

Structure and dynamics of ovalbumin gels: 1. Gel induced by high-temperature heat treatment

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Dynamic viscoelastic (DVE) measurements are made on seven ovalbumin (OVA) gel samples induced by thermal denaturation of aqueous suspensions of OVA with OVA concentration C ranging from 12 to 45 wt% at 160°C for 5 min and subsequent moulding under pressure, and also on four OVA gels with higher C prepared by slow evaporation of water from the 45 wt% gel. The storage and loss shear moduli, $G'(\omega)$ and $G''(\omega)$, of OVA gels with C in the range of 30–59 wt% show angular frequency ω dependence characteristic of the critical gel with the fractal structure, i.e. $G'(\omega) \sim G''(\omega) \sim \omega^n$ and $\tan \delta = \tan(n\pi/2)$ over the whole ω range of 0.14–100 rad s⁻¹ measured at three temperatures of 5.0, 25.0 and 45.0°C. This finding is confirmed by shear creep and creep recovery measurements as well as dynamic light scattering measurements on the same gels. The critical gel structure, however, is not formed either for gels with $C < 25$ wt% or for gels with $C > 59$ wt%.

(Keywords: ovalbumin gel; dynamic viscoelasticity; structure)

INTRODUCTION

Fibrous proteins such as polysaccharides, collagen, myosin and actomyosin easily form gels with a three-dimensional network upon cooling, mainly due to hydrogen bonding^{1–3}. On the other hand, gels are formed upon heating to 70–80°C for globular proteins such as glycinin, ovalbumin and cereal proteins^{4–9}. In this case, hydrophobic interactions between hydrophobic parts exposed on the surfaces of globular proteins due to their thermal denaturation is considered to play a dominant role in gel formation⁶.

As Tombs proposed¹⁰, two different types of gel structure are observed for thermally denatured globular proteins: one is a turbid gel composed of random aggregates of proteins^{11,12}, the other being a transparent gel whose network strands can be modelled as a linear string of protein beads^{13,14}. Some proteins are reported to form either type of gel, when proteins are denatured at moderate temperature varying the pH and ionic strength^{15–20}. One of the present authors, Doi, and his group have shown that the two-step heating method is not only useful as a preparation method of a transparent gel but also gives a clue to elucidation of gelling mechanism and resulting gel structure^{17,18,21}. Linear aggregates with high molecular weights of several millions can be obtained from the first-step thermal

denaturation of ovalbumin (OVA) in 20 mM sodium phosphate buffer of low ionic strength (pH 7.0) at $T \sim 80^\circ\text{C}$, as verified by intrinsic viscosity and static and dynamic light scattering measurements on their dilute solutions^{22–24}. Subsequent heating of the solution after addition of a fairly large amount of salt then gives rise to a transparent gel, for example, in the presence of NaCl as high as 150 mM, which contrasts with the fact that only a turbid gel is obtained by one-step heating of native OVA solution in the presence of 150 mM NaCl. The secondary structure of the heated OVA molecule is found to be similar to that of the native molecule. These results support the view that denatured proteins aggregate by the hydrophobic force, and that the electrostatic repulsive force prevents random aggregation of proteins at low ionic strength and enhances formation of more ordered linear polymers.

Kokini *et al.* attempted to draw state diagrams for cereal proteins plasticized by water on the basis of extensive rheological measurements²⁵. According to them, those proteins undergo the glass-to-rubber transition and start to flow with increasing temperature T as is frequently observed for synthetic flexible polymers in bulk or in the highly concentrated region²⁶. One of the characteristic features of biological polymers appears in the higher T region, named as the 'reaction zone' by them, where the storage and loss shear moduli G' and G'' increase almost 100-fold due to chemical reaction between thermally denatured proteins and ultimately

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take values characteristic of a three-dimensional gel network. Interestingly, with further increase in T , they observed decreases in G' and G'' , implying that softening or melting of the network may occur. Very recently, Doi and his associates succeeded in the preparation of a transparent gel from concentrated aqueous suspensions of globular proteins by thermal denaturation at high temperature of 160–180°C, subsequent moulding under pressure and quenching to room temperature²⁷. This gel has the property that it melts when reheated above the processing temperature, which distinguishes it from the heat-set gel obtained at $T \sim 80^\circ\text{C}$. They found that denatured proteins were not unfolded like random chains but almost retained their original secondary structure as revealed from circular dichroism (c.d.) measurements. Then we may ask what structure protein molecules take at high temperature, what force determines the gelling mechanism, what the gel structure is, what molecular motions are possible, and so on. Extensive physicochemical studies are clearly needed to answer these questions using various techniques such as spectroscopic, scattering and rheological methods.

In this first paper of a series of studies on the structure and dynamics of OVA gels, we shall present results of dynamic viscoelastic measurements on OVA gels induced by high-temperature heat treatment over a wide range of OVA concentration from 12 to 89 wt%. The storage and loss shear moduli, G' and $G''(\omega)$, of the OVA gels with C in the range of 30–59 wt% show angular frequency dependence characteristic of the critical gel with the fractal structure. This finding is confirmed by shear creep and creep recovery measurements as well as dynamic light scattering measurements on the same gels. The gel with $C = 89$ wt% has G' values of the order of 10^9 Pa so that it may be regarded as a new type of biodegradable plastic, and its practical application may be promising as a substitute for plastic made from synthetic polymers.

EXPERIMENTAL

Materials

Ovalbumin (OVA) was prepared from fresh egg white following the procedure described by Sorensen and Hoyrup²⁸. The protein was recrystallized four times and stored in ammonium sulfate solution at 5°C until use. Very pure OVA was obtained with this procedure⁶.

For preparation of OVA gels, the stock solution was exhaustively dialysed against distilled water containing 0.02% NaN_3 and 0.1 mM ethylenediaminetetraacetic acid (EDTA) at 5°C . After adjustment of the pH to 7.5 by addition of 0.1 N NaOH, the solution was thoroughly freeze-dried to obtain water-free OVA powder. A prescribed amount of OVA powder and distilled water was mixed and then poured into a cylindrical brass vessel (inner diameter 10 mm and height 40 mm) with two silicone rubber stoppers at the top and the bottom. The mixture was squeezed by applying pressure at both ends of the vessel, heated for 5 min at 160°C in a thermostated oil bath, and then quenched to room temperature. Seven transparent gel samples were prepared with this processing in the range of OVA concentration C of 12–45 wt%. Transparent gel-like samples were obtained for $C < 12$ wt%, but were found to be viscoelastic liquids. Four transparent gel samples

with $C > 45$ wt% were prepared from slow evaporation of water from the gel with $C = 45$ wt% in a desiccator. The disc-shaped samples with diameter of 10 mm and height of 5 mm were cut out from the middle of the gels using a sharp razor blade for viscoelastic and dynamic light scattering measurements.

Methods

Dynamic viscoelastic and shear creep measurements were performed with a stress-controlled rheometer CSL100 (Carri-MED, ITS Japan), using a parallel-plate geometry with a plate diameter of 1 or 2 cm. The gel samples were adhered to the plate surfaces by applying a very small amount of adhesive. The complex shear modulus was found to be independent of the strain applied when the strain was less than 1.2. The storage and loss shear moduli, $G'(\omega)$ and $G''(\omega)$, of the samples were measured at a strain of 0.3 over an angular frequency ω range of 0.14–100 rad s^{-1} at three temperatures T of 5.0, 25.0 and 45.0°C . The shear creep compliance, $J(t)$, and the recoverable compliance were measured for the samples with C of 15–89 wt% at the same temperatures as the viscoelastic measurements were made.

Dynamic light scattering was carried out with a photometer (ALV-125) equipped with a digital correlator (ALV-5000fast). A vertically polarized single-frequency 488 nm line of an argon-ion laser (Spectra Physics, Beamlock 2060) was used as the light source. The normalized time correlation function, $A_q(t)$, of the vertical component of the light intensity scattered from the gels was measured over a range of scattering angles from 20° to 150° at 25.0°C .

RESULTS AND DISCUSSION

Dynamic viscoelastic (DVE) behaviour

Figure 1 shows the ω dependences of $G'(\omega)$, $G''(\omega)$ and $\tan \delta (= G''/G')$ of the OVA gel with $C = 59$ wt% at $T = 5.0, 25.0$ and 45.0°C . As is clear from the figure, G' and G'' are both proportional to ω^n over the whole ω range of 0.14–100 rad s^{-1} with the same value of the exponent $n = 0.14 \pm 0.01$ at the three temperatures measured:

$$G'(\omega) \sim G''(\omega) \sim \omega^n \quad (1)$$

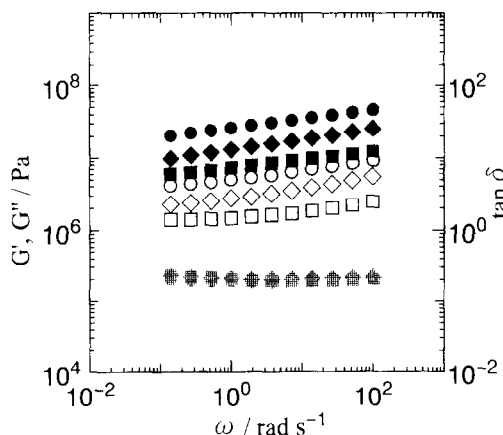


Figure 1 A plot of the storage and loss shear moduli, $G'(\omega)$ and $G''(\omega)$, and $\tan \delta$ against angular frequency ω of the 59 wt% OVA gel at three temperatures: $T = 5.0$ (circles), 25.0 (diamonds) and 45.0°C (squares). The black, white and grey symbols are for G' , G'' and $\tan \delta$, respectively

From the definition, then, $\tan \delta$ became independent of ω and was found to satisfy the equation:

$$\tan \delta = \tan(n\pi/2) \quad (2)$$

The applicability of equation (2) indicates that, on the basis of the Kramers–Kronig relationship, the power law may hold over a much wider ω range than that measured²⁶. Indeed, the respective G' and G'' data at the three temperatures in *Figure 1* can be superimposed on each other by a horizontal shift along the ω axis alone, which gives a composite straight line spanning over about eight decades. An objection may be raised to this superposition procedure, since we did not make the conventional temperature correction to G' and G'' (ref. 26). If this correction had been applied, we would obtain a straight line over nearly ten decades in the ω scale. It should be kept in mind that the time–temperature superposition principle applies to a system in which the mobility of constituent molecules varies with T but whose structure does not vary. We shall discuss the timescale in which the power law is applicable for the G' and G'' data of this gel in more detail later. The gels with $C = 30, 35$ and 45 wt% showed dynamic viscoelastic behaviours quite similar to those shown in *Figure 1* except that the exponent n decreased slightly with decreasing C .

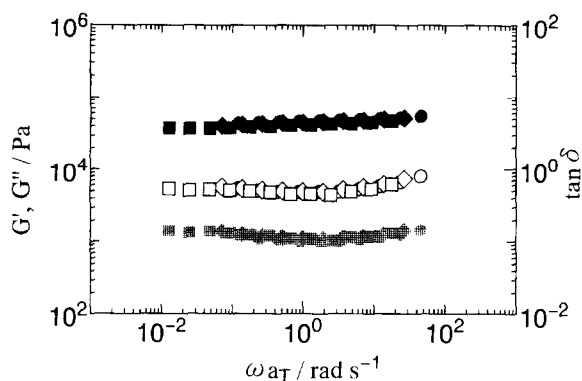


Figure 2 Superposition of the storage and loss shear moduli, $G'(\omega)$ and $G''(\omega)$, and $\tan \delta$ data of the 15 wt% OVA gel at three temperatures using the shift factor a_T calculated from the viscosity of water. The symbols are the same as in *Figure 1*

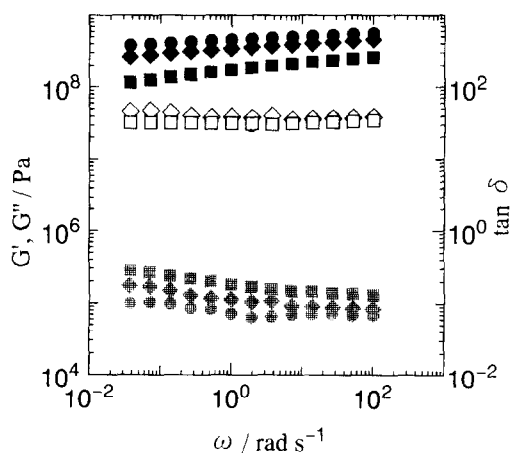


Figure 3 A plot of the storage and loss shear moduli, $G'(\omega)$ and $G''(\omega)$, and $\tan \delta$ against angular frequency ω of the 81 wt% OVA gel at three temperatures. The symbols are the same as in *Figure 1*

Winter and his associates were the first to show that the dynamic viscoelastic method was useful for a study on the gelling process as well as the critical dynamics of condensed polymeric systems near the sol–gel transition point^{29,30}. They postulated that the gel point could be accurately determined as the point where equation (1) held for $G'(\omega)$ and $G''(\omega)$ over a wide ω range and equation (2) for $\tan \delta$ and the exponent n . Since then, the critical behaviour of various gelling systems has been discussed in comparison with theories developed on the basis of the self-similar structure of the critical gel, i.e. the fractal structure, by many research groups^{31–60}. In referring to the above well established results, we may postulate that OVA gels prepared by thermal denaturation at high temperature take the critical gel structure in the range of C from 30 to 59 wt%.

The lowest OVA concentration at which the homogeneous gel can be obtained by the heat treatment described in the ‘Experimental’ section was 12 wt%. The values of G' and G'' of the OVA gel with $C = 15$ wt% only slightly decreased with increasing T at the same ω . Therefore the data at the three temperatures were superimposed on each other by a horizontal shift, with the shift factor a_T put equal to the ratio of the solvent viscosity of water at two temperatures. *Figure 2* shows that the time–temperature superposition principle is applicable for the gel with $C = 15$ wt%: $G'(\omega)$ appears to obey a power law, but $G''(\omega)$ data cannot be represented by a straight line. Thus, $\tan \delta$ is not constant but dependent on ω , indicating that the gel does not possess the critical gel structure.

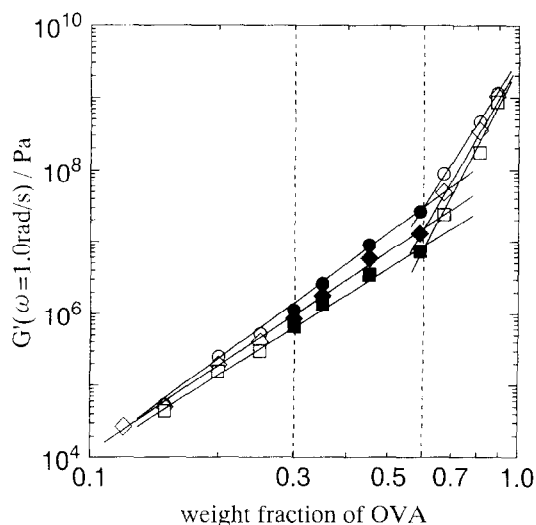
The highest OVA concentration of the transparent gel attained by slow evaporation of water from the 45 wt% gel was found to be 90 wt%. Further evaporation of water from the sample became very difficult even by the use of a vacuum rotary pump. We observed that the gel was no longer transparent above 90 wt% and could not hold its shape because of cracking. This observation suggests that water present in the gel may be classified into two types: the major portion of water is likely to be present in a state similar to free water; while the minor portion seems firmly bound to the protein surface for stabilization of ionic amino acid residues. In this connection, we had better note that three gels of $C = 15, 25$ and 45 wt% swell in pure water up to $C = 5.6, 20$ and 34 wt%, respectively. The chemical potential of water in the gel is, thus, always lower than that of pure water, but a small swelling ratio of 1.36 at $C = 45$ wt% is not incompatible with the above classification of water in the fractal structure.

Figure 3 shows the frequency dependence of G' and G'' of the 81 wt% OVA gel at three temperatures. Again, G' appears to obey a power law with the exponent n slightly increasing from 0.07 ± 0.01 to 0.10 ± 0.01 with increasing T . On the other hand, G'' hardly depends on ω , so that $\tan \delta$ always decreases with an increase in ω . The G' and G'' behaviours of 69 and 89 wt% gels were similar to those shown in *Figure 3*, though not shown. Values of the exponent n in $G' \sim \omega^n$ are listed in *Table 1*.

The DVE measurements revealed that OVA gels had the fractal structure intrinsic of the critical gel in the limited C range of 30–59 wt%. This range is shown as the area between the two vertical dotted lines in *Figure 4*, where G' at 1.0 rad s^{-1} is plotted against C at three temperatures. The C dependence of G' at each

Table 1 Experimental results on n and m at three temperatures^a

OVA conc.		5°C	25°C	45°C
15%	n	0.08	0.08	0.07
	m	0.07	0.08	0.07
20%	n	0.09	0.08	0.09
	m	0.09	0.09	0.08
25%	n	0.09	0.09	0.09
	m	0.09	0.10	0.09
30%	n	0.09	0.09	0.08
	m	0.10	0.10	0.09
35%	n	0.09	0.10	0.09
	m	0.11	0.11	0.10
45%	n	0.13	0.13	0.13
	m	0.13	0.14	0.14
59%	n	0.14	0.14	0.14
	m	0.15	0.14	0.15
67%	n	0.12	0.14	0.16
	m	0.12	0.16	0.17
81%	n	0.07	0.09	0.10
	m	0.07	0.09	0.12
89%	n	0.03	0.04	0.05
	m	0.04	0.05	0.06

^a Each entry in table ± 0.01 **Figure 4** A plot of G' at $\omega = 1 \text{ rad s}^{-1}$ against weight fraction of OVA at three temperatures. The concentration area where the fractal structure is observed is between the two dotted lines. The concentration dependence of G' is well represented by two straight lines intersecting at $C = 60 \text{ wt}\%$, irrespective of T

temperature can be approximately represented by two straight lines intersecting at $C = 60 \text{ wt}\%$, when data reproducibility of about 10% has been taken into account. A similar dependence was observed for G' at other ω . One straight line in the low C region indicates that G' data alone cannot distinguish the critical gel structure from another type of gel structure. Since the two slopes are dependent on T , detailed discussion about their absolute magnitudes may not be sound. However, we would like to point out that, first, values of the slope on the low-concentration side are about 4, which is much larger than the value of 2–2.5 obtained for rubber-like materials. If OVA molecules could be denatured to a coil form by the high-temperature heat treatment and could form a conventional three-dimensional network by physical crosslinks, the slope should become close to 2. Therefore, it is improbable that the OVA gel structure including the fractal structure realized in the C range of

15–59 wt% is composed of random chains. Secondly, G' increases sharply at higher C with slopes of about 10, and attains an order of magnitude of 10^9 Pa at $C = 89 \text{ wt}\%$, being close to the glass modulus. In spite of this high modulus, the gel is not yet brittle but tough. Also, the gel can be easily moulded to any shape when processed at around 160°C . Thus the gel may offer a new type of biodegradable and disposable plastic as a substitute for conventional ones made from synthetic polymers.

Viscoelastic quantities such as G' and the steady flow viscosity η of synthetic flexible polymer solutions are known to increase monotonically with C (ref. 26). Especially, a sharp increase is observed at very high C , when the bulk polymer reaches the glassy state at the measured temperature. Such behaviours have been successfully interpreted by the free-volume theory based on the idea that decrease in the free volume with increasing C makes the micro-Brownian motion of polymer chains sluggish, because the solvent usually has larger free volume than the polymer segment. The strong C dependence of G' at higher C in Figure 4 may be explained in terms of the free-volume theory in consideration of the large G' values.

Upon drying of the 45 wt% gel, the critical gel structure could not be maintained above 59 wt%. If the protein molecules could be thermally denatured so as to keep their nearly spherical rigid shape close to the native one, the result could be qualitatively related to the packing fraction. Formation of the fractal structure by association of spherical particles may need relatively large free space provided by the presence of water as solvent. Respective protein molecules start to come into contact with other surrounding molecules with increasing C and may reach some critical concentration C_f above which the fractal structure cannot be preserved any more. C_f may be taken as the concentration at the intersection of the two straight lines in Figure 4 and is read as 60 wt% irrespective of T . The packing fractions of cubic, b.c.c. and f.c.c. crystals are known to be 0.52, 0.68 and 0.74, respectively. The packing fraction corresponding to C_f is calculated as 0.53 using the value of the specific volume of native OVA ($0.75 \text{ cm}^3 \text{ g}^{-1}$). The figure of 0.53 seems reasonable taking into account the amorphous nature of the fractal structure. Above C_f , the protein molecules must be deformed so as to accommodate them in a restricted narrow space, with water localized on the protein surface filling the gap, which inevitably leads to a distribution of relaxation times different from that of the critical gel. It should be remarked that the 45 wt% gel prepared by swelling of the 81 wt% gel reproduced G' and G'' data exactly the same as those of the original 45 wt% gel. The extent of association is unaffected by the de-swelling and subsequent swelling procedure.

Shear creep behaviour

We pointed out in the previous section that the power law $G' \sim G'' \sim \omega^n$ might hold over an ω range much wider than the three-and-a-half decades experimentally accessible with our rheometer, whenever the OVA gels have the fractal structure. In order to examine this conjecture, we performed shear creep and creep recovery experiments on ten OVA gels with $C = 15\text{--}89 \text{ wt}\%$ at 5.0, 25.0 and 45.0°C. Figure 5 shows raw data of the 59 wt% gel at three temperatures, as an example. The

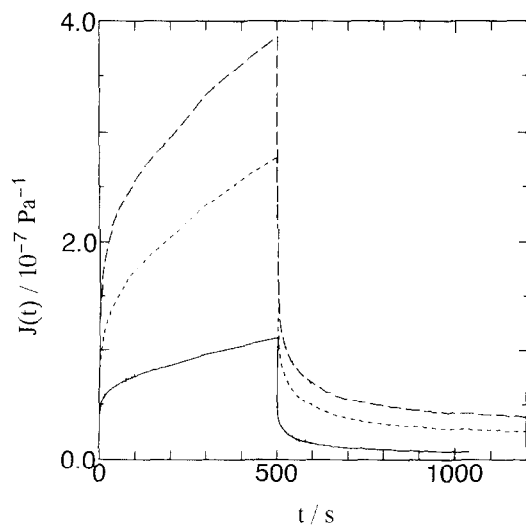


Figure 5 Shear creep and creep recovery data of the 59 wt% OVA gel at three temperatures: $T = 45.0, 25.0$ and 5.0°C from the top, respectively

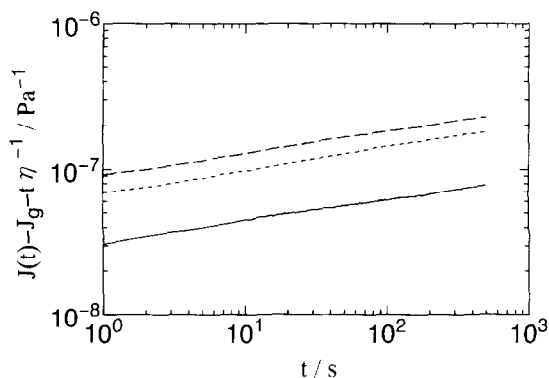


Figure 6 The retardation term $\phi(t) = J(t) - J_g(t) - t/\eta$ estimated from the data in Figure 5 plotted against t . Curves for T are the same as in Figure 5

shear creep compliance $J(t)$ was measured until $t = 500$ s elapsed and then the creep recovery was made after instantaneous release of the shear stress. $J(t)$ is generally expressed as:

$$J(t) = J_g + \phi(t) + t/\eta \quad (3)$$

where J_g is the instantaneous compliance, $\phi(t)$ is the retardation term and t/η represents the contribution of the viscous flow. J_g can be easily estimated from linear extrapolation of $J(t)$ to $t = 0$, and t/η from residual strain of the creep recovery data. The viscosity of the gelling system should diverge at the gel point from its definition that the largest cluster must be fully developed from one side to the other side of the sample volume. The data show that the 59 wt% OVA gel has finite viscosity. However, values of η estimated were as large as the order of 10^{10} Pa s, more precisely 6.3×10^{10} , 1.7×10^{10} and 1.2×10^{10} Pa s at $T = 5.0, 25.0$ and 45.0°C , respectively. $J(t)$ was found to be completely recoverable at higher C .

The retardation term $\phi(t) = J(t) - J_g - t/\eta$ calculated from the data shown in Figure 5 is plotted logarithmically against t in Figure 6. All the data can be well represented by the power law $\phi(t) \sim t^m$ with the same exponent m irrespective of T over the entire time range measured. The power law was found to be applicable for

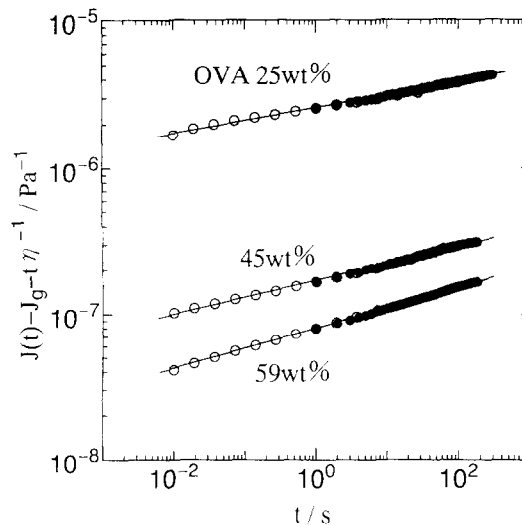


Figure 7 Superposition of $J(t)$ calculated from $G^*(\omega)$ data onto $J(t)$ data directly obtained from creep measurements for three OVA gels with $C = 25, 45$ and 59 wt%

$\phi(t)$ of all other OVA gels within experimental uncertainty. Furthermore, m and n are in excellent agreement with each other as shown in Table 1. This agreement may indicate that the relaxation spectrum $H(\tau)$ calculated from the storage modulus $G'(\omega)$ is reciprocally proportional to the retardation spectrum $L(\tau)$ from the creep compliance $J(t)$ ²⁶. Generally, short-time viscoelastic processes associated with the local gel structure are more revealed in H , and the long-time processes associated with the global structure in L . The result is thus consistent with the fractal nature of the OVA gel. Nonetheless it should be noted that the distribution of relaxation times is more sensitively reflected in the ω dependence of the loss modulus $G''(\omega)$ than $G'(\omega)$, and that equations (1) and (2) must be simultaneously satisfied for the critical gel.

Conversion of the dynamic modulus data to creep compliance data was made as follows. First, the storage and loss shear compliances, $J'(\omega)$ and $J''(\omega)$, were calculated from $J^*(\omega) = 1/G^*(\omega)$, and then $J(t)$ was estimated by the method of Ninomiya and Ferry, from the equation (equation (49) of ch. 4 of ref. 26):

$$J(t) = J'(\omega) + 0.40J''(0.4\omega) - 0.14J''(10\omega)|_{\omega=1/t} \quad (4)$$

As Figure 7 shows, $J(t)$ calculated from $G^*(\omega)$ is nicely superimposed onto the corresponding curves directly obtained from creep measurements, and is likely to extend the time range where the power law holds to more than four decades at least. The superposition also assures that reliable dynamic and static measurements have been made on the OVA gels in the linear viscoelastic region.

Values of $n (= m)$ are between 0.09 and 0.14 for the OVA gels with the fractal structure and are considerably smaller than 0.67 obtained for the ideal gelling system at the gel point formed by the end-linking chemical reaction of linear flexible polymers⁴⁸. The latter value may be related to the flexibility of a Gaussian chain whose molecular motions can in principle be described by the normal-mode analysis. Therefore, a protein molecule denatured by high-temperature heat treatment is not unfolded like a random chain, and adhesion of globular-shaped molecules due to the hydrophobic interaction,

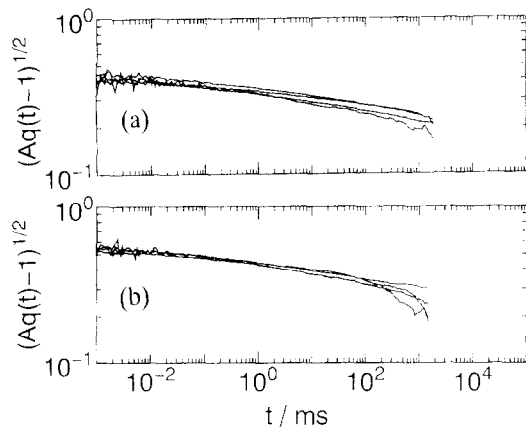


Figure 8 The time correlation function of the scattered electric field of the 45 wt% OVA gel at $T = 25.0^\circ\text{C}$. The scattering angle θ is (a) 30° and (b) 150°

which leads to a distribution of relaxation times widely separated along the time axis, may be a better representation for the gelling process of thermally denatured OVA.

Dynamic light scattering

Light scattering is also known as a useful technique for investigation of the gelling process near the sol-gel transition⁶¹⁻⁶⁶. We performed dynamic light scattering (DLS) measurements on the OVA gels, which showed the viscoelastic behaviour characteristic of the critical gel, to obtain the normalized time correlation function of light intensity $A_q(t)$. Figure 8 shows time profiles of $(A_q(t) - 1)^{1/2}$, i.e. the time correlation function of the electric field, $g_q^{(1)}(t)$, scattered from the 45 wt% OVA gel at two scattering angles of 30° and 150° at $T = 25.0^\circ\text{C}$ in a double logarithmic scale. The pairs of curves at each angle in the figure are obtained from repeated DLS measurements on the same gel by displacing the sample cell up and down, and good reproducibility seems to reconfirm the homogeneity of the transparent OVA gels used in this study.

As is clear from the figure, the data follow the power law:

$$g_q^{(1)}(t) \sim t^{-p} \quad (5)$$

with the exponent p as small as 0.05 ± 0.01 . Equation (5) was found to be applicable for $g_q^{(1)}(t)$ measured at other scattering angles. The $g_q^{(1)}(t)$ of the gelling system, which shows a unimodal distribution of the decay rate before the onset of chemical reaction, is better represented by a stretched exponential type of decay function accompanied with formation of larger and larger clusters as the chemical reaction proceeds, and finally tends to exhibit the power-law type of behaviour in the very vicinity of the gel point⁶⁴⁻⁶⁶. Therefore, the applicability of equation (5) to the DLS behaviour of the OVA gel strongly supports our conclusion that, when aqueous OVA suspensions are denatured by high-temperature heat treatment, the gels with the fractal structure can be formed in the limited concentration range.

At present we have no explanation of the fractal structure characterized by the small values of the exponents n and p , though we vaguely imagine that the

fundamental unit of the gel is a nearly spherical rigid particle. Direct observation of the gel structure by electron microscopy is in progress, and also small-angle X-ray scattering measurements seem useful for elucidation of local arrangements of protein molecules. We are attempting to prepare OVA gels using organic solvents and their mixtures with water to examine the effect of hydrophobicity of the medium on the ability of gel formation as well as the gel structure along with d.s.c. measurements for thermodynamic properties of the gels, which is based on our presumption that the strength of the hydrophobic force between denatured proteins plays a crucial role in the gel structure. The results will be reported in a subsequent paper soon.

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