-Lg formed the gel(Table 1). It means that the NaCl alone did not influence the gelation of α-La in case of no GSH. Furthermore, the combination of GSH and NaCl effected the gelation of α-La and β-Lg.

Multiple regression coefficient for prediction of the logarithmic gel strength of the mixed proteins are presented in Table 2. A regression equation could be calculated using the regression coefficient for each parameter. The coefficient of determination (R^2) provides a measure of the correlation between predicted and observed response values. The value of R^2 for the mixed α-La and β-Lg in ratio 2:8, 5:5, and 8:2 was obtained 0.91, 0.83, and 0.74 respectively. These values showed that the regression equations explained a significant contribution of GSH and NaCl ($p < 0.01$) to form the gel.

The optimum conditions for the gel strength of the mixed α-La and β-Lg in ratio 2:8, 5:5, and 8:2 were obtained at GSH 0 mM and NaCl 86 mM, GSH 43 mM NaCl 116 mM, and GSH 45 mM and NaCl 83 mM, respectively (Table 2).

Figure 2 shows the effects of mixing ratio of α-La and β-Lg on their gel strength in the presence of 25 mM GSH or absence of GSH. The gel strength was also determined at different protein contents, that is, some portion of α-La or β-Lg in the mixed proteins solution was replaced by distilled water.

In case of no GSH, a maximum gel strength of 253 x 10^4dyne/cm^2 was obtained for the 8% β-Lg (in the absence of α-La). The gel

strength decreased with decreasing the proportion of β-Lg , and no gelation was observed in the mixed α-La and β-Lg in ratio 5:5. However, when β-Lg alone, the gel strength drastically decreased with decreasing the concentration. The gel strength of α-Lg at 6.4% was one-hundredth of that of mixed protein (α-La, β-Lg:1.6%,6.4%). Even if the β-Lg has a more dominant role, the α-La also can promote the gel formation of the mixed proteins. α-La was considered to has negative influence on the gelation properties of other proteins (Paulsson et al, 1986), but, it can be suggested that α-La play the significant role on the heat-induced gelation of mixed protein in the present studies.

In the presence of 25 mM GSH resulted the high gel strength for the large amounts of α-La of the mixed proteins. A maximum gel strength of 375 x 10^4dyne/cm^2 was obtained in the 8% α-La. The gel strength slightly decreased for the mixed α-La and β-Lg in ratio 6:4, and then decreased with decreasing the amounts of α-La. Conversely, when α-La alone, the gel strength sharply decreased with decreasing the concentration. The gel strength of α-La at 6.4% was one-fifth of mixed protein (α-La, β-Lg:6.4%,1.6%). Of this phenomenon indicated that in the presence of GSH, α-La may play a more important role in the gel formation of the mixed proteins than β-Lg, and β-Lg can assist the gel formation. It is considered that α-La and β-Lg can interact each other in the course of gelation both in the presence and absence of GSH. It is under investigating how α-La and β-Lg interact.

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Table 1 Gel strength of the mixed proteins as functions of NaCl and GSH

Treatment	NaCl	GSH	Gel strength x 10^{-4} dyne/cm^2		
			α:β = 2:8	α:β = 5:5	α:β = 8:2
1	1	1	87	0	0
2	1	2	79	125	112
3	1	3	9	44	36
4	1	4	7	31	28
5	2	1	145	0	0
6	2	2	127	181	188
7	2	3	18	65	41
8	2	4	5	41	33
9	3	1	178	0	0
10	3	2	154	181	202
11	3	3	36	74	32
12	3	4	9	53	30
13	4	1	134	0	0
14	4	2	101	212	335
15	4	3	15	76	36
16	4	4	6	49	35
17	5	1	120	0	0
18	5	2	99	294	342
19	5	3	16	76	37
20	5	4	6	22	19
21	6	1	115	0	0
22	6	2	80	273	246
23	6	3	25	71	30
24	6	4	11	21	15

Table 2 Multiple regression models for prediction of the logarithmic gel strength

Dependent variable	α:β = 2:8	α:β = 5:5	α:β = 8:2
Constant	2.17	-3.03	-3.03
NaCl	0.12	0.35	0.19
GSH	-0.11	3.35	3.54
NaCl x NaCl	-0.02	-0.02	-0.02
GSH x GSH	-0.07	-0.56	-0.62
NaCl x GSH	0.01	-0.07	-0.03
R^2	0.91	0.83	0.74
Probability	0.0001	0.0001	0.0001

Optimum condition	NaCl		GSH	
	level	(mM)	level	(mM)
α:β = 2:8	3.44	86	1	0
α:β = 5:5	4.64	116	2.72	43
α:β = 8:2	3.32	83	2.8	45

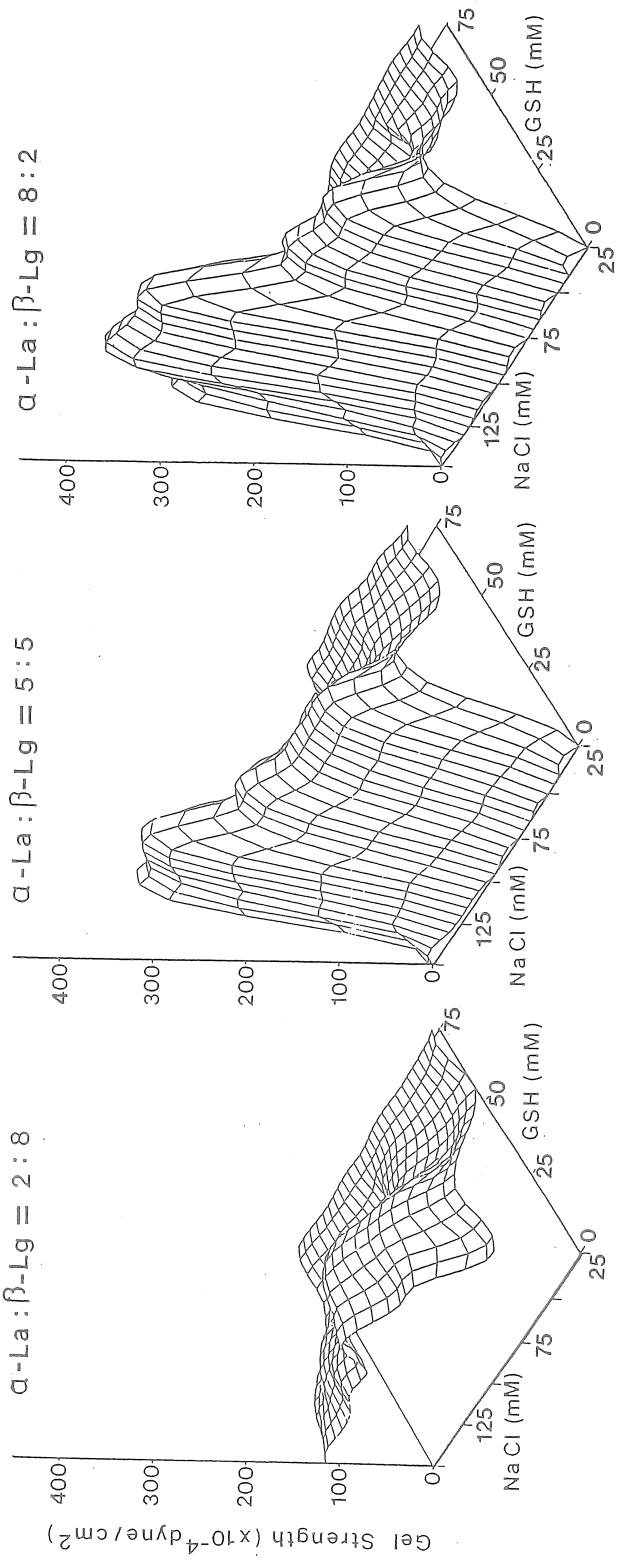

Fig.1 Three Dimensional Surface Plots of Gel Strength of the mixed proteins as functions of NaCl and GSH concentration.

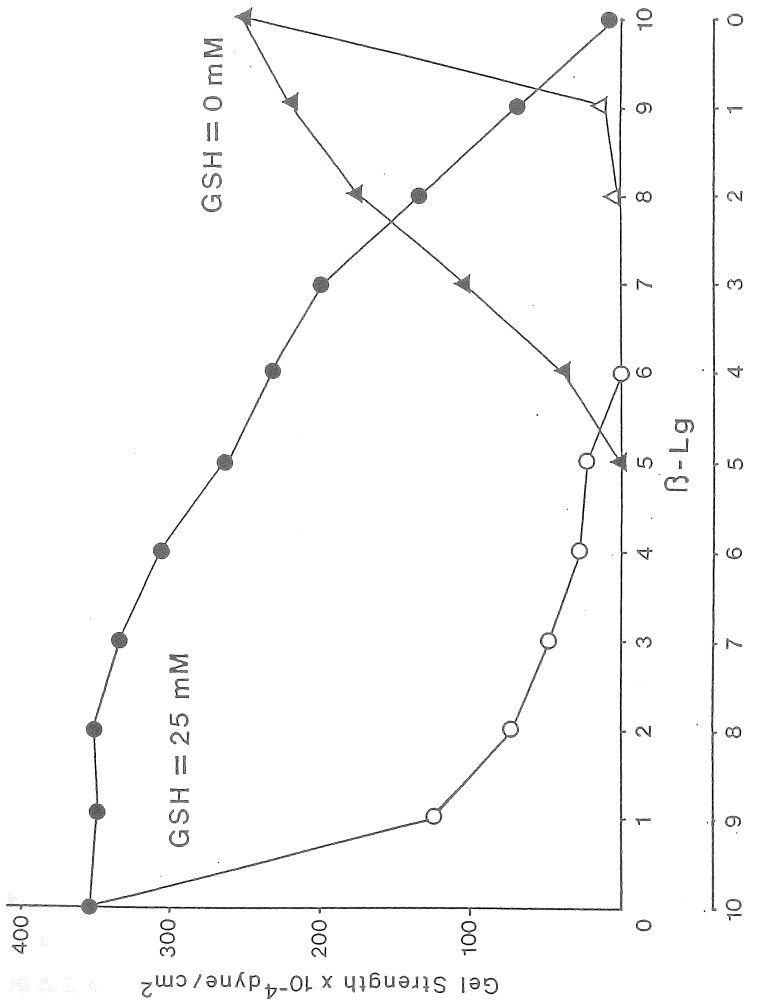

Fig.2 Effects of mixing ratio of α-La and β-Lg on gel formation with 25 mM GSH (●,○) or without GSH (▲,△). Gelling conditions: heating at 80°C, 15 min; pH 6.3; NaCl 86 mM; protein 8%.
○ = α-La alone. △ = β-Lg alone.