Colloid Science

Thermotropic gelation of ovalbumin 1. Viscoelastic properties of gels as a function of heating conditions and protein concentration at various pH values

N. V. Grinberg, T. M. Bibkov, V. Ya. Grinberg, and V. B. Tolstoguzov

Institute of Organo-Element Compounds, USSR, Academy of Sciences, Moscow, U.S.S.R.

Abstract: A study has been undertaken of stress relaxation in ovalbumin thermotropic gels with a concentration of 8–20 %, depending on time and temperature of heating (respectively, 20–60 min, 70°–110°C), at pH 2.5–10.0. In all instances, the dependence of the initial gel elasticity modulus on heating has a single maximum. Gelation conditions corresponding to this maximum are considered optimal. Optimal gelation time is 30 min, regardless of pH. On the other hand, the optimal heating temperature depends on pH. To the right and left of the isoelectric point of protein ($2.5 \leq pH < 4.0$ and $5.5 < pH \leq 10.0$) the optimal temperature is 80°. However, in the vicinity of the isoelectric point ($4.0 \leq pH \leq 5.5$) the optimal temperature rises considerably. Gels produced to the right and left of the isoelectric point belong to the homologous group A, while gels produced in the point vicinity belong to another homologous group B. Light scattering data proves group A gels to be more homogeneous optically. These gels may be supposed a single-phase system, while group B gels are two-phase-systems. For each group, the dependence of the relaxation modulus (G) of gels on heating conditions, pH and protein concentration (X_1, X_2, X_3, X_4), as well as on time of relaxation (t) may be generally described as $G(X_1, X_2, X_3, X_4, t) = G_e(X_1, X_2, X_3, X_4)\, f(t)$, where G_e is the equilibrium value of the elasticity modulus, and $f(t)$ the relaxation function. Thus, a change in the parameters only affects the value of the equilibrium elasticity modulus, and exerts no effect on the relaxation time spectrum. For this reason, all the relaxation curves obtained may be transformed into two normalized relaxation functions:

$$\tilde{f}(t) = f(t)/f(1) = G(X_1, X_2, X_3, X_4, t)/G(X_1, X_2, X_3, X_4, 1)$$

Each of these normalized functions corresponds to one of the homologous groups. Rheological similarity of gels in each homologous group evidently points to their structural similarity. Invariance of the gel relaxation properties with regard to protein concentration, leads to a concentration dependence of the equilibrium modulus at various pH values. These dependences are curvilinear on a double logarithmic scale. The slope of the curve exceeds 2 in the entire concentration interval studied. In other words, the dependences obtained cannot be described by the usual "law of squares". On the other hand, they adequately match Hermans' theoretical relation for a network formed by random association of identical polyfunctional particles without cyclization. This simple model evidently gives a true picture of the major regularities of thermotropic gelation for ovalbumin. An agreement between this theory and experiment was achieved for a protein concentration of $C^* = 6.0 \pm 1.0$ % at the gel point regardless of pH. Invariance of gel-point position with regards to pH demands further confirmation.

Key words: Ovalbumin, thermotropic gels, stress relaxation, reduced variables method, viscoelasticity, heating, protein concentration, rheological similarity, structure of gels.

List of Symbols

T_h, t_h	= heating temperature and time;	C^*	= protein concentration in gel-point;
T_h^*, t_h^*	= optimal heating temperature and time;	G	= relaxation modulus;
C	= protein concentration;	G_e	= equilibrium modulus;

$f(t)$ = relaxation function;
t = time of relaxation;
$\tilde{f}(t)$ = normalized relaxation function;
$\tilde{f}_A(t), \tilde{f}_B(t)$ = normalized relaxation functions of groups A and B;
$\tilde{G}_1$ = T_h, t_h-reduced modulus;
$\tilde{G}_2$ = T_h, t_h, pH-reduced modulus;
$\tilde{G}_3$ = C-reduced modulus;
b_1 = T_h, t_h reduction parameter of modulus;
b_2 = pH reduction parameter of modulus;
b_3 = C reduction parameter of modulus;
W_g = gel-fraction.

Introduction

Thermotropic gelation is the main functional property of proteins allowing the use of them in the production of various foodstuffs. However, the main regularities, as well as the nature and mechanism of this phenomenon are still to be subjected to careful study.

The results of a systematic study on phenomenology of formation and viscoelastic properties of soybean globulin (SBG) fraction gels were described by Bibkov et al. [1]. The study showed the SBG gel network to be composed of aggregates of protein molecules. It also provided an SBG gel network model that illustrates the relaxation behaviour peculiar to these gels.

It is known [2, 3] that SBG is a protein mixture. Its main components, 7S and IIS globulins are of a complex structure [2–5], being made up of various subunits. It has been proved that during gelation, different subunits are not divided randomly between sol and gel fractions [6].

A study of thermotropic gelation of the simpler proteins should yield more accurate data on the nature of this phenomenon. Therefore it should prove interesting to investigate ovalbumin, one of the major proteins used in the food industry.

Ovalbumin (OA) is a monomer globular glycophosphoprotein with a molecular weight of 45 kD [7, 8]. It is represented by three forms A_1, A_2, A_3 in a ratio of approximately 85 : 12 : 3, respectively, which are distinguishable in phosphate group content (from 2 to 0). The OA molecule has a disulfide bond and 4 sulfhydryl groups. Sulphydryl groups react differently with specific reagents. They are accessible only via denaturation in urea and concentrated solutions of guanidinium hydrochloride. A characteristic of OA is the fact that almost half of its amino acid residues is hydrophobic.

Van Kleef et al. studied the elastic properties and swelling of OA thermotropic gels in 6M urea [9]. Based on the compression modulus of elasticity and an equilibrium swelling ratio, the number of junctions per protein macromolecule was determined in gels. This number equals the amount of sulfhydryl groups in the OA molecule. This leads to the conclusion that OA thermotropic gel in 6M urea is a network of flexible polypeptide chains, cross-linked by disulfide bonds. This study, however, ignored urea decomposition and protein chemical modification at a temperature over 60 °C [10].

Egelandsdal [11] studied the influence of pH (from 2.5 to 7.5), ionic strength (from 0.002 to 0.3) and β-mercaptoethanol (1 % conc) on OA thermotropic gelation. At low ionic strengths (0.052), gel elasticity dependence on pH has two maxima to the left and right of the isoelectric point (pI) and a minimum at pI. The maximum in the acid region is higher and possesses a higher absolute value of the mean charge of the protein molecule. An increase in ionic strength affects both maxima. From this, the conclusion has been drawn that OA thermotropic gelation is largely controlled by electrostatic interaction. It should be mentioned that gelation conditions (temperature and heating time) remained the same, while the above mentioned factors were changed. Therefore, to investigate the influence of these factors on viscoelastic properties of gels, it is necessary to take into account the influence of these factors on optimal gelation conditions. It is only possible to compare viscoelastic characteristics of gels if they were obtained under optimal conditions.

The present paper deals with the influence of pH on formation and viscoelastic properties of ovalbumin thermotropic gels. By a systematic approach, using the shear modulus as indicator of the degree of conversion during gelation, optimal gelation conditions were determined, i. e. temperature (T_h^*) and heating time (t_h^*), for each pH value. Relaxation properties of thermotropic gels at various pH, obtained under optimal conditions, have also been compared.

Experimental

Materials

The work was carried out with once crystallized sedimentationally homogeneous OA preparation ("Biokhimreaktiv", USSR). The preparation has the following characteristics: protein content −96.2 ± 1.2 %; ash content −2.0 %; water solubility −93.0 ± 1.0 %; sedimentation coefficient −3.28 S.

Methods

Preparation of solutions and gels

The OA preparation was dissolved in distilled water by mixing. The solution was centrifuged (30.000 g, 1 h) to remove insoluble

substances. The precise concentration was determined by drying to a constant weight at 105 °. Concentrated solution was used to prepare the solutions at required concentrations and pH values. OA solutions were then titrated with 0.1–0.2 M NaOH. The protein solutions were poured into special moulds [12]. The filled moulds were centrifuged in a swing-out rotor using the T-23 centrifuge (MLW, G.D.R.) (600 g, 1–2 min) in order to deaerate them, and then heated in a water or glycerine bath. After heating, the moulds were cooled to room temperature in a stream of air for 30 min and placed in a thermostat with a temperature of 4 ± 0.5°. The gel samples were removed from the moulds 20 h later and were held at room temperature for 3 h to relax internal stress.

Rheological measurements

Rheological measurements were taken with a spherical indenter in the linear region at a temperature of 20 ± 0.2° by the penetration method using a "TM-S-L" dynamometer (Instron) [12]. Each test involved at least three parallel measurements, with standard deviation of stress values not exceeding ± 5 %. The sample surface was covered with a thin layer of liquid silicone to prevent water evaporation. Relaxation shear modulus values ($G(t)$) were calculated according to the semiempirical equation [12],

$$G(t) = 0.0986\, P(t) R^{-0.5} h^{-1.5} \qquad (1)$$

where $P(t)$ is a load, $R = 0.275$ cm indenter radius, h – penetration depth, t – time. The equation differs from the theoretical equation of Lee-Radoka only by the factor [13]. The initial modulus of gels (G_o) was determined from the slope of the linear section $P = f(h^{1.5})$ at constant loading speed (0.5 cm/min).

Nephelometry

Light scattering intensity ($\lambda = 546$) in ovalbumin gels was measured in cylindrical solder-sealed tubes (⌀ 12 mm) at an angle of 90° on a universal "SPEKOL" spectrophotometer (Carl Zeiss, Jena), using the "TiMi" nephelometric microtitration attachment. Photocell EMF of the spectrophotometer was measured with a G1B1 recorder ("MLW", G.D.R.). The spectrophotometer was calibrated in relative units against a glass turbidity standard (Carl Zeiss, Jena).

Velocity sedimentation

The OA preparation homogeneity was controlled according to velocity sedimentation data at 50000 rpm, 20° on the ultracentrifuge 3170B ("MOM", Hungary). The OA sedimentation coefficient was determined at a protein concentration of 0.98 % in 0.05 M acetate buffer with pH 4.4, containing 0.15 M NaCl [14].

Results

pH dependence of optimal gelation conditions

The dependence of the initial modulus of OA thermotropic gels on temperature and heating time has a single, weakly defined maximum. It is assumed that heating conditions corresponding to the maximum modulus can be considered optimal.

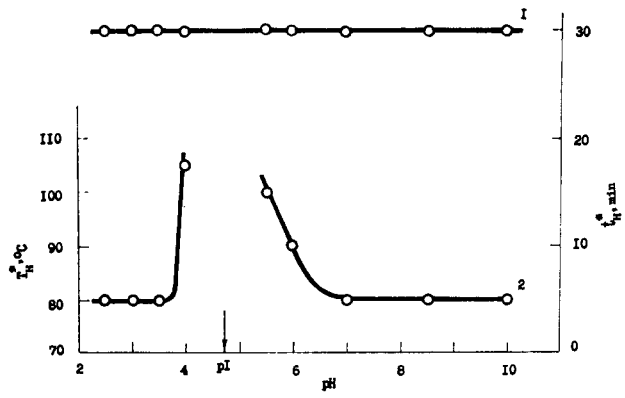

Fig. 1. pH influence on optimal conditions of ovalbumin gelation. 1: optimal heating time (right ordinate); 2: optimal heating temperature (left ordinate). Protein concentration, 15 %; isoelectric point of ovalbumin is marked by the arrow

Optimal gelation conditions that correspond to the maximum initial shear modulus were determined as for planning an extreme multifactorial experiment [15]. Figure 1 shows the dependence of optimal temperature (T_h^*) and heating time (t_h^*) on pH. It is evident that t_h^* equals 30 min and does not depend on pH. At pH 2.0 – 3.5 and 7.0 – 10.0, T_h^* equals 80 ° and does not depend on pH. In the vicinity of the isoelectric point (pI 4.7), T_h^* rises abruptly.

Figure 2 shows the dependence of the initial shear modulus on pH for a 15 % gel under optimal conditions. The modulus decreases with increasing distance

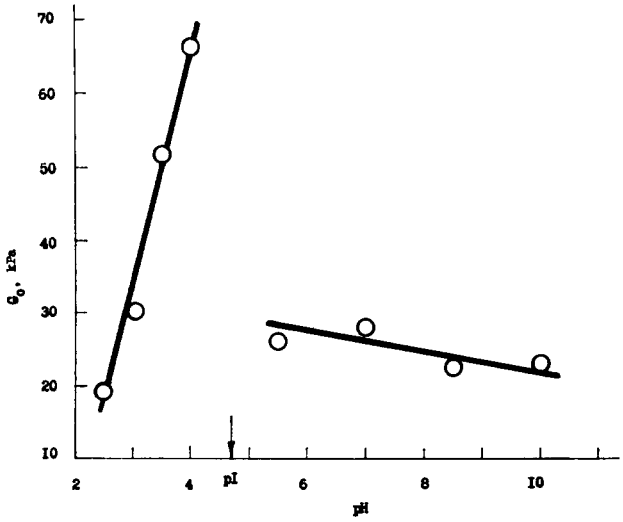

Fig. 2. pH dependence of the initial modulus for thermotropic gel produced under optimal conditions (15 % ovalbumin); isoelectric point is marked by the arrow

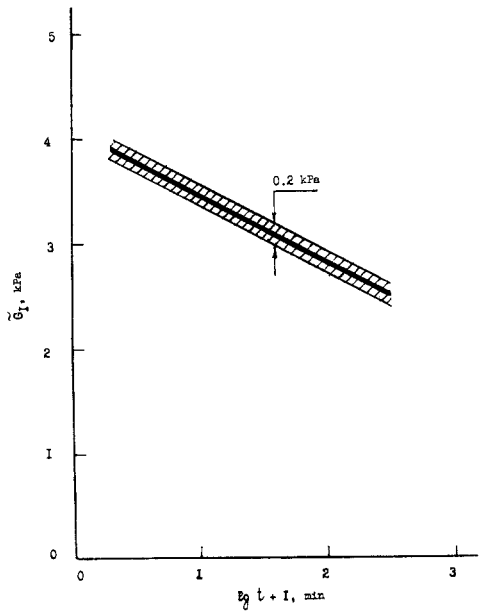

Fig. 3. T_h, t_h-invariant time dependence of relaxation modulus for a thermotropic gel (12 % ovalbumin) at pH 7.0, based on a reduction of relaxation modulus time dependences for 17 gel samples, obtained under varying heating conditions ($20 \leq t_h \leq 60$ min, $70° \leq T_h \leq 110°$). The dependence is approximated (straight line, heavily drawn) for 180 points by the least square method. Thin lines mark maximum point deviation (± 0.1 kPa)

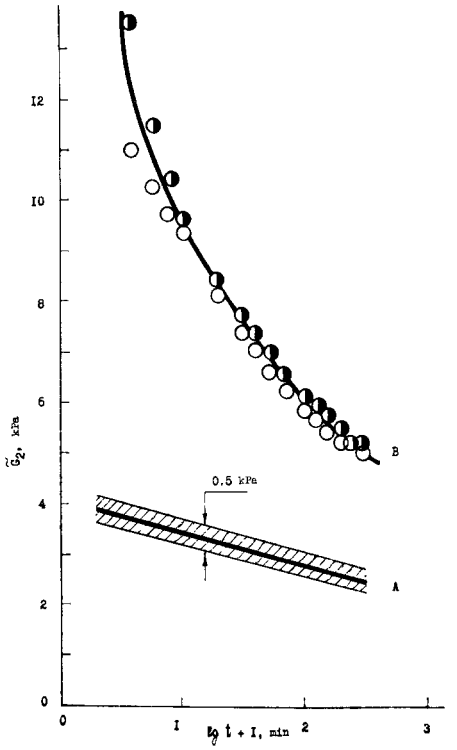

Fig. 4. T_h, t_h, pH-invariant dependences of relaxation modulus for ovalbumin thermotropic gels. Relation A results from reduction of seven T_h, t_h-invariant time dependences of relaxation modulus, for two pH intervals, from 2.5 to 3.5 and from 6.0 to 10.0 (group A, standard conditions - pH_o 7.0). The relation is approximated (straight line, heavily drawn) for 56 points by the least square method. Thin lines mark maximum point deviation (± 0.25 kPa). Relation B results from reduction of two T_h, t_h-invariant time dependences of relaxation modulus for pH 4.0 (–◑–) and pH 5.5 (–○–) (group B, standard conditions – pH_o 4.0)

from pI. This dependence is evidently asymmetrical. The slope on the acid side is larger than in alkaline medium. In other words, under conditions of equal deviation of pH from pI, state of the gels undergoes more changes in the acid region than in the alkali region. It seems probable that the OA gel will show maximum rigidity at the isoelectric point.

Influence of gelation conditions on relaxation properties at constant pH

At constant pH, relaxation curves of gels obtained under different conditions (T_h and t_h variable), are similar in form and can be transformed into a condition-invariant curve $\tilde{G}_1(t)$ via modulus reduction, where the reduced modulus

$$\tilde{G}_1(t) = G(t, T_h, t_h)/b_1(T_h, t_h) \qquad (2)$$

and the reduction parameter

$$b_1(T_h, t_h) = G(t, T_h, t_h)/G(t, T_h^*, t_h^*)\,. \qquad (3)$$

Figure 3 shows such an exemplary invariant curve at pH 7.0. Such T_h, t_h-invariant curves have been obtained for every pH value (2.5, 3.0, 3.5, 4.0, 5.5, 6.0, 7.0, 8.5, 10.0). These curves can be divided in to two groups. The first group (A) comprises curves at pH 2.5, 3.0, 3.5, 6.0, 7.0, 8.5, and 10.0; the second comprises curves at pH 4.0 and 5.5. For both groups, a pH, t_h, T_h-invariant relaxation curve $\tilde{G}_2(t)$ can be obtained by modulus reduction. The reduced modulus $\tilde{G}_2(t)$ is then determined as follows:

$$\tilde{G}_2(t) = \tilde{G}_1(t, \mathrm{pH})/b_2(\mathrm{pH}), \qquad (4)$$

and the modulus reduction parameter

$$b_2(\mathrm{pH}) = \tilde{G}_1(t, \mathrm{pH})/\tilde{G}_1(t, \mathrm{pH}_o) \qquad (5)$$

where pH_o is a standard pH value in a given interval.

Figure 4 shows pH, t_h, T_h-invariant relaxation curves $\tilde{G}_2(t)$ for both groups. They differ considera-

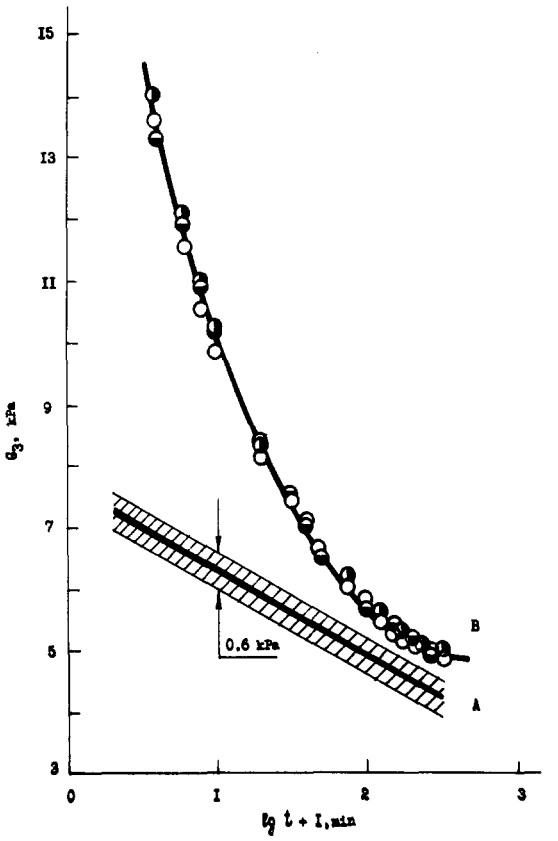

Fig. 5. Concentration invariant time dependences of relaxation modulus for ovalbumin thermotropic gels at pH 7.0 and 8.5 (group A) and at pH 4.0 (group B), under optimal conditions. Relation A results from reduction of 11 time dependences of relaxation modulus for gels with concentration of 8 % to 20 % (standard state – 12 % gel). The relation is approximated (straight line, heavily drawn) for 121 experimental points by the least square method. Thin lines show the maximum point deviation (± 0.3 kPa). Relation B results from reduction of 3 time dependences of relaxation modulus for gels with concentrations of 11 % (–◒–), 15 % (–○–) and 16 % (–◑–) (standard state, 15 % gel). It is shifted upwards by 2 kPa

bly. These data prove that the same relaxation mechanism operates within each group, whereas the relaxation mechanisms related to groups A and B are very different.

Concentration dependence of viscoelastic properties of gels

Relaxation curves of gels of different concentrations (C) from 8 % to 20 % under optimal conditions at pH 3.0, 7.0, 8.5, 10.0 (group A) and at pH 4.0 and 5.5 (group B) can be transformed (within each group) to a concentration-invariant curve $\tilde{G}_3(t)$ by modulus reduction

$$\tilde{G}_3(t) = G(t, C)/b_3(C), \tag{7}$$

with

$$b_3(C) = G(t, C)/G(t, C_o) \tag{8}$$

where C_o is a standard concentration.

As shown in Figure 5, the concentration-invariant curves of groups A and B are essentially different.

The b_3 reduction parameter is by definition proportional to the gel equilibrium modulus G_e [16]. Therefore, the concentration dependence of this parameter hints at a concentration dependence of the equilibrium modulus. To describe concentration dependence of the equilibrium modulus of gels formed due to random association of macromolecules, Hermans [17] proposed using the method of curve superposition. According to this method, an experimental concentration dependence of the equilibrium modulus is constructed in double logarithmic coordinates. Into this plot, a theoretic relation is introduced by

$$G_e \sim [(2 - W_g)(C/C^*) - 2]\,(C/C^*)W_g \tag{9a}$$

where

$$W_g \sim \{1 - \exp[-W_g(C/C^*)]\} \tag{9b}$$

C is the polymer concentration, while C^* is the polymer concentration in the gel-point, resulting from an extension of the simplest variant of the Flory-Stockmayer statistical theory of gelation for the random reversible association of macromolecules. Then, by parallel translation of experimental dependence along both axes, its optimal coincidence with the theoretical curve is achieved. The C^* gel-point is thereby determined as an antilogarithm of the displacement value along the concentration axis.

This procedure was used to describe concentration dependence of the equilibrium modulus G_e of the QA gels obtained under optimal conditions at pH 3.0, 7.0, 8.5, 10.0 (group A) and at pH 4.0 (group B), as well as in 6M urea (data by Van Kleef et al. [9]), on the basis of $G_e \sim b_3$ (Eq. [8]) (Fig. 6). In all cases, theory and experiment coincided at the same concentration in the gel point $C^* = 6.0 \pm 1.0$ %. Thus, in a first approximation, solvent quality (strong solvent 6 M urea, good solvent – group A, and poor solvent –

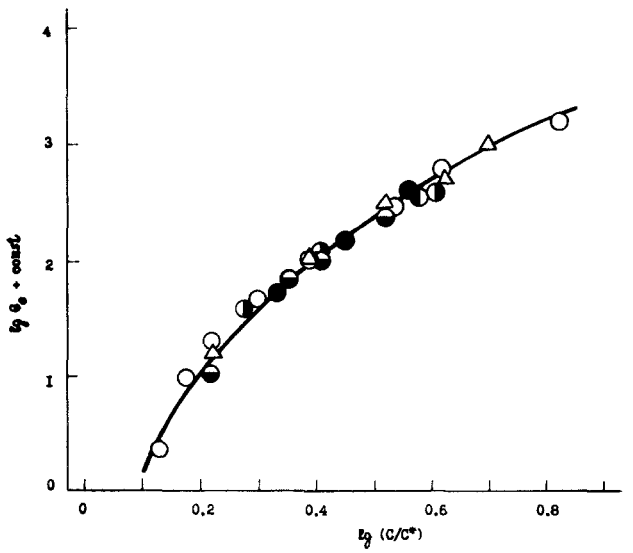

Fig. 6. Generalized concentration dependence of equilibrium modulus for ovalbumin thermotropic gels. Gels obtained under optimal conditions at pH 3.0 (—◐—), 4.0 (—◑—), 7.0 (—○—), 8.5 (—◒—), 10.0 (—●—) and in 6M urea (—△—) [9]. Solid line marks Hermans' theoretical relation [9a, b]

group B) exerts no effect upon critical gelation conditions.

Discussion

The study of the influence of pH on optimal temperature of geling OA solutions shows (Fig. 1) this temperature to be practically independent of pH to the right and left of the protein isoelectric point. Nevertheless it is known [18], that the denaturation temperature has a clear maximum around the isoelectric point. An increase in protein charge at increasing distance from the isoelectric point leads to a destabilization of the protein molecule which reduces the denaturation temperature. Thermal denaturation is thus a necessary, though insufficient, gelation condition. It is noteworthy that OA denatured molecules form gels in concentrated urea solution [9]. In this case, heating is also necessary for gelation. Minimum temperature is 60 °C, but the optimal is 85 °C. A stronger definition of necessary and sufficient conditions for OA gelation demands additional investigation.

Data on the influence of pH on mechanical properties of thermotropic gels show (Fig. 2) that electrostatic interaction of protein molecules has a large influence on the process of gelation. This influence is more pronounced at pH below the isoelectric point. The dependence of initial modulus of OA gels on pH probably has one maximum at the isoelectric point (pI 4.7). On the other hand, Egelandsdal found that the dependence of the rigidity of OA gels on pH has two maxima at the left and right of the isoelectric point [11]. This disagreement can evidently be explained by the fact that the results described in this work characterize gels that had been produced unter optimal heating conditions at each pH while Egelandsdal [11] studied the mechanical properties of the gels which have been formed in a fixed time-temperature mode (heating at a constant speed of 2.6 °/min to 90 °C with further cooling to 23 °C at a speed of 1 °/min), regardless of pH. However, as Figure 1 shows, optimal heating temperature increases in the isoelectric point region by at least 25 °C, evidently due to an increase in thermal stability of the native protein. This illustrates that the gelation conditions in [11] do not allow the process to proceed completely in the isoelectric point region. This is the reason for the two maxima at the right and left of the isoelectric point. It may be assumed that the deviation of pH from the isoelectric point retards gelation due to the action of coulomb repulsion forces between protein molecules. If an analogy between the coagulation of hydrophobic colloids and thermotropic gelation of proteins is possible, it may be deduced that coulomb repulsion forces act as an activation barrier in the gelation process and retard gelation. It is for this reason that, at a fixed heating time, the degree of cross-linking, and therefore gel modulus, should decrease with increasing charge of protein molecules at increasing distance from the isoelectric point.

The data show that the modulus of OA thermotropic gel (G) depends on thermal denaturation conditions (T_h, t_h), pH, concentration (C) and time of relaxation experiment (t). Within each group of gels (A or B) it is possible to unify these dependences and produce T_h, t_h, pH, C-invariant time dependences on the relaxation modulus by universal modulus reduction as the ratio of gel moduli obtained under different conditions does not depend on the time of the relaxation experiment

$$G(T_h, t_h, \mathrm{pH}, C, t)/G(T_h^*, t_h^*, \mathrm{pH}_o, C_o, t) \neq F(t)\,. \quad (10)$$

If the relaxation modulus is a product of the equilibrium modulus (G_e) on the relaxation function $f(t)$ [19], then for group A ($2.5 \leq \mathrm{pH} < 4.0$ and $5.5 < pH \leq 10.0$):

$$G(T_h, t_h, \mathrm{pH}, C, t) = G_e(T_h, t_h, \mathrm{pH}, C) \cdot f_A(t) \quad (11)$$

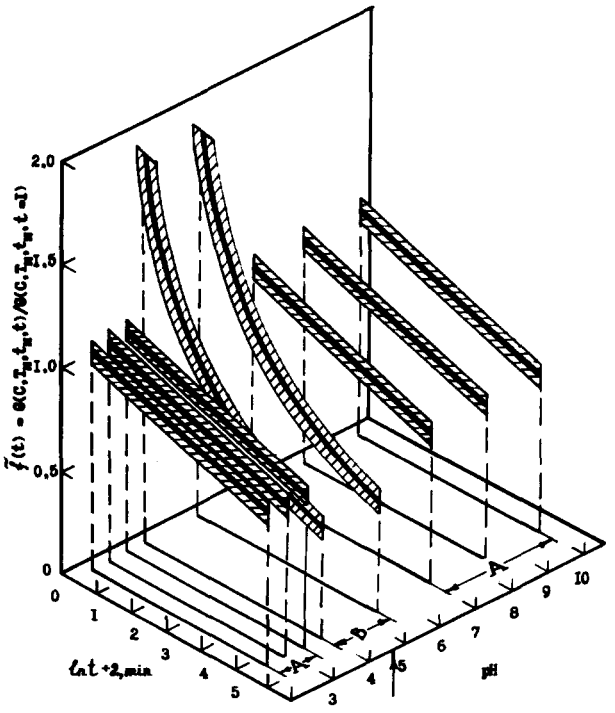

Fig. 7. Normalized relaxation functions of group A (360 points; standard deviation ± 0.02) and group B (54 points; standard deviation ± 0.02) ovalbumin thermotropic gels

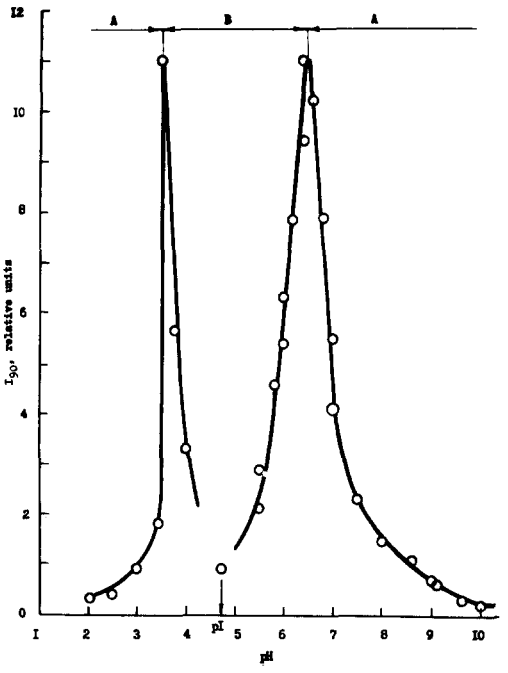

Fig. 8. pH dependence of light scattering intensity for 10 % ovalbumin gels at an angle of 90°. Gels produced under optimal conditions. A sharp drop in intensity in region B, points to an abrupt increase in back scattering, due to considerable turbidity of the system. Dependence maxima probably correspond to boundaries between zones of existence of homologous groups A and B of ovalbumin gels

and for group B ($4.0 \leq \mathrm{pH} \leq 5.5$)

$$G(T_h, t_h, \mathrm{pH}, C, t) = G_e(T_h, t_h, \mathrm{pH}, C) \cdot f_B(t) \quad (12)$$

while

$$f_A(t) \neq f_B(t) . \quad (13)$$

Thus, in each particular group of gels, the relaxation function does not depend on thermal denaturation conditions, pH, or protein concentration. Nevertheless, relaxation functions of different groups, i. e. $f_A(t)$ and $f_B(t)$, vary distinctly.

The entire plurality of obtained relaxation data may be reduced to two normalized relaxation functions (Fig. 7) for groups A and B:

$$\tilde{f}_A(t) = G(T_h, t_h, \mathrm{pH}, C, t)/G(T_h, t_h, \mathrm{pH}, C, t = 1) = f_A(t)/f_A(t = 1) \quad (14)$$

and

$$\tilde{f}_B(t) = G(T_h, t_h, \mathrm{pH}, C, t)/G(T_h, t_h, \mathrm{pH}, C, t = 1) = f_B(t)/f_B(t = 1) . \quad (15)$$

The fact that gels produced under varying conditions have the same relaxation spectrum within each group, proves them to be structural homologs, i. e. structures made up of identical elements. A change in gelation conditions affects the density of cross-links of structural elements; the elements themselves, however, remain unchanged from a dynamic point of view. Internal viscosity of structural elements does not depend on gelation conditions.

The difference in relaxation properties of gels of groups A and B is probably explained by the fact that their structures are different. Nephelometry data reveal that group A gels are optically more homogeneous than gels of group B (Fig. 8). It may be assumed that group A gels are single-phase systems, whereas group B gels are two-phase systems. The two-phase character of group B gels is explained by denser cross-linking of the structural elements, due to an accelearation of the gelation process at the isoelectric point, as a result of weakened coulomb repulsion forces between protein molecules. Ordinary polymer gels have also

been known to be two-phased when cross-linking density is high [20].

It is common knowledge that the concentration dependence of equilibrium modulus may be used to procure indirect information on the mechanism of gelation. In our case, experimental data on the influence of protein concentration on equilibrium elasticity of gels studied at every pH can be adequately described by the theory of Hermans' [9a, 9b]. From this, we can conclude that the model of random cross-linking of identical macromolecules without cyclization adequately corresponds to the essential process of thermotropic gelation of OA. It should be mentioned that experiments carried out for three substances: carboxymethylcellulose [17], soybean globulins [16] and ovalbumin have already proved this simple model of gelation to be realistic.

The theoretical description of concentration dependences for equilibrium modulus of OA gels made it possible to determine gel-points at different pH values. pH, at a first approximation, has no effect on gel-point location. The concentration $C^* = 6.0 \pm 1.0$ % corresponds to it. This result is difficult to understand as the location of the gel-point is determined by the functionality of particles that must change with a change in their charge. A possible reason of this contradiction is probably a low accuracy of gel-point determination by the curve superposition method. This makes it necessary to determine experimentally the OA gel-point at various pH values.

Conclusion

1. The existence of two homologous groups of ovalbumin thermotropic gels has been established. Group A is made up of single-phase gels, and group B, of two-phase gels. Group A gels are produced at pH ranging from 2.5 to 3.5 and from 6.0 to 10.0. Group B gels are produced at pH ranging from 4.0 to 5.5. Within each homologous group, gels have the same spectrum of relaxation times. This remains unchanged under varying thermal denaturation conditions, pH and protein concentrations. These variations affect the equilibrium elasticity of gels, but not their dynamic properties.

2. A characteristic of OA thermotropic gels is that their relaxation properties may be invariant to protein concentration. Evidently, relaxation of stress in gels is caused by dynamic processes at the level of individual structural elements, i. e. their internal viscosity. In this respect, OA thermotropic gels are not unlike soybean globulin thermotropic gels.

3. At a first approximation, OA thermotropic gelation can be regarded as a process of random association of identical polyfunctional particles without cyclization.

4. To appreciate the significance of a particular factor in the viscoelasticity of protein thermotropic gels, it is necessary to take into account the influence it has on optimal thermal denaturation conditions which correspond to maximum elasticity modulus value. It is only through comparison of optimally-produced gels that the true regularities may be obtained.

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Authors' address:

V. Ya. Grinberg
Institute of Organo-Element Compounds, USSR
Academiy of Sciences
Vavilov-Str. 28
117 312 Moscow V-312, U.S.S.R.