Gelation Phenomena of Soybean Globulins. III. Protein-Lipid Interactions

N. CATSIMPOOLAS and E. W. MEYER, Laboratory of Protein Chemistry, Central Soya Research Center, Chicago, Illinois 60639

ABSTRACT

The major factors, other than temperature, pH, and concentration, affecting the gelation of soybean globulins in the presence of lipids were determined to be (a) the length of the aliphatic chain of the glyceride, (b) the degree of unsaturation of the glyceride, and (c) the number of unesterified hydroxyl groups in the glycerol component. The apparent viscosities of the progel and gel were increased either by decreasing the fatty acid chain length of the glyceride or decreasing the esterification of the hydroxyl groups of glycerol. Saturated fats produced higher gel viscosity than unsaturated ones. Phospholipids and cholesterol also enhance gelation of soybean globulins.

Many natural and processed foods contain lipids dispersed in hydrated protein systems wherein the nature of the dispersion and the existence of protein-lipid interactions are fundamental in defining the textural characteristics of a given food item. With recent emphasis on the utilization of unconventional proteins for the fabrication of analogs which simulate conventional food products, an understanding of specific protein-lipid interactions becomes important in achieving familiar, accepted food characteristics. Although much effort has been devoted to the development of food analogs derived from soy proteins, there exists no published information on the nature of the interaction of lipids with these proteins in relation to their structure-forming properties.

The study of protein-lipid complexes as indicated by work on biological membranes (1,2,3) is complicated by the presence of a multi-phase system. Undoubtedly, some of the factors affecting the intermolecular contacts between protein and lipid will be the protein conformation, protein-protein interactions, and the spatial arrangement of the lipid phase as a result of lipid-lipid interactions. The intermolecular bonding forces are expected to be of noncovalent nature such as hydrophobic, electrostatic, and hydrogen bonds (4). The absence of covalent bonds is suggested by the extraction of lipids from lipoproteins with alcohol-ether solvent mixture and by other experimental work (5,6).

Work in this laboratory (7,8,9) has indicated that the rheological properties of soybean globulin gels can be utilized for the study of soybean protein-protein and soybean protein-solvent interactions. This report is concerned with a study of the influence of various lipids on the gel-forming properties of the acid-precipitable globulins of the soybean.

The gelation of soybean globulins was investigated in relation to the chain length of aliphatic acid in the glyceride, the degree of unsaturation, and the number of unesterified hydroxyl groups of the glycerol component of the glyceride.

MATERIALS AND METHODS

Soybean Globulins

Soy globulins were prepared from hexane-defatted soybean flakes by aqueous extraction, clarification of the extract by centrifugation, and acidification to pH 4.5. The precipitated globulins were washed thoroughly with water, neutralized, and spray-dried (7). The lipids, because of the large quantities required for gelation studies, were of practical or technical grade and were purchased from Eastman Organic Chemicals and Matheson Coleman & Bell Co.

Gelation

Protein-lipid dispersions were prepared by adding the protein to the water-lipid mixture and stirring for 3 min. with a Sorvall Omni-Mixer operated at full speed at room temperature. The entrapped air was removed by centrifugation at 250 g. for 1 min. The dispersions were placed in 25 X 150-mm. stoppered test tubes and heated at the specified temperatures in a water bath. After heating, the samples were removed immediately and cooled at 4°C. (8).

Viscosity Measurements

Viscosities of the sol, progel, and gel were determined with the Brookfield Synchro-Lectric viscometer model LVT. With suitable calibration, the readings are readily converted to apparent viscosities in poises, corresponding to a 200-fold range of shear rates as expressed in relative units referred to a given spindle. A Helipath stand was used because of the thixotropic effects of soybean protein gels (8).

RESULTS AND DISCUSSION

Effect of Lower Triglycerides on Gelation

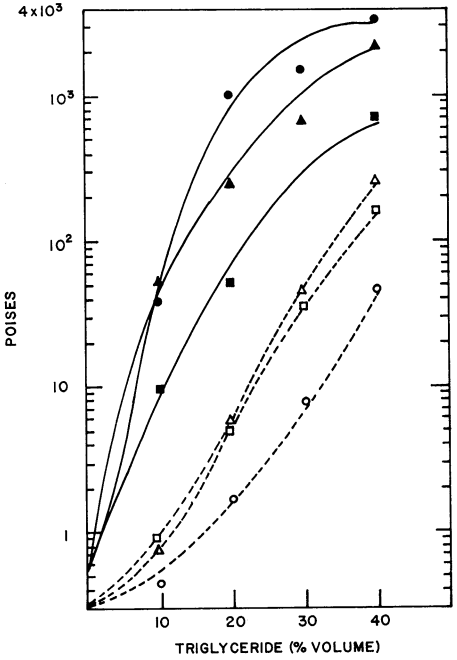
Soy globulin dispersions (6% w./v.) containing from 10 to 40% (v./v.) triglyceride were heated at 80°C. for 30 min. and then cooled at 4°C. for 1 hr. Viscosities were determined before and after gelation. The following triglycerides were used: triacetin, tripropionin, and tributyrin. The viscosities of both the unheated and gelled dispersions increased with increasing concentration of triglyceride. Gelation was influenced by the chain length of the fatty acid (Fig. 1), i.e., the shorter the chain length, the higher the viscosity. Thus the decreasing order triacetin > tripropionin > tributyrin was established. Hydrophobic bonds between the aliphatic chain of the fatty acid and nonpolar protein groups, although important as will be discussed later, did not play the major role in the observed effect. Nonpolar contacts are strengthened by increasing the length of the aliphatic chain (10). In this case, it appears that the length of the chain may have imposed a steric hindrance (11) to the potential interactions of the lipid carbonyl groups which are capable of forming hydrogen bonds with polar protein groups (12). Since hydrogen bonds are preferentially formed during cooling, the gel viscosity may have been affected by negation of these interactions.

The amount of lipid present in the unheated protein dispersions had a significant effect on viscosity. This phenomenon could be explained as an increased monomolecular arrangement of the protein molecules around the lipid micelles

(13, 14). When the lipid concentration is low in respect to the protein, only a part of the molecules of the latter are at the lipid-water interface, whereas most of them are surrounded by water. As the lipid concentration increases, more protein molecules may be oriented around the micelles and the viscosity may rise because of facilitated protein-lipid and protein-protein interactions. Unfolding of the protein associated with lipid-water interface has been the subject of considerable biological interest (15). However, no final conclusions have been drawn as to the configuration of the polypeptide chain when spread on a surface and as to the position of the various functional groups relative to the interface.

Comparative Effects of Mono-, Di-, and Triglycerides

The same experimental conditions as described above were used to study the



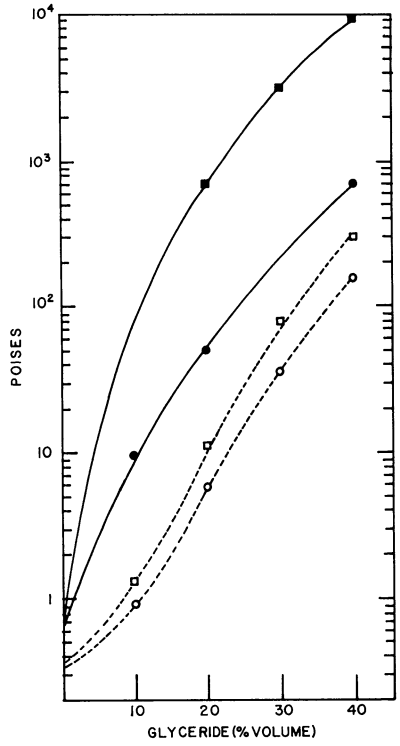


Fig. 1 (left). Effect of lower triglycerides on gelation; 6% soybean globulins unheated in the presence of the following triglycerides: open circles, triacetin; open triangles, tripropionin; open squares, tributyrin; 6% soybean globulins heated at 80°C. for 30 min. and cooled at 4°C. for 1 hr.: solid circles, triacetin; solid triangles, tripropionin; solid squares, tributyrin.

Fig. 2 (right). Comparative effects of lower di- and triglycerides on gelation; 6% soybean globulins unheated in the presence of the following glycerides: open squares, dibutyrin; open circles, tributyrin; 6% soybean globulins heated at 80°C.. for 30 min. and cooled at 4°C. for 1 hr.: solid squares, dibutyrin; solid circles, tributyrin.

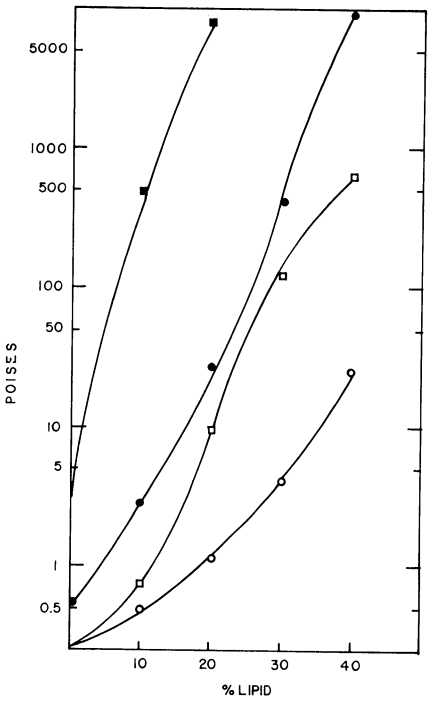


Fig. 3 (left). Comparative effects of mono- and triglycerides on gelation; 6% soybean globulins unheated in the presence of the following glycerides: open squares, monostearin; open circles, tristearin; 6% soybean globulins heated at 80°C. for 30 min. and cooled at 4°C. for 1 hr.: solid squares, monostearin; solid circles, tristearin.

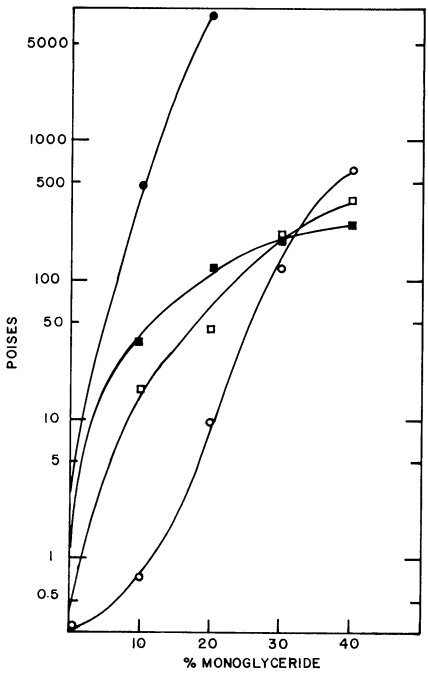


Fig. 4 (right). Effect of aliphatic chain unsaturation on gelation; 6% soybean globulins unheated in the presence of the following monoglycerides: open circles, monostearin; open squares, mono-olein; 6% soybean globulins heated at 80°C. for 30 min. and cooled at 4°C. for 1 hr.: solid circles, monostearin; solid squares, mono-olein.

effect of unesterified hydroxyl groups of the glycerol component of lipids. Figure 2 shows comparative data on di- and tributyrin. In both the unheated and gelled dispersions, the diglyceride resulted in a higher viscosity than did the triglyceride, especially in the gelled state. Similar results were obtained with mono- and tristearin (Fig. 3). It appears that unesterified hydroxyl groups have a significant effect on the gelation of soybean globulins. Increased hydrogen bonding could account for these results (11). It is also believed that hydrogen bonds contribute to the strong emulsifying power of monoglycerides.

Effect of Aliphatic Chain Unsaturation

With the exception of monostearin in the protein gel which exhibited significantly higher viscosities than did mono-olein, the effect of unsaturation of the fatty acids on gelation seems to be complicated (Figs. 4 and 5). In unheated dispersions, mono-olein showed higher viscosity than mono-stearin, but this

phenomenon was reversed at concentrations above 30% of the monoglyceride. Similar reversion was observed at the 30% concentration level when triolein and tristearin were compared in the gel state. However, in unheated dispersions, triolein gave higher viscosities than tristearin. At 25°C., triolein is liquid and its spreadability and site of contacts with the protein will be probably greater than that of tristearin which is solid. Thus, it is reasonable to assume that the state of the lipid (solid, liquid, or intermediate equilibrium phase between solid and liquid depending on the temperature) will play a major role in protein-lipid interactions. Apparently, in biological systems unsaturated lipids are expected to be more functional in relation to interactions than saturated fats.

Effect of Temperature and pH on Protein-Triglyceride Interactions

Soybean globulin dispersions (10% w./v.) containing 10% (w./v.) triglyceride were heated at the rate of 0.5°C. per min. in the range of 25° to 90°C. in a water bath. The following triglycerides were used: triacetin, tripropionin, tributyrin, and triolein. Mono-olein was also used for comparative purposes. At each experimental temperature, a tube was removed from the water bath and the viscosity was determined immediately while at the same time an identical sample was placed at 4°C. and cooled for 1 hr. The viscosity of the "hot" dispersion was taken as that of the progel state and the viscosity of the "cooled" dispersion as representative of the

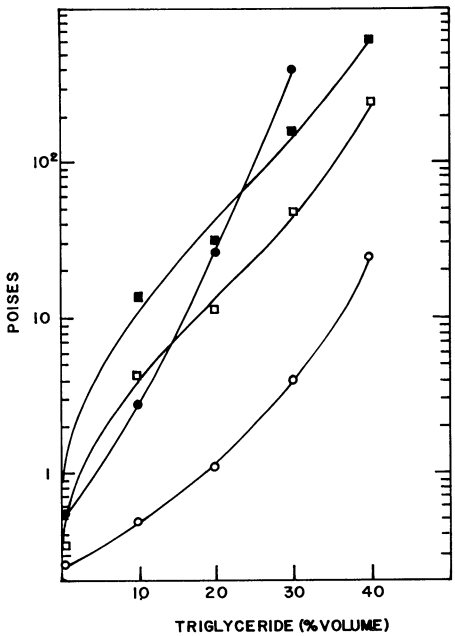
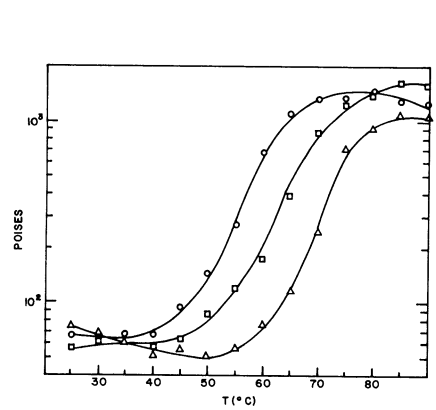


Fig. 5. Effect of aliphatic chain unsaturation of gelation; 6% soybean globulins unheated in the presence of the following triglycerides: open circles, tristearin; open squares, triolein; 6% soybean globulins heated at 80°C. for 30 min. and cooled at 4°C. for 1 hr.: solid circles, tristearin; solid squares, triolein.



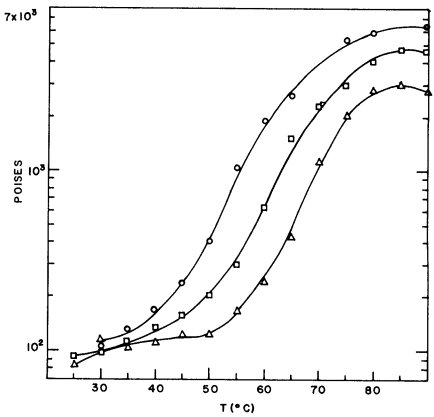
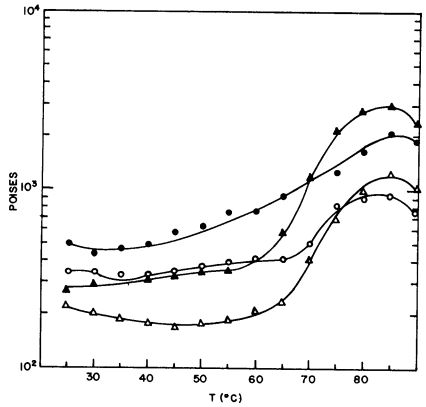


Fig. 6 (left). Effect of triglycerides on progel viscosity as a function of temperature; 10% soybean globulins, 10% triglyceride, pH 7: circles, triacetin; squares, tripropionin; triangles, tributyrin.

Fig. 7 (right). Effect of triglycerides on gel viscosity as a function of temperature; 10% soybean globulins, 10% triglyceride, pH 7: circles, triacetin; squares, tripropionin; triangles, tributyrin.



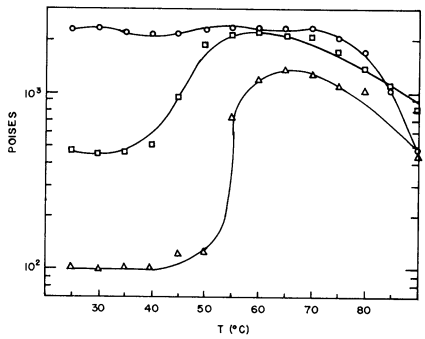


Fig. 8 (left). Effect of unsaturated glycerides on progel and gel viscosities as a function of temperature; 10% soybean globulins, 10% lipid, pH 7: open circles, mono-olein, progel; solid circles, mono-olein, gel; open triangles, triolein, progel; solid triangles, triolein, gel.

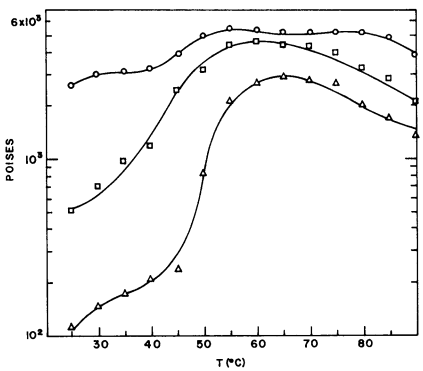
Fig. 9 (right). Effect of triglycerides on progel viscosity at pH 1.5 as a function of temperature; 10% soybean globulins, 10% triglyceride: circles, triacetin; squares, tripropionin; triangles, tributyrin.

gel state. The difference between the gel and progel viscosity, designated as ΔG, indicates the viscosity gained during the cooling process. Figure 6 shows the formation of progel in the presence of triacetin, tripropionin, and tributyrin, as a function of temperature at pH 7. The effectiveness of triglycerides in increasing progel viscosity was of the order: triacetin > tripropionin > tributyrin. Thus, the shorter the chain of the aliphatic acid, the more effective is the triglyceride as a

progel inducer. The maximum viscosity of the progel was not significantly different among the various triglycerides. However, the maximum gel viscosities were of decreasing order with increasing chain length of the fatty acid (Fig. 7). Triolein has no effect on progel formation, but mono-olein was effective even at low temperatures (Fig. 8). At pH 1.5, the temperature range of progel formation in the presence of triglycerides is lowered. Triacetin causes maximum progel viscosity to be obtained at 25°C. with a significant drop above 70°C. (Fig. 9). Tripropionin also caused high progel viscosities at low temperatures, but this effect was less pronounced in the case of tributyrin. Gel viscosities (Fig. 10) followed a similar temperature range pattern as the progel viscosities. However, the flat parts of the progel curves observed at low temperatures did not appear in the gel curves. It seems that the additional viscosity gained during cooling of the progel derives from hydrogen bonding of the redistributed polar groups of the protein during the heating process. Maximum progel and gel viscosities were much lower at pH 1.5 than at neutral pH. Inhibition of certain hydrogen bonds, at acidic pH, due to the lack of ionization of carboxyl groups, is one explanation offered for this phenomenon. The bonds inhibited could be between the carboxylate-phenolic group (16) and carboxylate-protonated amino group (17).

Effects of Temperature and pH on Protein-Cholesterol and Protein-Phosphatide Interactions

The effects of cholesterol and phosphatides (soybean lecithin) on progel formation as a function of temperature and at two pH values—neutral and acidic—are shown in Fig. 11. The concentration of protein was 10% (w./v.) and the concentration of phosphatides and cholesterol 2 and 10%, respectively. A low



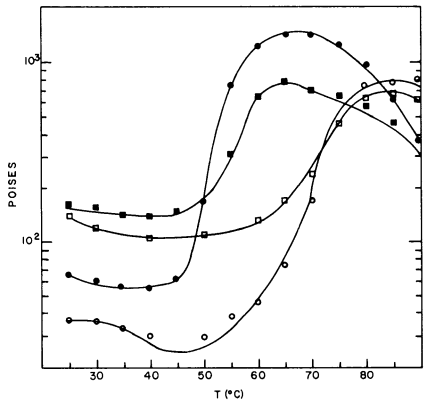


Fig. 10 (left). Effect of triglycerides on gel viscosity at pH 1.5 as a function of temperature; 10% soybean globulins, 10% triglyceride: circles, triacetin; squares, tripropionin; triangles, tributyrin.

Fig. 11 (right). Effect of cholesterol and phosphatides on progel viscosity as a function of temperature and pH; 10% soybean globulins, 10% cholesterol, 2% phosphatides: open circles, cholesterol, pH 7; solid circles, cholesterol, pH 1.7; open squares, phosphatides, pH 7; solid squares, phosphatides, pH 1.7.

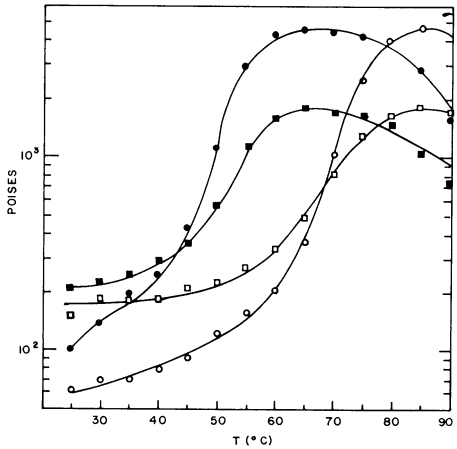


Fig. 12. Effects of cholesterol and phosphatides on gel viscosity as a function of temperature and pH; 10% soybean globulins, 10% cholesterol, 2% phosphatides: open circles, cholesterol, pH 7; solid circles, cholesterol, pH 1.7; open squares, phosphatides, pH 7; solid squares, phosphatides, pH 1.7.

concentration of phosphatides was used to avoid viscosities beyond the measuring range of the Brookfield viscometer. The experiment was carried out in a manner similar to the one described above for triglycerides. At pH 7, the maximum viscosity caused by the presence of cholesterol or phosphatides was at the same range of maximum temperature. At low temperatures the phosphatides produced higher viscosities than did cholesterol (although at much lower concentration). The acidic pH (1.7) had more effect on the cholesterol progel than on the phosphatide progel. The temperature of maximum progel formation was much lower at pH 1.7 than at pH 7 both in the presence of cholesterol and phosphatides.

Gel formation is shown in Fig. 12. The absence of pH effect on maximum viscosity is noted, but the temperature of maximum gel formation is different for neutral and acidic pH values. The pH effect is not significant even at this phase of gelation where hydrogen and electrostatic bonds predominate. It is possible that the speculated carbonyl-carboxylic acid hydrogen bond is not formed because of the long chain of fatty acid components of the phosphatides. Electrostatic bonds between the side chain carboxylate anions of the protein and the positively charged groups of the phosphatides may not be of any significance, since at acidic pH, where the carboxylic acid group is unionized, the viscosity remains the same as at pH 7. Therefore, during the cooling process, the most probable bonds that may be formed are hydrogen bonds between the polar groups of the phosphatides and side chain groups of the protein or the peptide backbone. The existence of strong hydrogen bonds between phosphatides and proteins was also suggested by Therriault and Taylor (18), studying the binding of phosphatidyl serine by human serum albumin. The effect of cholesterol on gelation could be attributed to hydrogen bonds or van der Waals forces. Westphal (19) studied extensively the interaction of proteins with a number of steroids and his data on the free energy of binding are of a magnitude typical of noncovalent bonds.

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