Excellent Gelation of Egg White Preheated in the Dry State Is due to the Decreasing Degree of Aggregation

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Molecular weights of heat-induced soluble aggregates of a diluted solution of dry heated egg white proteins (DEW) were determined by high-performance gel chromatography using a TSK gel G3000SW column. Elution was monitored by a low-angle laser light scattering photometer and a differential refractometer. The average molecular weight of DEW aggregate decreased greatly with increases of preheating time in the dry state and the gel strength greatly increased. The molecular weight distribution curve shifted toward lower molecular weight and became sharper with increases in preheating time in the dry state. In addition, the expanded structure of polymer formed from preheated DEW was predicted from the plots of molecular weight vs retention time of heat-induced DEW aggregates. Good correlation was observed between decreases in molecular weight and increases in gel strength of dry-heated DEW in various gelation temperatures.

INTRODUCTION

Intermolecular interaction of denatured molecules is a common event involved in heat-induced gelation of egg white proteins. Many investigators have been concerned with elucidating the mechanism of thermal aggregation (Holme, 1963; Seideman et al., 1963; Nakamura et al., 1978; Hegg et al., 1979; Shimada and Matsushita, 1980; Egelandsdal, 1980; Ma and Harwalker, 1987; Kato and Takagi, 1987, 1988) or gelation (Kitabatake et al., 1987, 1988; Hatta et al., 1986; Hegg et al., 1982; Kato et al., 1989, 1990) of egg white proteins. Protein gels may be defined as three-dimensional matrices or networks in which polymer–polymer and polymer–solvent interactions occur in an ordered manner resulting in the immobilization of large amounts of water by a small proportion of protein (Flory, 1974; Hermansson, 1979; Harwalker, 1985; Tombs, 1970, 1974). The mechanisms underlying the formation of the three-dimensional networks of protein gels are not fully understood and have not been systematically studied. The overall gelation process requires that the proteins unfold or undergo some conformational changes initially and that the second aggregation step proceeds relatively more slowly than the first to allow the denatured protein molecules to orient themselves and interact at specific points, thus forming a three-dimensional network (Hermansson 1979). On the other hand, when aggregation occurs very rapidly, a coagulum is obtained. Coagulum occurs essentially when random protein–protein interactions predominate to form large molecular weight aggregates.

Our recent papers have revealed that the gel strength of dried egg white proteins (DEW) greatly increases with increases in preheating time in the dry state (80 °C). This phenomenon is suitable for the elucidation of the gelation mechanism of proteins, because various conformational states can be obtained by using the same protein. When DEW is preheated in the dry state, stability significantly decreases, resulting in the formation of a strong and stable three-dimensional gel network. However, there is no information regarding the molecular size required for gelation. It should be of great interest to elucidate the relationship between gel strength and extent of aggregation of egg white proteins upon heat-induced gelation.

The present approach will introduce a better understanding of the influence of dry denaturation on the gelation–aggregation relationship of egg white proteins. Particularly, the development of the low-angle laser light scattering technique makes it possible to obtain a precise value of the molecular weight of soluble aggregate (progel).

MATERIALS AND METHODS

Dried egg white (DEW), spray-dried at 60–70 °C after decarbohydrate treatment, was provided by Q. P. Corp., Tokyo.

Heat treatment of DEW was done as follows: 5 g of DEW was put in a test tube, which was tightly sealed and then incubated at 80 °C for various periods of time (days) in the dry state (7.5% moisture). As soon as the sample was heated to a given time, the tube was taken from the incubator and cooled at room temperature (25 °C), and subsequently thermal gelation was carried out for different percentages of protein (see below). Consequently, protein concentration was adjusted spectrophotometrically. However, we found that the absorbance values at 280 nm of the samples before and after filtration were essentially the same.

The thermal gelation of dried egg white proteins was done by the method described previously (Kato et al., 1989). Ten percent heat-treated dry powdered egg white solution was made to determine its gel strength. Of this solution, 4 mL was put into an aluminum tube, and the tube was tightly sealed. The gelation was carried out in a water bath at a controlled temperature of 80 °C for 20 min. After a given time, the tubes were taken from the hot bath and cooled at room temperature (25 °C) for 30 min. The gel strength was immediately measured by using a tensility tester (Tensilon/UTM-II, Toyo Baldwin Co.). The gel strength was expressed as breaking stress (g) exerted by a cylindrical plunger with a flat section (0.5-cm diameter).

Heat-induced DEW aggregation was done as follows: Diluted DEW solutions (0.15% native of dry heated in water) were passed through a membrane filter (0.22 μm), with subsequent adjustment of protein concentration spectrophotometrically. Two milliliters of DEW solution was put into a test tube, and the tube was immersed in a water bath kept at a certain temperature (80 °C, except in Figure 7, which was at various temperatures) for 20 min and subsequently cooled at 25 °C for 30 min. The DEW solution thus prepared was applied to a high-performance liquid chromatography system, consisting of a TSK gel G3000SW column (Toyo Soda Co.; 0.75 × 30 cm), at a flow

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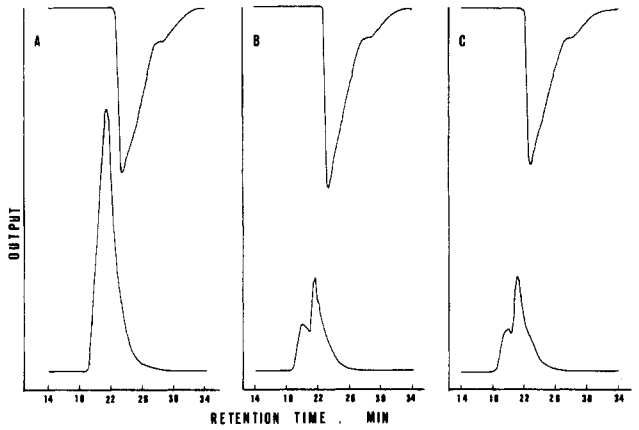

Figure 1. Elution patterns of heat-induced DEW aggregates obtained with a low-angle laser light scattering photometer (bottom) and a precision refractometer (top). (A) Nonpreheated, (B) preheated for 5 days, and (C) preheated for 10 days in the dry state. The gain of the LS photometer was set at 1 and that of the refractometer at 64.

rate of 0.3 mL/min of the elution buffer of 10 mM sodium phosphate, pH 6.9, containing 0.1% sodium azide. Elution from the column was monitored with a low-angle laser light scattering photometer (LS-8, Toyo Soda) and then with a precision differential refractometer (RI-8, Toyo Soda Co.).

The molecular weight of heat-induced DEW aggregates was estimated from the ratio of the output of a low-angle laser light scattering photometer, $(output)_{LS}$, to that of a refractometer $(output)_{RI}$, by eq 1 (Takagi and Hizukuri, 1984; Maezawa et al., 1983)

$$MW = K(output)_{LS}/(output)_{RI} \quad (1)$$

where K is a constant depending on the instrumental and experimental conditions and is determined by using standard protein.

The average molecular weight of DEW aggregates was determined by eq 2 (Takagi and Hizukuri, 1984)

$$MW = K(area)_{LS}/(area)_{RI} \quad (2)$$

where $(area)_{LS}$ and $(area)_{RI}$ are the total areas in the elution peak of the LS photometer and the refractometer, respectively.

RESULTS

Figure 1 shows typical elution patterns of heat-induced DEW aggregates monitored by a low-angle laser light scattering photometer (LS) and a refractometer (RI). The peaks of DEW aggregates detected with the refractometer were similar at any preheating time in the dry state. On the other hand, LS peaks of aggregates formed from nonpreheated DEW were sharper and higher than those of samples dry heated for 5 or 10 days. This result indicates that the molecular weight of nonpreheated DEW aggregates was much greater than that of pre-dry-heated DEW aggregates.

Each pair of elution curves in Figure 1 was converted to a corresponding molecular weight distribution curve. The molecular weight distribution curves of DEW aggregates were estimated according to the method of Kato and Takagi (1987). Figure 2 shows the molecular weight distribution curves thus obtained. The curve of nonpreheated DEW in the dry state manifested a wide molecular weight distribution. On the contrary, the preheated DEW in the dry state indicated sharp and narrow molecular weight distribution of the heat-induced aggregates and shifted toward low molecular weight. This suggests a rapid progress of aggregation with heating for gelation in the native DEW, while a decrease in the degree of polymerization occurs in the preheated DEW. Therefore, dry heating of DEW seems to result in a decrease of the tendency of aggregation upon gelation. This may play a critical role in the formation of strong and stable gels of dry-heated DEW.

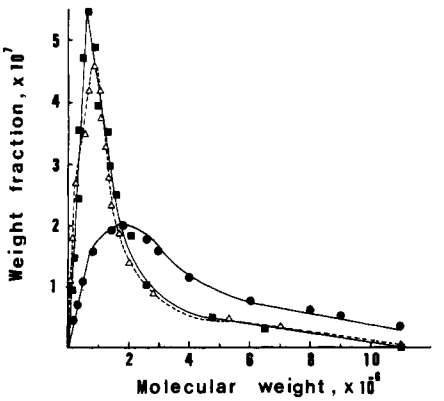

Figure 2. Molecular weight distribution curves of heat-induced DEW aggregates. (●) Nonpreheated, (■) preheated for 5 days, and (△) preheated for 10 days in the dry state. Curves were drawn from the pairs of elution patterns in Figure 1.

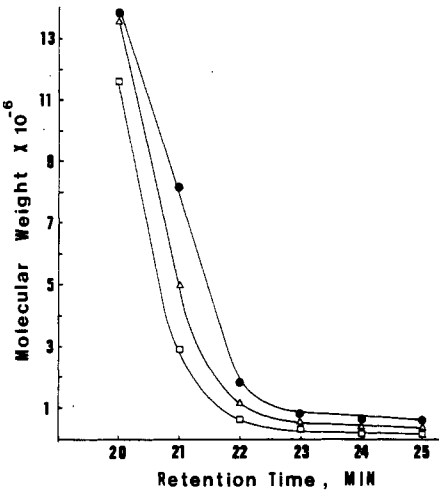

Figure 3. Relationship between retention time and molecular weight of heat-induced aggregates of preheated DEW in the dry state for various periods of time: (●) nonpreheated; (△) preheated for 5 days; (□) preheated for 10 days.

More details of the nature of DEW aggregates can be detected from plotting molecular weight against retention time of the polymerized forms, because the retention time reflects the size and mode of aggregates eluted at a particular retention time. Figure 3 shows the plots of molecular weight vs retention time of heat-induced DEW aggregates. These curves were obtained by converting the elution patterns in Figure 1. There is a remarkable shift to shorter retention time for DEW aggregates with an increase in the preheating time in dry state. This suggests that dry heating led not only to a decrease in the molecular weight of DEW aggregates but also to the formation of uncompact structure of aggregates. This uncompactness of aggregates increased progressively with an increase in preheating time in the dry state.

Figure 4 shows the effect of protein concentration on the molecular weight of heat-induced DEW aggregates as a function of preheating time in the dry state. The molecular weight of DEW aggregates progressively increased with an increase in protein concentration. But this progress in molecular weight of DEW aggregates was more pronounced in the nonpreheated DEW than in the preheated DEW in the dry state. It should be noted that

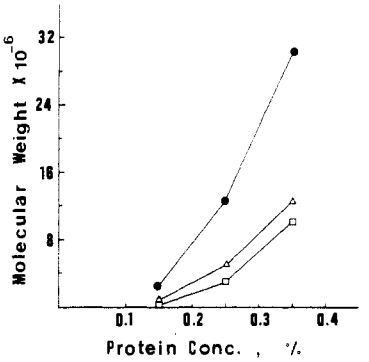

Figure 4. Relationship between protein concentration and molecular weight of heat-induced aggregates of DEW preheated in the dry state for various periods of time. Symbols as in Figure 3.

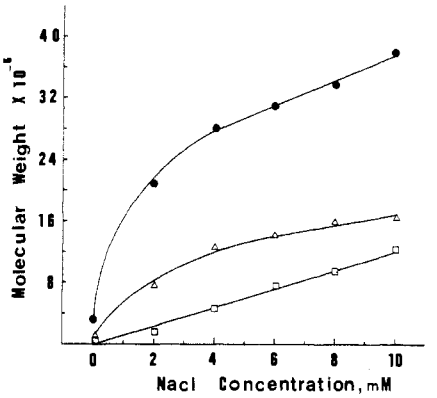

Figure 5. Relationship between salt concentration and molecular weight of heat-induced aggregates of DEW preheated in the dry state for various periods of time. Symbols as in Figure 3.

the molecular weight of nonpreheated DEW aggregates was much greater than that of preheated DEW aggregates at high protein concentration where gelation occurs.

Figure 5 shows the effect of salt concentration on the molecular weight of heat-induced DEW aggregates as a function of preheating time in the dry state. Adding salt to the medium (from 2 to 10 mM NaCl) caused progressive and rapid increase in the molecular weight of heat-induced DEW aggregates of nonpreheated DEW in the dry state. In contrast, the heat-induced DEW aggregates of preheated DEW in the dry state showed relatively slower response to the increase of salt concentration. However, the molecular weight of nonpreheated DEW aggregates was much higher than that of preheated DEW aggregates in the dry state at any salt concentration. It can also be noted that, at any salt concentration used, the molecular weight of heat-induced DEW aggregates was significantly decreased with an increase in preheating time in the dry state. The data suggest that the apparent decrease in the degree of polymerization of the preheated DEW in the dry state upon gelation might be attributed to surface charge factors, since salt is known to rupture ionic attraction between protein molecules and indirectly enhance hydrophobic interactions (Nandi and Robinson, 1972).

The effect of heating temperature on the molecular weight of polymerized forms and gel strength of preheated DEW in the dry state for various lengths of time is shown in Figures 6 and 7, respectively. Figure 6 shows the changes in molecular weight of heat-induce DEW aggregates under various temperatures (from 70 to 80 °C). The molecular weight of nonpreheated DEW aggregate decreased rapidly with an increase in heating temperature up to 76 °C, where it reached a constant value. In contrast, the molecular

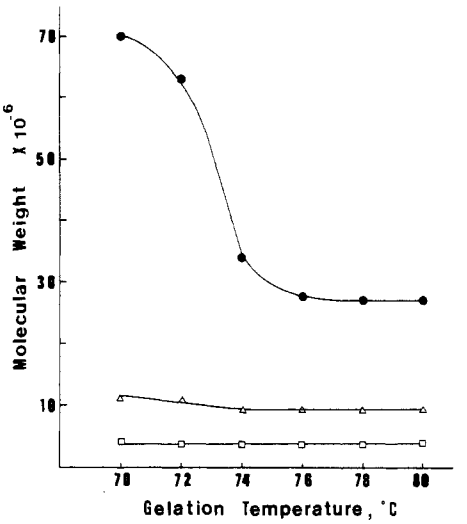

Figure 6. Relationship between heating temperature and molecular weight of heat-induced aggregates of DEW preheated in the dry state for various periods of time. Symbols as in Figure 3.

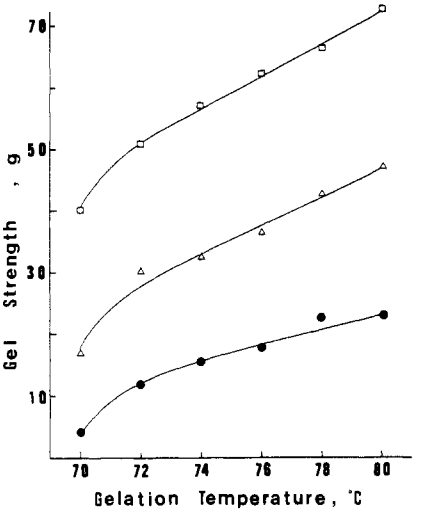

Figure 7. Relationship between heating temperature and gel strength of DEW preheated in the dry state for various periods of time. Symbols as in Figure 3.

weight of heat-induced DEW aggregate formed from DEW preheated for 5 days emerged only slightly decreased with the increase of heating temperature up to 74 °C, while the molecular weight of heat-induced DEW aggregate formed from DEW preheated for 10 days did not alter at any heating temperature used. It is clearly shown that the molecular weight of nonpreheated DEW aggregate is much greater than that of preheated DEW aggregates at any heating temperature.

The effect of heating temperature on gel strength of DEW preheated in the dry state for various periods of time is shown in Figure 7. Increase in heating temperature resulted in a marked increase in gel strength at any heating temperature for all samples, indicating the high dependency of the gel strength of DEW on heating temperature. The gel strength of DEW preheated in dry state was much higher than that of nonpreheated DEW. As a result, it seems likely that the higher the tendency of aggregation, the lesser the gel strength. Therefore, these data led us to conclude that one of the most effective roles of dry heating on gel strength of egg white proteins is the reduction of the degree of polymerization during gelation, leading to the formation of a strong three-dimensional gel network.

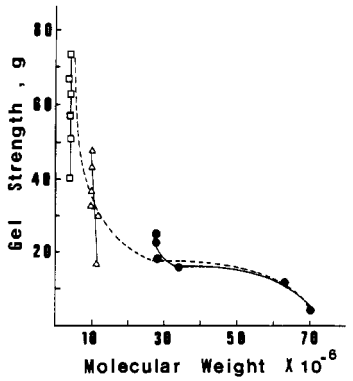

Figure 8. Relationship between gel strength and molecular weight of heat-induced aggregates of DEW preheated in the dry state for various lengths of time, subsequently heated for gelation at different temperatures. Symbols as in Figure 3.

Interestingly, when the data of Figures 6 and 7 were converted into plots of gel strength vs molecular weight of aggregates of DEW preheated in the dry state for various lengths of time as shown in Figure 8, a regression curve (dashed line) was observed between gel strength and molecular weight. The higher gel strength values were obtained from aggregates ranging in molecular weight from 2000K to 10000K, while the weak gels were obtained from aggregates that exceed these values of molecular weight.

DISCUSSION

The mechanism of gelation of egg white proteins is similar to that of other globular proteins, with an initial denaturation step followed by interaction to form a gel matrix. However, there is little information regarding the aggregation behavior when the protein gel is formed. Fortunately, the molecular weight of heat-induced aggregates (progel) can accurately be determined by using a low-angle laser light scattering technique combined with HPLC. We have found that dry denaturation is a critical step in the formation of excellent gels from egg white proteins (Kato et al., 1989, 1990). In such case, egg white proteins were subjected to two steps of denaturation, one in the dry state and the other in the gelation process. From the results of the present study, it was found that exposure of egg white proteins (DEW) to dry heating prior to heating for gelation reduced dramatically the molecular weight of aggregates and simultaneously increased progressively the gel strength under any condition of gelation. Interestingly, there was a remarkable shift in the plots of molecular weight of DEW aggregates toward short retention time with an increase in preheating time in the dry state prior to gelation. This indicates that the aggregate formed from preheated DEW was more expanded than that from nonpreheated DEW. This expanded form might be compatible for the formation of a strong and stable gel network which harbors an adequate amount of water molecules.

Gelation and aggregation largely depend on both protein and salt concentration, occurring more rapidly at higher concentrations of either. Thus, the data in Figures 4 and 5 are fully consistent with this fact. In this sense, we postulate that some conformational changes in the DEW occurred during the preheating step in the dry state that lowered the sensitivity to NaCl. These changes might be attributed to surface charge factors.

Thus, we have come to several important conclusions that the gel strength of DEW could be determined by factors determining the state of aggregation. A possible explanation for this behavior of dry-heated DEW upon heat-induced gelation is that, in the case of preheated sample, uniform matrices composed of fine strands of various DEW proteins were present. As a result, the gel consists of a fine network of protein particles. Presumably, the partially unfolded soluble aggregates formed by preheating in the dry state behaved as an association unit for the formation of the preferable three dimensional network. On the contrary, since a severely compacted aggregate and collapsed network are present in the nonpreheated proteins, the formation of strong and stable gel might be interfered.

This work introduces a convincing concept that dry denaturation decreases the extent of aggregation upon gelation by which an excellent three-dimensional gel network is formed. Also, it gives supplementary information that points directly to how a certain degree of aggregation on gelation affects the quality of the resultant gel network.

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