Fitch, W. L.; Dolinger, P. M.; Djerassi, C. *J. Org. Chem.* **1974**, *39*, 2974-2979.
Keeler, R. F. *Teratology* **1973**, *7*, 31-35.
Keeler, R. F. *Lloydia* **1975**, *38*, 56-86.
Keller, W. J., unpublished data, 1984.
Keller, W. J.; Hatfield, G. M. *Phytochemistry* **1982**, *21*, 1415-1417.
Keller, W. J.; Zelenski, S. G. *J. Pharm. Sci.* **1978**, *67*, 430-431.
Kinghorn, A. D.; Selim, M. A.; Smolenski, S. J. *Phytochemistry* **1980**, *19*, 1705-1710.
Kingsbury, J. M. "Poisonsous Plants of the United States and Canada"; Prentice-Hall: Englewood Cliffs, NJ, 1964; pp 333-341.
Mears, J. A.; Mabry, T. J. In "Chemotaxonomy of the Leguminnosae"; Harborne, J. B., Boulter, D., Turner, B. L., Eds.; Academic Press: London and New York, 1971; pp 73-178.
Shupe, J. L.; Binns, W.; James, L. F.; Keeler, R. F. *Am. Vet. Med. Assoc.* **1967**, *151*, 198-203.
Steinegger, E.; Wicky, K. *Pharm. Helv. Acta* **1965**, *40*, 610-619.
Von Euler, H.; Erdtmann, H. *Liebigs Ann. Chem.* **1935**, *520*, 1-7.

Received for review February 4, 1985. Accepted May 20, 1985. This research was supported, in part, by a grant from the Northeast Louisiana University (NLU) Faculty Research Fund.

A Study on Gelation of Soybean Globulin Solutions. 5. The Effect of Protein Concentration on the Extent of Conversion in Gelation Process According to Data of Sol-Analysis

T. Bikbov, V. Grinberg,* A. Danilenko, T. Chaika, I. Vaintraub, and V. Tolstoguzov

A study has been undertaken on the effect of protein concentration, C, on gel yield, G, in the process of thermotropic gelation of three soybean globulin preparations (5% of β-conglycinin + 95% of glycinin; 85% of β-conglycinin + 15% of glycinin; 50% of β-conglycinin + 50% of glycinin) at pH 7. G equals zero until a certain concentration C^* and then increases with concentration to some constant level. The master concentration dependences of G vs. $\tilde{C} = C/C^*$ for the preparations are the same at low $\tilde{C}$ and are different at high $\tilde{C}$. The initial slope of these dependences equals 2. Sol fractions of all preparations consisted of acidic subunits of glycinin. It has been suggested that gelling ability of proteins should be estimated by the value of the average hydrophobicity parameter $H\phi_{av}$ according to Tanford-Bigelow. The gelling ability of the monomeric forms of main soybean globulins diminishes in the series 2.8S globulin > B > β > α > A, where A and B are acidic and basic subunits of glycinin and α and β are heavy and light subunits of β-conglycinin.

INTRODUCTION

At the present time a thermotropic gelation of proteins is being studied widely in connection with the great significance of this phenomenon for food technology. A number of studies (Babajamopoulus et al., 1983; Mori et al., 1982a,b; Nakamura et al. 1983, 1984a,b; Utsumi et al., 1983) was devoted to the elucidation of the molecular mechanism of thermotropic gelation. One can also mention the works of van Kleef et al. (1978) and Richardson and Ross-Murphy (1981a,b), where the methodology of modern physical chemistry of polymers was applied to study some interesting aspects of protein gelation. In all of these works only some properties of gel networks were considered. However, it is well-known that the gelation of polymers leads to the formation not only of networks but also some soluble products (the finite clusters) (Flory, 1953). He calls these products the sol-fraction of the gel. The investigation of qualitative and quantitative aspects of the sol-fraction formation permits us to understand some important details of the gelation mechanism (Irzshak et al., 1979).

In this report the qualitative and quantitative aspects of sol-formation for thermotropic gelation of soybeans globulins are considered. Since this approach to studying thermotropic gelation of proteins is being stated for the first time, some general information from the physical chemistry of polymers is cited in the paper to facilitate understanding the data obtained.

The most important characteristics of the gelation process appear to be the content of a soluble sol-fraction (S) in the gel and its equilibrium elasticity modulus (E_e) (Flory, 1953). Sol-fraction content and the modulus have similar information but not identical. They outline the extent of conversion in this process. The lower the content of sol-fraction and the greater the modulus the higher is the extent of conversion. The value (1 - S), which is termed as gel yield (G), may be identified to a larger degree with the extent of conversion, while the modulus depends not only upon the extent of conversion, but also upon the gel structure.

The statistical theory considers gelation as a result of the formation of multiple random bonds between the particles that can be either polyfunctional monomers or macromolecules. It provides a connection between gel yield of the elasticity modulus and the extent of conversion for random bonds (p). Analysis of experimental dependences $G(p)$ and $E_e(p)$ makes it possible to study the gelation mechanism in the given system.

In the case of thermotropic gelation of polymers, the extent of conversion in the reaction of association or aggregation of macromolecules is usually unknown. It directly relates to the concentration of a polymer in com-

Institute of Organo-Element Compounds, USSR Academy of Sciences, 117312 Moscow, USSR (T.B., V.G., A.D., and V.T.), and Laboratory of Protein Chemistry, State University, Kishinyov, USSR (T.C. and I.V.).

0021-8561/85/1433-0912$01.50/0

pliance with the laws of chemical equilibrium or chemical kinetics. However, this relation is difficult to find in its qualitative form.

Hermans (1965) suggested that the extent of conversion in the process of association is proportional to polymer concentration (C), i.e.,

$$p \propto C \quad (1)$$

He substantiated this assumption for a specific case, when the formation of a gel network junction appears to be a bimolecular equilibrium process.

In this case, at a low extent of conversion

$$p = (Kf/M)C \quad (2)$$

where K is the constant of bond formation between two functional groups, f is the number of functional groups in a macromolecule, and M is its weight.

Proceeding from the indicated assumption, Hermans extended the theory of cross-linking identical macromolecules (Flory, 1953) as applied to thermotropic gelation of polymers. The main result of this theory is a master dependence of the equilibrium modulus of gel on the reduced concentration of polymer

$$\tilde{C} = C/C^* \quad (3)$$

where C^* at gel point ($E_e = 0$ at $C \leq C^*$):

$$E_e \propto [(2 - G)\tilde{C} - 2]G\tilde{C} \quad (4)$$

and

$$G \propto [1 - \exp(-G\tilde{C})] \quad (5)$$

Comparison of the experimental dependence $E_e(C)$ with the master dependence $E_e(\tilde{C})$ may be made by the curve superposition method in a logarithmic scale.

In the previous reports (Bikbov et al., 1979b, 1981) we employed this approach for analyzing the concentration dependence of the equilibrium modulus for thermotropic gels of soybean globulin fraction. As a result, we obtained very good agreement between the experiment and Hermans' theory over a wide range of concentrations ($7.0 \leq C \leq 58.0\%$) with a reasonable value of gel point $C^* = 6\%$. This agreement formed the basis for considering, as a first approximation, thermotropic gelation of globulin fraction as a process of cross-linking of identical macromolecules.

For a more comprehensive substantiation, it was deemed necessary to study the effect of protein concentration on the other fundamental characteristic of the gelation process—gel yield $G = (1 - S)$, which reflect more unambiguously the extent of conversion in this process. Apart from this, it seemed of interest to elucidate the role of the basic components of globulin fraction, 2.8 S globulin, β-conglycinin, glycinin, or their subunits in the gelation process. The results of this investigation by the methods of sol-analysis and gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol are presented in the present report.

EXPERIMENTAL SECTION

Materials. Glycinin (preparation 1) and β-conglycinin (preparation 2) were isolated by the methods of Thanh and Shibasaki (1976), soybean globulin fraction (preparation 3) by precipitation at the isoelectrical point at pH 4.5 (Wolf, 1972), and globulin with a sedimentation constant 2.8 S (preparation 4) by the method of Vaintraub and Shutov (1969). The composition of the preparations was determined by the planimetry method on the base of deconvaluation of the sedimentograms in series of Gaussians (Table I). Preparations 1, 2, and 3 can be regarded as mixtures of glycinin and β-conglycinin of different composition. It may be expected that the behavior of preparation 1 is comparable to that of glycinin, while the behavior of preparation 2 is to that of β-conglycinin.

Table I. Sedimentation Composition of Soybean Globulin Preparations (wt %)

	sedimentation components			
preparation	2 S	7 S	11 S	15 S
1		5	95	
2		85	15	
3[a]	7	42	42	9
4[b]	100			

[a] To simplify comparison between the behavior of preparations 1, 2, and 3, it was assumed that preparation 3 represents a 1:1 mixture of 7 S and 11 S components by weight. [b] Preparation 4 was also homogeneous according to data of gel chromatography and gel electrophoresis.

Thermotropic Gelation. Gels were obtained by heating protein solutions in distilled water (pH 7.0) on a boiling water bath for 30 min followed by cooling to room temperature and holding at a temperature 4 °C for 24 h. This procedure is optimal for a soybean globulin fraction in reference to the elasticity modulus value for gel (Bikbov et al., 1979a).

Sol-analysis. Sol-fraction was extracted from finely cut gel (weighing about 100 mg) with 12 mL of standard phosphate buffer (pH 7.2, ionic strength 0.5) for 15–18 h in a slowly rotating vessel at 20 °C. Longer mixing, preliminary grinding of gel, as well as replacement of the buffer with water exerted no effect whatsoever on the amount of the sol-fraction extracted. Protein content in the extract was determined after the centrifugation (20 000g, 30 min) by the microbiuret method (Itzhaki and Gill, 1964). The calibration curve was plotted by using glycinin. At least three gel samples were prepared for each protein concentration. The content of a sol-fraction was determined three times in each sample. A standard deviation in determining sol-fraction content was ± 0.015.

Gel Electrophoresis. Electrophoresis was carried out in a block of polyacrylamide gel with a concentration gradient from 7.0 to 42.0% at a pH 8.3 (tris-glycine buffer + 0.1% of sodium dodecyl sulfate) (Laemmli, 1970). Preliminary dissolution of thermotropic gels and denaturation of proteins were performed in a solution of sodium dodecyl sulfate and 2-mercaptoethanol with concentrations 2 and 0.5%, respectively, at a temperature of 100 °C for 2 min. The molecular weights of electrophoretic components were assessed by the mobility of reference proteins (bovine serium albumin, ovalbumin, carboxypeptidase, Kunitz' inhibitor of trypsin from soybeans) and glycinin subunits. The average molecular weights of basic and acidic subunits of glycinin are equal to 20.8 and 37.7 kD, respectively (Catsimpoolas et al., 1971; Badley et al., 1975).

RESULTS

Qualitative Composition of Sol-Fraction. The method of gel electrophoresis was employed to determine the qualitative composition of the initial preparations and sol-fractions of their gels with a 20% concentration. These data are schematically presented in Figure 1.

Preparation 1 contains two electrophoretic components which can be identified with basic (B) and acidic (A) subunits of glycinin.

Preparation 2 contains four basic electrophoretic components. The mobility of the two fast-moving components coincides with that of glycinin subunits (preparation 1). The presence of these components apparently results from glycinin contained in preparation 2. However, it is quite possible that the two fast-moving zones also contain low

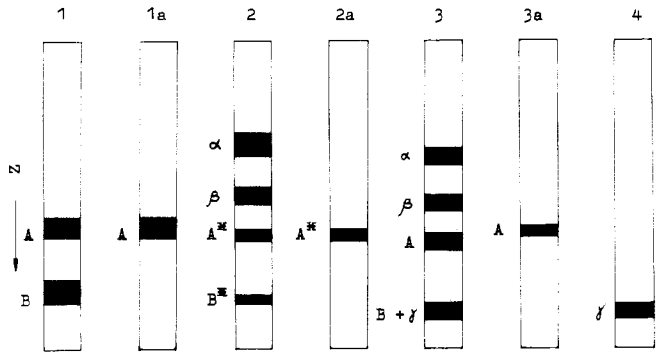

Figure 1. Schematic representation of data on qualitative analysis of the composition of initial soybean globulin preparations and their sol-fractions by gel electrophoresis in the presence of sodium dodecyl sulface and 2-mercoptoethanol. The scale is preserved in respect with migration axis Z. (1) Preparation 1; (1a) sol-fraction of preparation 1; (2) preparation 2; (2a) sol-fraction of preparation 2; (3) preparation 3; (3a) sol-fraction of preparation 3; (4) 2.8 S globulin. Designations of zones: A and B, acidic and basic subunits of glycinin; α and β, heavy and light subunits of β-conglycinin; γ, 2.8 S globulin. Admixed components are marked by an asterisk. Sol-fractions are extracted from 20% gels.

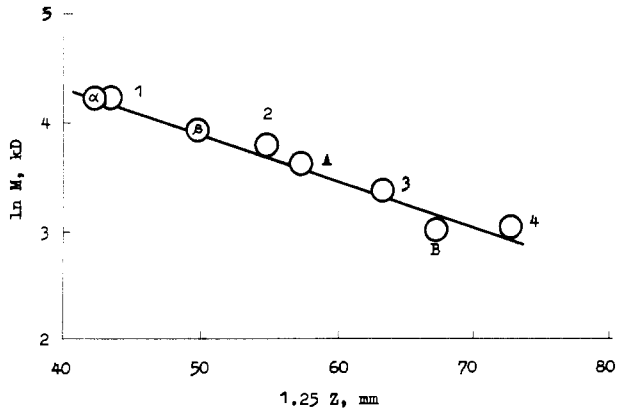

Figure 2. Correlation between molecular weight and mobility of electrophoretic components A, B, α, and β of preparation 2. The figure also presents data for reference proteins: ovalbumin (1), bovine serum albumin (2), carboxypeptidase (3), Kunitz' inhibitor of trypsin from soybeans (4).

molecular products of the partial posttranslation processing of α and β subunits of β-conglycinin (Chrispeels et al., 1982). The data on the molecular weights of β-conglycinin subunits appear to be contradictory (Koshiyama, 1972; Vaintraub and Shutov, 1972; Thanh and Shibasaki, 1977; Iibuchi and Imahori, 1978; Hoshi et al., 1982); this may be connected with the specific features of the original beans or inadequate experimental conditions. For this purpose, in order to identify the other two components in preparation 2, a calibration curve was plotted for the given sample of polyacrylamide gel by using A and B subunits of glycinin as internal standards (Figure 2). Thus, the molecular weights of the latter two electrophoretic components in preparation 2 were found to be about 68 and 52 kD, respectively. They are comparable with the molecular weights of α and β subunits of β-conglycinin, according to Iibuchi and Imahori (1978). Therefore, the first and second electrophoretic components of preparation 2 may be identified with α and β subunits of β-conglycinin.

The electrophoretic diagram of preparation 3 consists of four zones. The last three zones can be referred to α and β subunits of β-conglycinin and A subunits of glycinin. The first fast-moving zone obviously contains 2.8 S globulin in addition to B subunits of glycinin (compare electrophoretic diagrams 1, 3, and 4).

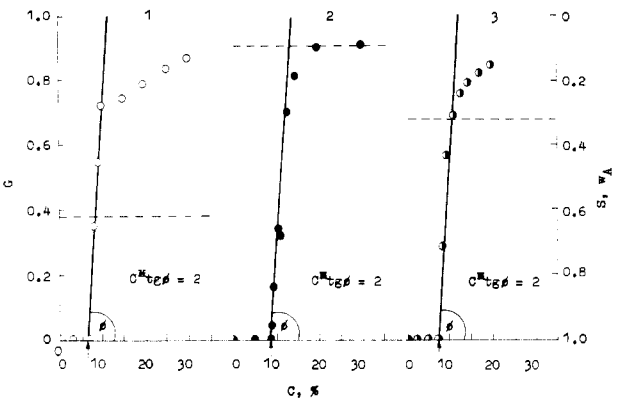

Figure 3. Dependences of gel (G) and sol (S) fraction yields on protein concentration in the process of thermotropic gelation of preparations 1, 2, and 3. Initial tangents of these dependences are designated by thin lines. Arrows designate gel points (C^*); dotted line designates weight fraction (w_A) of acidic subunits of glycinin in the given preparation (right ordinate).

Sol-fractions of all preparations predominantly contain A subunits of glycinin. Thus, among all the products of thermal decomposition of soybean globulins the A subunits of glycinin are characterized by the lowest conversion in the gelation process. Previously, the sol-fractions of soybean globulin fraction gels were found to contain a component with a sedimentation coefficient of about 2 S (Bikbov et al., 1981) which was taken for a 2 S component of globulin fraction. This reference does not seem unambiguous. According to gel electrophoretic data the component with a sedimentation coefficient of 2 S found in the sol-fraction is more likely a monomeric form of A subunits of glycinin.

The Effect of Protein Concentration on Gel Yield. The concentration dependences of gel yield in the process of thermotropic gelation of preparations 1, 2, and 3 are presented in Figure 3. They possess a number of common features characteristic of dependences $G(p)$ in the statistical theory of cross-linking macromolecules, assuming that $p \propto C$; they possess a number of peculiarities which have no analogies in the indicated theory.

Let us first consider the common features of the experimental dependences $G(C)$. They are characterized by a distinct gel point C^* (marked by an arrow). Up to this concentration the gel yield equals zero, while above this level it grows monotonically with concentration.

According to their gel point values the preparations under study can be arranged in a series $1 < 3 < 2$. Apparently, there is an inverse correlation between the gel point value and glycinin content in the preparation. Among soybean globulins, glycinin seems to have the lowest gel point and β-conglycinin the highest. The existence of a gel point appears to be one of the characteristic features of random cross-linking macromolecules (Gordon, 1974). In this connection it can be said that there is a similarity between the processes of thermotropic protein gelation and random cross-linking macromolecules.

Another characteristic feature of all the dependences $G(C)$ is that their reduced initial slope $C^*(dG/dC)_{C\to C^*} = C^*tg\varphi = 2$. Such a value of the initial slope is characteristic of the theoretical dependence $G(p)$ in the case of cross-linking identical macromolecules (Gonzalez and Daud, 1981).

At the end of the investigated range of concentrations, the experimental dependence $G(C)$ of the preparations under study are greatly different. The dependence $G(C)$ of preparation 2 is practically approximating a constant level ($G \simeq 0.9$) which corresponds to the content of A

Table II. The Value of Hydrophobicity Parameter $H\phi_{av}$ for Monomeric Forms of Main Soybean Globulins

protein	monomeric form	$H\phi_{av}$, cal/residue
2.8 S globulin		1171
β-conglycinin	α	908
	β	964
	A	857
glycinin	B	1030

subunits of glycinin in this preparation ($\bar{w}_A \simeq 0.1$). This is an unusual factor, since, by definition, the gel yield should tend toward 1 with increases in the degree of cross-linking or in polymer concentration. An impression is formed that A subunits of glycinin do not participate in the gelation of preparation 2.

The experimental dependence $G(C)$ of preparations 1 and 3 do not approach a constant level in the investigated range of concentrations. At the same time, they cross the level which corresponds to the content of low active A subunits of glycinin in them (marked by a dotted line on Figure 3). Consequently, A subunits participate in the gelation of preparations 1 and 3. Apparently, the extent to which A subunits participate in the gelation process increases in the series $2 < 3 < 1$. It may be also noted that the rate of the gel yield approaching to a constant level is decreasing with an increase of protein concentration.

DISCUSSION

Correlation between the Gelling Ability and the Effective Hydrophobicity of Soybean Globulin Monomeric Forms. It may be assumed that thermotropic gelation results from the amphiphilic nature of proteins. In this respect, it has a remote resemblance with renaturation. Above its denaturation temperature the protein molecule is apparently in the conformation of random coil since under these conditions water is a good solvent for its nonpolar units. The conformation of random coil is unstable at lower temperatures, when water is a poor solvent in respect to these units. Due to this fact, the protein molecule is spontaneously folded, i.e., it is renatured if protein concentration is sufficiently low. If this condition is not satisfied, the protein molecules in addition to folding are aggregated. The aggregation leads finally to gelation. Thus, the folding and aggregation of proteins are apparently characterized by moving forces of the same type. Both processes are thermodynamically equivalent to the transfer of nonpolar amino acid residues from aqueous medium into the hydrophobic interior of a globule or aggregate of protein molecules. Proceeding from the analogy between folding and the indicated transfer, Tanford suggested that the tendency of protein to folding or the stability of its globular conformation should be evaluated by the mean value of free transfer energy ($H\phi_{av}$) (Tanford, 1962; Bigelow, 1967). This value is usually calculated in approximation of independent group contributions on the basis of amino acid composition of protein and experimental data on the thermodynamics of transfer for various model compounds from ethanol to water (Tanford, 1962). This approach can apparently be employed for a comparative evaluation of protein tendency to thermotropic gelation, since there is a certain analogy between folding and gelation.

Computation of the value $H\phi_{av}$ for monomeric forms of the main soybean globulins included the use of data on their averaged amino acid composition, according to Catsimpoolas et al. (1971) and Iibuchi and Imahori (1978), and the values for group contributions of amino acid residues, according to Tanford (1962). The obtained values for the parameter $H\phi_{av}$ are presented in Table II. In terms of the value of the parameter $H\phi_{av}$ the monomeric forms of the main soybean globulins may be arranged in the following series: 2.8 S > B > β > α > A. The tendency to gelation in this series can be expected to diminish, i.e., 2.8 S globulin and B subunits of glycinin possess the greatest gelling ability, while A subunits of glycinin have the least. As far as A subunits are concerned, this conclusion is qualitatively consistent with the data on the composition of sol-fractions in the gels of preparations investigated by gel electrophoresis. Below, it will be qualitatively confirmed that the gelling ability of B subunits of glycinin actually surpasses that of α and β subunits of β-conglycinin. Thus, as a first approximation, the parameter $H\phi_{av}$ can be employed for a comparative evaluation of the gelling ability for at least related proteins. This approach is certainly as imperfect as evaluating the stability of the native conformation of globular proteins by the value of the parameter $H\phi_{av}$. It does not take into account such specific effects as partial restoration of the secondary structure, nor the role of thiol–disulfide exchange in the process of thermotropic gelation.

Comparing the Gel Points of Investigated Preparations. The gel points C^* of preparations 1, 2, and 3 equal 7, 9, and 8%, respectively. It has been suggested that the gel point should be evaluated as a boundary concentration of overlapping protein molecules (C_o) in the conformation of unperturned random coil; it is determined as an inverse value for the intrinsic viscosity of protein in this state (Bikbov et al., 1981). However, in the case of the investigated preparations the gel point is naturally higher than the boundary concentration of overlapping, i.e.,

$$C^* > C_o \tag{6}$$

[The method for determination C_o has been described in the report by Bikbov et al. (1981).] This result is consistent with physical sense, since it is above C_o, where macromolecules are considerably overlapped, there are necessary conditions for their multiple interactions which, strictly speaking, lead to gelation. Evidently, the statistical theory of cross-linking macromolecules will provide a more precise determination of gel point. A version of this theory for cross-linking macromolecules with different functionalities should apparently be applied to the investigated preparations, which prior to gelation appear to be mixtures of monomeric forms with different gelling abilities. In this case the critical extent of conversion, corresponding to the gel point, equals (Irzshak et al., 1979)

$$\bar{p}_c = (\sum_i \bar{w}_i f_i - 1)^{-1} \tag{7}$$

where w_i and f_i are the weight fraction and functionality of the i component. If, according to Hermans (1965), we assume that

$$\bar{p}_c = kC^* \tag{8}$$

where k is a certain constant, we can obtain the dependence of a gel point value on the mixture composition.

$$(C^*)^{-1} = k(\sum_i \bar{w}_i f_i - 1) \tag{9}$$

As a first approximation, the preparations under study can be regarded as mixtures of glycinin (1) and β-conglycinin (2). In this case

$$(C^*)^{-1} = k(\bar{w}_1 f_1 + \bar{w}_2 f_2 - 1) \tag{10}$$

Assuming that for pure components

$$(C_i^*)^{-1} = k(f_i - 1)$$

$$(i = 1, 2) \tag{11}$$

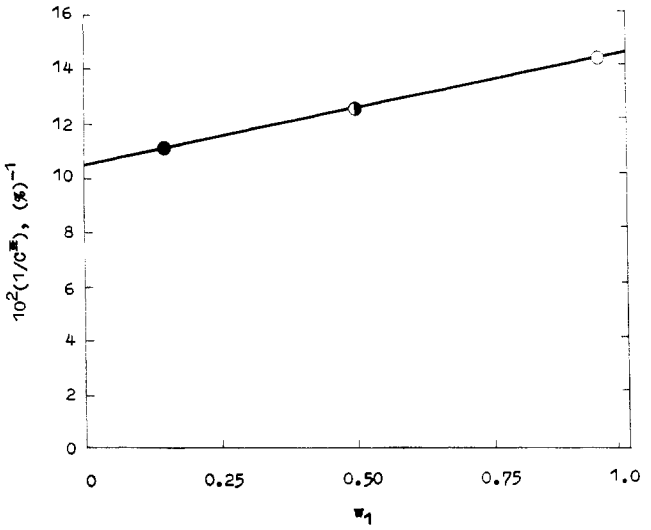

Figure 4. Correlation between the inverse value of gel point concentration (C^*) for the investigated soybean globulin preparations and weight fraction (w_1) of glycinin in them according to the eq 12. (○) Preparation 1; (●) preparation 2; (◑) preparation 3.

the gel point of the prepataion can be expressed through its composition and the gel points of the pure components

$$(C^*)^{-1} = \bar{w}_1(C_1^*)^{-1} + \bar{w}_2(C_2^*)^{-1} \quad (12)$$

This relationship is consistent with experimental data (Figure 4). It provides a means for assessing the gel point value for glycinin ($C_1^* = 6.8\%$) and β-conglycin ($C_2^* = 9.5\%$).

The relationship (9) is also applicable to pure glycinin and β-conglycinin, considering them as mixtures of subunits having certain functionalities. In the case of glycinin

$$(C_1^*)^{-1} = k(\bar{w}_A f_A + \bar{w}_B f_B - 1) \quad (13a)$$

where w_A and w_B are weight fractions of A and B subunits and f_A and f_B are their functionalities. On the other hand, in the case of β-conglycinin

$$(C_2^*)^{-1} = k(\bar{w}_\alpha f_\alpha + \bar{w}_\beta f_\beta - 1) \quad (13b)$$

where w_α and w_β are weight fractions of α and β subunits and f_α and f_β are their functionalities. Since

$$(C_2^*)^{-1} < (C_1^*)^{-1} \quad (14)$$

then

$$f_B - (\bar{w}_\alpha f_\alpha + \bar{w}_\beta f_\beta) > (f_\beta - f_A)\bar{w}_A \quad (15)$$

According to the data of gel electrophoresis the gelling ability of B subunits is higher than that of A subunits, i.e.,

$$f_B > f_A \quad (16)$$

Therefore

$$f_B > \bar{w}_\alpha f_\alpha + \bar{w}_\beta f_\beta = f_2 \quad (17)$$

i.e., the gelling ability of B subunits of glycinin is higher than the average gelling ability of β-conglycinin. This result agrees with the above-mentioned series for the gelling ability of monomeric forms of the main soybean globulins in the value of the parameter $H\phi_{av}$.

Analysis of Master Concentration Dependences of Gel Yield. Various versions of the statistical theory for cross-linking macromolecules determine gel yield as a certain function of the reduced extent of conversion, p/p_c (Irzshak et al., 1979). The form of this function reflects the specificity of the given model for gelation. The

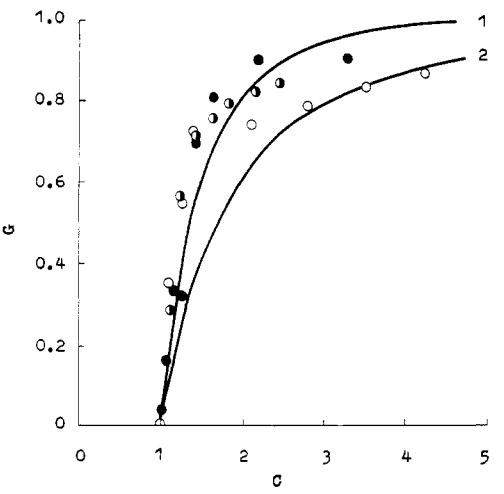

Figure 5. Master concentration dependences of gel yield in the process of thermotropic gelation of soybean globulin preparations. (○) Preparation 1; (●) preparation 2; (◑) preparation 3. Curve 1, theoretical master concentration dependence of gel yield for the process of random association of identical macromolecules (eq 18). Curve 2, theoretical master concentration dependence of gel yield for the process of random association of macromolecules identical chemically, but differing in functionality, with their functional distribution having the form of Flory's distributions (eq 19).

problem concerning the effect exerted by functional heterogeneity of macromolecules chemically identical on the form of the dependence $G(p/p_c)$ has been particularly well studied. Specifically, it is known that as the functional distribution grows wider as the initial slope of this dependence diminishes and its approximation to the asymptote $G = 1$ is slowed down. Consequently, at the given value of the reduced extent of conversion, the wider the functional distribution of macromolecules the lower is the gel yield.

In the case of thermotropic gelation, it is also possible to plot the master function for gel yield, if the reduced extent of conversion is replaced by its equivalent, that is by the reduced concentration $\tilde{C} = C/C^*$ (Hermans, 1965). Such dependences for the soybean globulin preparations under study are presented in Figure 5. This figure also displays the theoretical dependences $G(\tilde{C})$ for a model of cross-linking macromolecules identical chemically and functionally (curve 1, Irzshak et al., 1979)

$$G = 1 - \exp(-G\tilde{C}) \quad (18)$$

and for a model of cross-linking macromolecules identical chemically, but having rather wide continuous functional distribution, which is known as Flory's distribution (curve 2, Irzshak et al., 1979).

$$(1 - G) - (1 - G)^{0.5} = 2(\tilde{C})^{-1} \quad (19)$$

The experimental dependence $G(\tilde{C})$ of preparation 1 coincides in the initial section with theoretical curve 1 (monodisperse model) and in the final section with theoretical curve 2 (polydisperse model). This result contradicts the statistical theory of cross-linking chemically identical macromolecules. According to this theory, the heterogeneity of macromolecules in functionality is to manifest itself in the same way both in the initial and final sections of the dependence $G(\tilde{C})$. If the system is as heterogeneous in functionality as $G(\tilde{C} = 3) = 0.75$, the initial slope of the dependence $G(\tilde{C})$ should be significantly lower than 2 (Gonzales and Daud, 1981). This conclusion may also be reached by using more real discrete functional distribution of components of preparation 1. Assuming that it consists only of A and B subunits of glycinin having

the functionality f_A and f_B, the master concentration dependence of gel yield will have the following form (Flory, 1953).

$$G \simeq \bar{w}_A(1 - \tilde{C}G/f_1)^{f_A} + \bar{w}_B(1 - \tilde{C}G/f_1)^{f_B} \quad (20)$$

where $f_1 = w_A f_A + w_B f_B$ is a weight average functionality of the mixture. Analysis of this relationship shows that $G \simeq 0.75$ at $\tilde{C} = 3$ if the functionality of A and B subunits differ 5-fold. However, in this case the initial slope of the dependence $G(\tilde{C})$ should be equal to 1.2–1.3.

The dependence $G(\tilde{C})$ of preparation 2 also coincides with theoretical curve 1 only in the initial section. As a whole, the dependence approximates the asymptote more rapidly than the theoretical curve. It is important in this case that the asymptotic value of the experimental dependence is equal to about 0.9, while the theoretical curve tends to 1 at higher $\tilde{C}$. This behavior of preparation 2 may result from the presence of glycinin A subunits in it, which in this case hardly participate in gelation.

The dependence $G(\tilde{C})$ of preparation 3 approximates theoretical curve 1 to a larger extent than similar dependences of the other preparations. However in this case, the theory is only roughly consistent with experiment. Deviation of experimental points from the theoretical curve is rather significant. The experimental points are scattered relative to the theoretical curve more or less at random, whereas in the case of preparations 1 and 2 they lie in a certain order, either under theoretical curve 1, or above it.

Thus, according to sol-analysis, there is a certain analogy between the behavior of preparation 3 (soybean globulin fraction) and the model for cross-linking identical macromolecules. It is noteworthy that among the date on the concentration dependence of the equilibrium modulus for soybean globulin fraction thermotropic gels this anagy is manifested in a more precise sense (Bikbov et al., 1981). As a whole, the behavior of preparation 3 appears to be intermediate between the behavior of preparations 1 and 2. This regularity apparently arises from the fact that the content of A subunits in preparation 3 is not so high as in preparation 1, but also it is not so low as in preparation 2.

Summarizing the data on the behavior of the preparations under study within the framework of the statistical theory for cross-linking chemically identical macromolecules, it should be noted that this theory describes only qualitatively certain features of thermotropic gelation of these preparations near the gel point, i.e., when the extent of conversion in the association process is rather low. At the same time, it does not explain the behavior of the investigated preparations with a higher extent of conversion. It can be expected that a theory that takes into account the chemical heterogeneity of macromolecules participating in the gelation process would have been more successfully used. However, such a theory has not been developed yet in a complete form. Thus, Gonzalez and Daud (1981) employed the methods of percolation theory for describing the gelation of a mixture of two chemically nonidentical polymers. However, the theory of Gonzalez–Daud as well as the other percolation theories considers the behavior of the system only in proximity of the gel point, i.e., in the region which in our case is well described by the mean field theory of cross-linking according to Flory–Stockmayer.

However, certain qualitative considerations can be adduced concerning the role of chemical heterogeneity of macromolecules and the specificity of some intermolecular interactions connected with this in the investigated processes of thermotropic gelation.

Let us first consider the gelation of preparation 1 which can be conditionally represented as a mixture of A and B subunits of glycinin. Simplified schemes for elementary stages of the association processes in this system can be written in the following way:

$$A + A \xrightarrow{k_{AA}} A\text{–}A \quad (21)$$

$$A + B \xrightarrow{k_{AB}} A\text{–}B \quad (22)$$

$$B + B \xrightarrow{k_{BB}} B\text{–}B \quad (23)$$

where k_{AA}, k_{AB}, and k_{BB} are rate constants. Let us assume that

$$k_{AB} \sim k_{BB} > k_{AA} \quad (24)$$

This means that the first process, selfassociation of A subunits, proceeds much more slowly than the other two processes. For this reason, at relatively low conentrations (in proximity of the gel poir.t) the kinetically similar processes of self-association of B subunits and the formation of mixed associates of the AB type prevail. As a result, the system behaves as an ensemble of identical macromolecules subjected to random cross-linking. This is manifested by the fact that the initial slope of the master dependence $G(\tilde{C})$ equals 2. At high concentrations, as the availability of highly active B subunits is exhausted, the selfassociation of A subunits begins to manifest itself. The extent to which A subunits participate in gelation slowly increases with concentration due to a low rate constant k_{AA}. This is responsible for slow approximation of gel yield to 1.

In the case of preparation 2, two groups of single type processes may also be distinguished: rapid and slow. The rapid processes include

$$\alpha + \alpha \xrightarrow{k_{\alpha\alpha}} \alpha\text{–}\alpha \quad (25)$$

$$\alpha + \beta \xrightarrow{k_{\alpha\beta}} \alpha\text{–}\beta \quad (26)$$

$$\beta + \beta \xrightarrow{k_{\beta\beta}} \beta\text{–}\beta \quad (27)$$

$$\alpha + \beta \xrightarrow{k_{\alpha\beta}} \alpha\text{–}\beta \quad (28)$$

$$\beta + B \xrightarrow{k_{\beta\beta}} \beta\text{–}B \quad (29)$$

$$A + B \xrightarrow{k_{AB}} A\text{–}B \quad (30)$$

and the slow processes include

$$\alpha + A \xrightarrow{k_{\alpha A}} \alpha\text{–}A \quad (31)$$

$$\beta + A \xrightarrow{k_{\beta A}} \beta\text{–}A \quad (32)$$

$$A + A \xrightarrow{k_{AA}} A\text{–}A \quad (33)$$

Let us assume that

$$k_{\alpha\alpha} \sim k_{\alpha\beta} \sim k_{\beta\beta} \sim k_{\alpha\beta} \sim k_{\beta B} \sim k_{AB} = k_f \quad (34)$$

and

$$k_{\alpha A} \sim k_{\beta A} \sim k_{AA} = k_s \quad (35)$$

with

$$k_f >> k_s \quad (36)$$

Due to low k_s and relatively low content of A subunits in the preparation the slow processes are hardly manifested, therefore these subunits are accumulated in the sol-fraction. As a whole, the system behaves as a mixture of active and inactive polymers, with active polymer being composed of identical macromolecules. Apparently, such a mixture

should have a master dependence $G(\tilde{C})$ with the initial slope 2, which with increase in $\tilde{C}$ tends not to 1, but to the level corresponding to the content of inactive polymer in the mixture.

These considerations are also applicable to preparation 3. By the degree of participation of A subunits in gelation it occupies an intermediate position between preparations 1 and 2. It may be assumed that the participation of A subunits in gelation in this case stems from a relatively high content of B subunits in preparation, which have high affinity to A subunits.

CONCLUSION

The concentration dependences of gel yield in the process of thermotropic gelation of soybean globulins are similar in outline to the dependences of gel yield on the extent of conversion in the processes of random cross-linking macromolecules. They have a gel point, i.e., gel is formed only under conditions when the protein concentration is higher than a certain boundary value. Above the gel point, the gel yield increases monotonically with protein concentration. These regularities make it possible to regard the thermotropic gelation of globulins as a process of random cross-linking macromolecules, in which the extent of conversion is proportional to protein concentration in compliance with the laws of chemical equilibrium or chemical kinetics.

The characteristic feature of the investigated globulin preparations is a considerable heterogeneity of their components with respect to gelling ability. Acidic subunits of glycinin have the lowest gelling ability. The gelling ability of the monomeric forms of main soybean globulins appearantly correlates with the parameter of hydrophobicity $H\phi_{av}$ according to Tanford–Bigelow. The greater the value of this parameter the higher is the gelling ability.

Experimental master dependences of gel yield on the reduced concentration, determined as a ratio of protein concentration to its threshold value, have a more complex form than what results from the statistical theory of cross-linking macromolecules homologues, i.e., identical chemically, but having various numbers of functional groups. These deviations arise from chemical heterogeneity of main soybean globulin mixtures at the level of monomeric forms.

ACKNOWLEDGMENT

We thank the Head of the European Division of Central Soya International, Inc., J. F. Casey, for supplying deffated soybean flour, Dr. B. A. Reva for help in carrying out electrophoresis, and Dr. E. E. Braudo for a useful discussion.

LITERATURE CITED

Babajimopoulus, M.; Damodaran, S.; Rizvi, S. S. H.; Kinsella, F. D. *J. Agric. Food Chem.* **1983**, *31*, 1270.
Badley, R. A.; Atkinson, D.; Hauser, H.; Oldani, D.; Green, J. P.; Stubbs, J. M. *Biochim. Biophys. Acta* **1975**, *412*, 214.
Bigelow, C. C. *J. Theor. Biol.* **1967**, *16*, 187.
Bikbov, T. M.; Grinberg, V. Ya.; Tolstoguzov, V. B. *Nahrung* **1979a**, *23*, 487.
Bikbov, T. M.; Grinberg, V. Ya.; Antonov, Yu. A.; Tolstoguzov, V. B.; Schmandke, H. *Polym. Bull. (Berlin)* **1979b**, *1*, 865.
Bikbov, T. M.; Grinberg, V. Ya.; Schmandke, H.; Chaika, T. S.; Vaintraub, I. A.; Tolstoguzov, V. B. *Colloid Polym. Sci.* **1981**, *259*, 536.
Catsimpoolas, N.; Kenney, J. A.; Meyer, F. W.; Szupaj, B. F. *J. Food. Agric.* **1971**, *22*, 448.
Chrispeels, M. I.; Higgins, T. J. V.; Spencer, D. *J. Cell Biol.* **1982**, *93*, 306.
Flory, P. J. "Principles of Polymer Chemistry"; Cornell University Press: New York, 1953; Chapter IX.
Gonzalez, A. E.; Daud, M. *J. Phys. A. Math. Gen.* **1981**, *14*, 2441.
Gordon, M. *Disc. Faraday Soc.* **1974**, *57*, 201.
Hermans, J., Jr. *J. Polym. Sci.* **1965**, *A3*, 1859.
Hoshi, I.; Yamauchi, F.; Shibasaki, K. *Agric. Biol. Chem.* **1982**, *46*, 2803.
Iibuchi, C.; Imahori, K. *Agric. Biol. Chem.* **1978**, *42*, 31.
Irzshak, V. I.; Rosenberg, B. A.; Enikolopjan, N. S. "Polymer Networks"; Science: Moscow, 1979; Chapter 4.
Itzhaki, R. F.; Gill, D. M. *Anal. Biochem.* **1964**, *9*, 401.
Koshiyama, J. *Agric. Biol. Chem.* **1972**, *35*, 385.
Laemmli, U. K. *Nature (London)* **1970**, *227*, 680.
Mori, T.; Utsumi, S.; Inaba, H.; Kitamura, K.; Harada, K. *J. Agric. Food Chem.* **1981**, *29*, 20.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Food Sci.* **1982a**, *47*, 26.
Mori, T.; Nakamura, T.; Utsumi, S. *J. Agric. Food Chem.* **1982b**, *30*, 828.
Nakamura, T.; Utsumi, S.; Mori, T. *J. Agric. Food Chem.* **1983**, *31*, 503.
Nakamura, T.; Utsumi, S.; Kitamura, K.; Harada, K.; Mori, T. *J. Agric. Food Chem.* **1984a**, *32*, 647.
Nakamura, T.; Utsumi, S.; Mori, T. *J. Agric. Food Chem.* **1984b**, *32*, 349.
Richardson, R. K.; Ross-Murphy, S. B. *Int. J. Biol. Macromol.* **1981a**, *3*, 315.
Richardson, R. K.; Ross-Murphy, S. B. *Br. Polym. J.* **1981b**, March 11.
Tanford, C. *J. Am. Chem. Soc.* **1962**, *84*, 4240.
Thanh, V. H.; Shibasaki, K. *J. Agric. Food Chem.* **1976**, *24*, 1117.
Thanh, V. H.; Shibasaki, K. *Biochim. Biophys. Acta* **1977**, *490*, 370.
Utsumi, S.; Nakamura, T.; Mori, T. *J. Agric. Food Chem.* **1983**, *31*, 503.
Vaintraub, I. A.; Shutov, A. D. *Biokhimia (USSR)* **1969**, *34*, 984.
Vaintraub, I. A.; Shutov, A. D. *Proc. USSR Acad. Sci.* **1972**, *203*, 1200.
Van Kleef, F. S. M.; Boskamp, J. V.; Van den Tempel, M. *Biopolymers* **1978**, *17*, 225.
Wolf, W. J. In "Soybeans. Chemistry and technology. Proteins"; AVI Publishing Co., Inc.: Westport, CN, 1972; Vol. 1, p 93.

Received for review August 9, 1984. Revised manuscript received April 22, 1985. Accepted June 16, 1985.