An ultrasonic Study on Gelation Process in Egg White Protein

(초음파에 의한 egg white의 gel화의 연구)

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ABSTRACT

Ultrasonic measurement was made at 3MHz in the egg white gel to study the properties of the natural protein. The gelation process caused by heat was studied by the change in the velocity and absorption. An increase in absorption and a decrease in velocity were observed with a time lapse at several aging temperatures ranging from 60 to 75℃. Hysteresis phenomenon was also observed by varying the aging temperature as a parameter. This phenomenon is associated with the memorizing effect of the network structure of protein in the gel. It was demonstrated that the ultrasonic technique was useful for monitoring the gelation process.

요 약

천연단백질인 egg white의 gel화의 과정을 초음파측정에 의하여 연구하였다. Egg white는 60℃에서 gel화 하고, 60℃ 이상의 어떤 온도에서 aging 측정을 하면 gel화의 진행에 따라 흡수는 증가, 음속은 감소하였다. Egg white의 gel화는 60℃와 75℃에서 크게 진행하였고, egg white gel의 비가역성을 반영해서 음속과 흡수 모두 이력현상이 관측되었다.

Ⅰ. INTRODUCTION

Egg white, containing mainly water(88%) and protein(10%), has attracted the attention of bioscientists as one of the typical substances of natural protein, and has been investigated by several means[1-3]. Gelation of egg white has been studied by several techniques : viscosity measurements, electrophoresis, light scattering, and other ch emical analyses[4]. These techniques have certain limitations : the sample can only be used for a single experiment, and many samples are required to observe the total gelation process. The optical measurement cannot be made in the gel region where the sample is opaque. The ultrasonic method, on the other hand, can monitor the total process from sol to gel nondestructively in the same sample. Thus, the ultrasonic method may provide a valuable tool for investigating the gelation process.

In this paper, we reported the results at 3MHz of ultrasonic absorption and velocity measurements

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which were made in egg white below and above the gelation temperature. We noted two phenomena. First, an increase in absorption and a decrease in velocity were observed with the gelation. Secondly, a remarkable hysteresis effect was found in the velocity and absorption of egg white gel with respect to temperature.

Ⅱ. EXPERIMENT

The specimen was egg white taken from the eggs of white leghorn chickens which had been kept at 5℃ for two day after laying. Egg white was carefully filtered with 1mm mesh screen, and only the thin portion was used as a sample after being degassed under vacuum. The sample volume required was 20ml which was taken from three eggs. We measured the ultrasonic absorption in the sol state of the egg white and found no aging effect and no observable differences between the thin and the thick portions of egg white[3]. The experimential method used for this measurement is essentially the pulse-echo overlap method. This system is, however, partially modified in our laboratory to give the absorption as well as the velocity. In the present study, this system was used to measure absorption and velocity at 3MHz in the temperature range from 10 to 75℃.

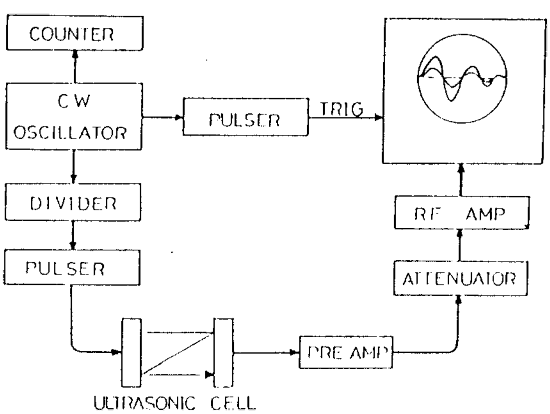

Fig. 1 Block diagram of the apparatus.

Figure 1 illustrates the block diagram of the apparatus used in the present study. Two transduces are of X-cut quartz with a fundamental frequency of 3MHz. The entire front face of the transducers is grounded. They have exactly same dimensions and are fixed on both sides of the cell of stainless steel with path length 62.04mm. The cell wall was made taking special care that the cell ends be parallel ; therefore, no extra mechanism to adjust the parallelism between the quartz plates. Ultrasonic pulse is excited by sharp de voltage rising up within a time shorter than 10ns. The rf amplifier can be turned to 3MHz and pulse at the fundamental frequency is received and observed. Only the first arriving pulse and the second echo are made visible on CRT[Trio CS-2070]. Repetition rate of CW oscillator[Trio AG-203] is adjusted so that the two pluse are overlapped and matched up cycle for cycle. The period of the oscillator which is measured by counter [Hewlett-Packared 5315A] gives a round-trip time of the pulse and yields phase velocity with the known value of path length. The absorption was obtained from the ratio of the pulse amplitude between the first arriving pulse and the second echo. The ratio of two amplitudes determined with a variable step attenuator [Tamagawa Electronics TRA-601D] which allows amplitude adjustment with 0.1-dB accuracy. The waveform of the echo was deformed by the reflection at the half wavelength quartz plates, and this deformation introduced apparent attenuation over the intrinsic absorption. This effect was strictly corrected by relative measurement made with water as a reference liquid and the additional loss was appropriately subtracted from the observed value. The water showed no relaxation effect in the 3MHz frequency range, and the acoustic impedence of the water is close to that of egg white. The calculation of the sound diffraction loss was also made by using the equation of Schoch[5-6] numerically with an effective radius (5mm) of the source and f=3MHz. The relative sensitivities of the absorption and the velocity measurement were 1% and 0.001%, respectively. This systemwas very handy and convenient for simultaneous measurements of the velocity and the absorption, and was suitable for the present experiment which should have been done at many different temperatues. To investigate the aging effect in the gel state, a series of measurements was carried out at intervals of 5minutes after the temperature was set at 60, 62, 64, 66, 70, and 75℃. The temperature was controlled within 1/50℃ by immerging the cell in a thermostated bath [Jeio Tech RC10V].

Ⅲ. RESULTS AND DISCUSSION

1. Ultrasonic Hysteresis

The study of aging effect of egg white gel showed a marked increase in absorption and slight decrease in velocity at 60~75℃. Figure 2 shows a typical example of the time variation of absorption and velocity observed at 75℃. The temperature was raised from 70℃ at the rate of 0.2℃/min. The results are well fitted to the exponential curves represented by the solid lines. The time constant was 220(±20) minutes for both corves. The time constants obtained at different temperatures agree well with each other as shown in Fig. 3. This value was found to be independent of the

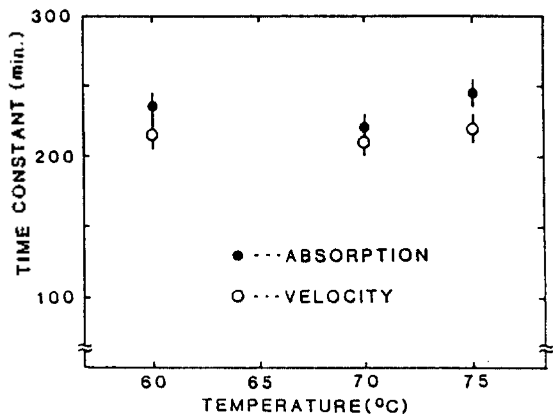

Fig. 3. Time constant of the variation of Fig. 2. The characteristic time is 220 min.

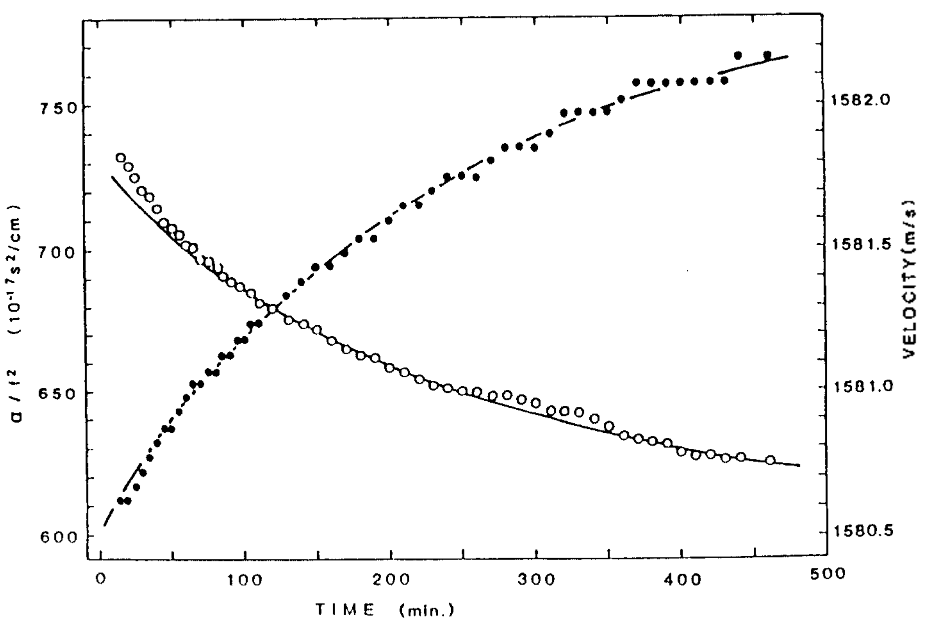

Fig. 2. Ultrasonic absorption(●) and velocity(○) in egg white gel observed in the aging experiment at 75℃. The solid lines repersent the exponential curves fitted to the experimential values

aging temperature, while it may have been dependent on the rate of temperature rise.

The first stage of the experiment started in the sol state from 10℃ and on. Then, the sample was heated up to 60℃ which is the gelation point, and kept at a constant temperature for 7 hours while the aging experiment was conducted. This procedure is indicated by a→b* in Fig. 4. The asterisk denotes the aging experiment. The data was taken periodically for six or seven hours until the absorption and velocity almost reached the saturation values. The sample was then cooled down to 10 ℃ and the measurements were repeated in the gel state during increasing temperature from 10 to 60℃. The second aging experiment was made at 62℃ as expressed by c→b→d*. The subsequent experimental procedure is represented by e→d→f*, g→f→h*, h→i*, j→i→k*, and l→k*.

The results of these experiments are summarized in Figs.4 and 5 for the absorption and the velocity, respectively. The absorption decreased with increasing temperature in the sol state as shown by the closed stars. This decrease reflects a natural trend common to every solution of protein as well as other molecules[6-7]. The absorption began to increase rapidly upon gelation. The closed triangles denote the final values in the aging experiment. Other symbols represent the values in the gel aged at different temperatures. At temperatures of 62, 64, and 66℃ no gelation process was observed. The absorption showed reversible change with temperature along the curve b→c, unless the temperature was raised newly beyond the aging temperature of the gel. The hystersis phenomenon was thus observed with respect to temperature : the absorption depended not only on the temperature at that time but also on the highest temperature the gel has experienced. A quite simlar hysteresis was observed also in the case of velocity. It should be noted, however, that the velocity maximum

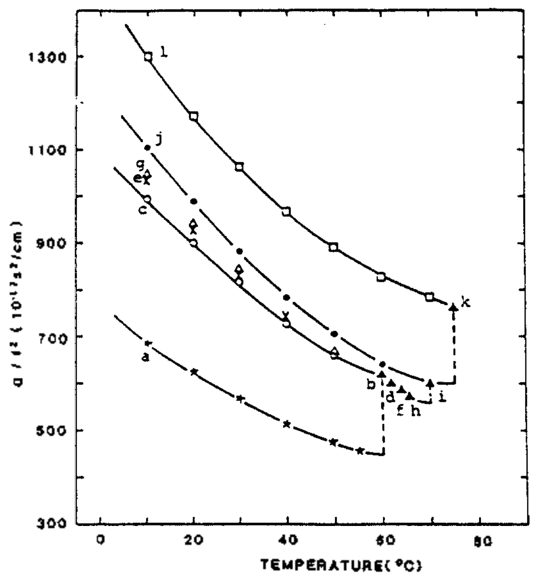

Fig. 4. Hysteresis of ultrasonic absorption. The closed stars indicate the experiment values in the sol state. The colsed triangles denote the final values in the aging experiment. The symbols ○, ×, △, ● and □ represent the values obtained after aging at 60, 62, 64, 70 and 75℃, respectively.

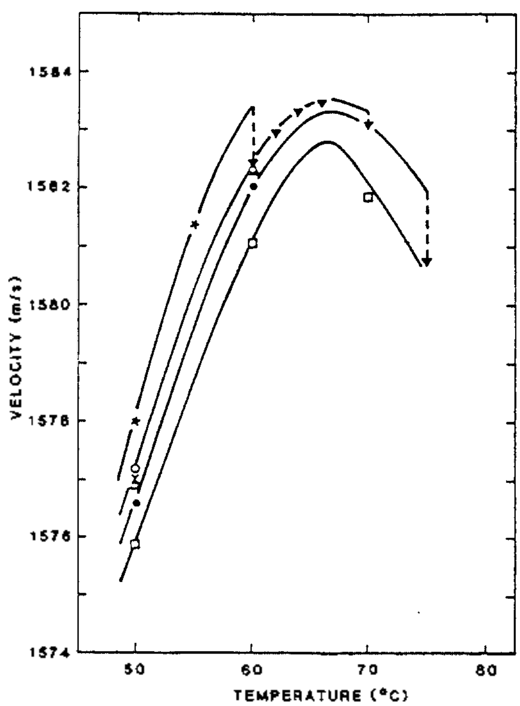

Fig. 5. Hysteresis of ultrasonic velocity. All symbols have the same meaning as those in Fig. 4.

in Fig. 5 is not associated with the gelation. The velocity in water, which is the major composition of the sample, had a maximum value at 74℃[6] and this peak was shifted down by the effect of protein molecules as a solute. The temperature of the velocity maximum in the gel state was 66℃.

These hysteresis phenomena were explained in terms of the molecular aggregation in a gel. Above the gelation temperature, protein molecules in egg white were denatured and aggregated to make a network structure[4][9], which caused the change in the absorption and the velocity. If the gel was at a certain temperature T_m for a sufficiently long time, the network formation continued until it arrives at the final equilibrium state at T_m. This process was monitored through the ultrasonic properties as shown in Fig.2. The final state of aggregation is retained stable as long as the temperature is kept below T_m, and therefore the absorption and the velocity show the reversible change. The first time the gel was heated up over T_m, aggregation still proceeded and a finer network was built. The network in gel became "harder" and the ultrasonic properties had different values. Thus, the thermal history strongly affected the values of absorption and velocity in the egg white gel.

2. Gelation Process

The total change in absorption and velocity in the aging experiment measured the network growth advanced during the temperature increase, and is plotted as a function of temperature in Figs.6 and 7. The bars indicate the differences between the final and initial values obtained when the temperature was raised. The open circles represent the accumulated values of the bars, and the solid lines drawn through the open circles are the estimated direct measurements of the degree of gelation at that temperature. A close resemblance was found

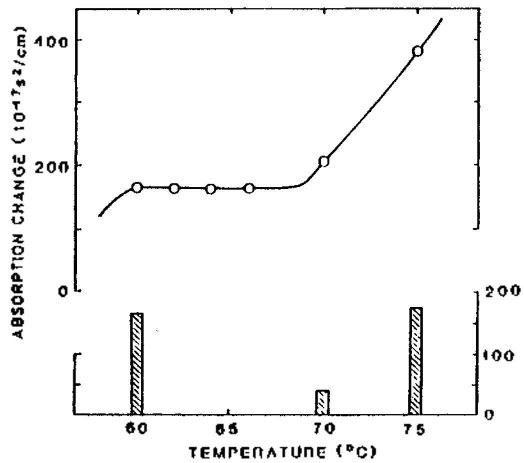

Fig. 6. The total increase in absorption in the aging experiment (bars) and its accumulated values (circles). The latter give the degree of gelation of egg white.

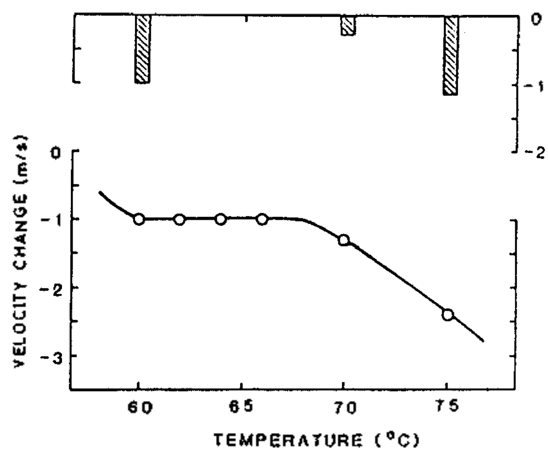

Fig. 7. The total decrease in velocity in the aging experiment (bars) and its accumulated values (circles). The latter give the degree of gelation of egg white.

Table. 1. Composition of thin portion of egg white.

	Amount in thin egg white(%)		Molecular weight(g)
Water	88		
Protein	10		
	Ovalbumin	5.4	45,000
	Conalbumin	1.3	80,000
	Ovomucoid	1.1	28,000
:	:		:
Etc	2		

between Figs.6 and 7 excetp for the difference in signs. The change in the absorption and the velocity are no doubt of the same origin. The change is prominent at 60 and 75℃.

Table 1 shows main constituent the concentration and molecular weight of the thin protion of egg white[10]. The change at 70 and 75℃ in Figs. 6 and 7 may be attributed to the aggregation of ovalbumin, the main constituent of egg white protein. Kato *et al*[11] has studied the agregation of ovalbumin in a diluted solution using a light scattering technique. They have reported that ovalbumin aggregated rapidly near 76℃ where the thermal denaturation of the ovalbumin easily occurs. This agrees with our results. The ovomucoid is known not to be denatured under common heating condition[9]. Therefore, the change at 60℃ may be attributed to the aggregation of conalbumin, the second major protein, which is known to be denatured at a lower temperature than ovalbumin[9]. The protein molecules undergo heat--induced denaturation and then these denatured molecules aggregate with each other to form a network. The second process is possibly the rate determining step of the observed time dependence in Fig.2.

The mechanism of the increase in absorption and the decrease in velocity is not clear at the present time. The network-solvent interaction is likely to cause the additional absorption, as suggested by Gormally *et al*[12]. The decrease in velocity is explained as a longitudinal modulus change. The velocity is expressed by $C=(M/\rho)^{1/2}$, where C is velocity, ρ is density in gel state, and M is longitudinal modulus. The density is not likely to change much with gel process. The longitudinal modulus is $M=K+4/3\times G$, where K is bulk modulus and G is shear modulus. When egg white changes from a liguid to a gel state, the constructed network introduces a finite value of shear modulus which may increase the longitudinal modulus[13]. However, the structure of the network would be very soft and the bulk modulus of its own is much smaller tan that of the solution. The overall longitudinal modulus would decrease as the network grows.

Ⅳ. SUMMARY AND CONCLUSION

Ultrasonic measurements were carried out to investigate gelation process in egg white at 3MHz by pulse-echo overlap which was partially modified in our labortory. The increase in absorption and the decrease in velocity caused by the gelation process of egg white were prominent at 60 and 75℃. The change at 60 and 75℃ was attributed to the aggregation of conalbumin and ovalbumin, respectively. The increase in absorption was described by the interaction between the solvent and the network structure made of protien The decrease in velocity is explained as the longitudinal modulus decrease. Hysteresis phenomenon was observed by varying the aging temperature as a parameter. We have demonstrated that the ultrasonic technique was useful for monitoring the gelation process occurring in egg white.

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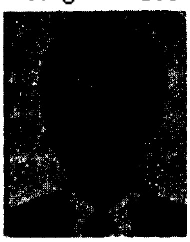

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