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OBSERVATIONS ON THE MICROSTRUCTURE AND RHEOLOGY OF OVALBUMIN GELS

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Abstract

Understanding the gelation behaviour of proteins is of importance in order to be able to influence the properties of many food systems and it may lead to proposals for product or process improvement. In this context the formation of heat-set ovalbumin gels, in different media, has been studied by microstructural, rheological and conformational observations. An ovalbumin/water gel, prepared at pH 5, shows a granular, inhomogeneous microstructure. At this pH there are both many inter- and intramolecular interactions, and network formation occurs via aggregates of folded, globular protein chains. On the other hand, gels prepared at pH 10 or in urea solution (6 or 8 mol/l), show a uniform, homogeneous microstructure. Under these conditions, network formation occurs via flexible, unfolded protein chains. The ultimate properties of the gels are well in accordance with these microstructural observations: with respect to the deformation at break it is found that the network composed of flexible unfolded protein chains (pH 10, urea) can be extended further without breaking, leading to a higher breaking stress, than the network composed of spherical aggregates of strongly interacting protein molecules (pH 5). In the latter case the regions of low protein concentration will act as weak points and consequently these gels will break at a lower stress than the gels with a more homogeneous microstructure. A study to determine whether glutaraldehyde acts as a proper fixative revealed that this compound very effectively maintains the original gel structure.

Introduction

Proteins play an important role in many food systems. Insight into structure formation and into the relationship between molecular properties, microstructure and macroscopic properties of these structured materials, may stimulate the proper use of proteins and lead to proposals for product or process improvement.

From time immemorial, ovalbumin has been known to form an irreversible gel on heating in aqueous solution to 100°C and subsequent cooling to room temperature (boiled egg white). An investigation has now been made into the heat induced gelation of ovalbumin under different conditions of pH and solvent. These parameters induce conformational changes in the protein molecules and consequently influence the intra- and intermolecular forces between the protein molecules and thus the rheology and the microstructure of the gelled material. In this context urea solutions were used in order to distinguish between formation of covalent and non-covalent crosslinks during gelation. It was envisaged that in the presence of urea, the protein molecules behave like randomly coiled polymers that show little if any intra- and thus intermolecular interaction such as hydrogen bonding or hydrophobic interaction. Consequently if a protein/urea solution shows thermally induced gelation it is clear that gelation is caused by the formation of covalent crosslinks.

In the present work ovalbumin gels heat-set at 100°C were examined by scanning and transmission electron microscopy and rheologically characterized by tensile experiments, to determine the ultimate (breaking) properties. 1H-NMR was used to measure conformational changes in the protein molecules.

As during the sample preparation for electron microscopy, glutaraldehyde is used as a fixative, it has also been investigated how effective this compound is in fixing properly the ovalbumin structure in the different media.

Materials and Methods

Materials

Pure ovalbumin (grade V ex Sigma) was added with stirring as a dry powder to distilled water and to urea solution (6 or 8 mol/l) to a final concentration of 20 g/100 g. The pH was adjusted by adding dropwise aqueous NaOH (0.1 mol/l) or HCl (0.1 mol/l) until the required pH was obtained. The solutions were clarified by centrifugation and de-aerated prior to heat-setting.
Rheological measurements

Tensile experiments were carried out on an Instron Universal Testing Instrument 1122. Ovalbumin solution was poured between two parallel polyester coated copper plates which were placed in a boiling water bath for 20 min. The sheet of gel so formed was removed from between the two plates. The thickness of a typical gel sheet was about 3 mm. Tensile strips were made from the sheet with the aid of a Zwick Stanzpresse using a DIN 53504 S 5 blade module. The strips were fixed between the clamps of the Instron, the strips being wetted with liquid paraffin in order to prevent evaporation. The original length of the tensile strip was 2.75 cm and the width 4 mm. Results of the tensile experiments are expressed in terms of $\lambda$ and the true stress $\tau$ defined as: $\tau = F/A_0 \times \lambda$, in which $F$ is the force applied on the force transducer of the Instron and $A_0$ the original surface of the cross section of the tensile strip ($12 \times 10^{-3}$ m$^2$).

It turned out to be impossible to perform tensile experiments with gels made from heat-set solutions of ovalbumin in urea solutions, because of severe slipping of the gel sheet from the clamps of the Instron. Therefore the mechanical properties of ovalbumin/urea gels were characterized at large deformations, by means of simple shear using the Weissenberg Rheogoniometer model R18. Detailed information on these rheological measurements can be found in Ref. 9.

Electron microscopy

The gel slices prepared for tensile measurements were also used for the microstructural observations by electron microscopy. A three-dimensional impression of gel structures can best be obtained using scanning electron microscopy (SEM). To this end the gel was sliced into small pieces by a rotor blade and fixed in glutaraldehyde solution (15 or 30 g/l) for 1 h at room temperature in cacodylate (0.1 mol/l) buffer at pH 6.6. After fixation, the gel slices were washed with distilled water to remove all water soluble substances, such as urea in the case of urea treated gels. Excess water was removed with filter paper. The gel samples were rapidly frozen in melting nitrogen (nitrogen slush) and subsequently freeze-dried in a Balzers freeze-etching unit, avoiding any temperature rise above $-80^\circ$C. Alternatively critical point drying was used. After fixation, the gel was dried in a graded series of water/ethanol mixtures and finally with carbon dioxide in a Balzers critical point dryer. After the drying procedure (freeze drying as well as critical point drying) fresh fracture surfaces were obtained by fracturing the dried material. The dried samples were mounted on a copper specimen plate with silver paint and coated with 0.05 $\mu$m platinum. Photographs were taken using the scanning device of a JEOL 100C Tenscan in the secondary electron image mode at 40 kV.

Samples for transmission electron microscopy (TEM) were prepared using the thin sectioning technique. Small gel pieces (volume about $1 \times 10^{-9}$ m$^3$) were fixed by successive immersion in buffered solutions of glutaraldehyde (15 g/l) and osmium tetroxide (10 g/l). This treatment was followed by staining in aqueous uranyl acetate (10 g/l) for 2 h. Samples were then washed and dehydrated with ethanol and propylene oxide and embedded in Epon. Thin sections of about 60 nm were cut and collected on electron microscope grids. The sections were poststained with lead tartrate solution for 90 seconds and viewed in a JEOL 100C transmission electron microscope, operating at 80 kV.

![Fig. 1. TEM micrograph of ovalbumin gel (20 g/100 g), pH 10. Dark area represents protein.](image)

NMR measurements

NMR spectra of ovalbumin in D$_2$O (1 g/100 g) were measured in 5 mm sample tubes at 70, 80 and 90°C. Chemical shifts were referenced to the $^1$H-resonance of TSS (2,2,3,3-tetradecuter-o-3-trimethylsilyl-propionic acid sodium salt). NMR spectra were recorded on a Bruker WP 200 (200MHz) spectrometer. The resonance from residual HDO was suppressed by the gated decoupling technique. The pH values were obtained by adding 0.4 units to the normal pH-meter reading. The aliphatic proton intensity (I) (indication of the mobility of the hydrophobic groups) was determined from the ratio between the integrated signal from about 3–0.5 ppm and that of the external TSS standard.

Results

The results on water gels prepared at pH 5, pH 10 and in urea solution can be seen as extremes: at pH 5 the ovalbumin molecule is very near to its IEP and thus maximally globular, at pH 10 the most elastic gel is formed without protein denaturation. Electron micrographs were only taken at these pH values. Rheological characterizations were carried out at other pH values as well (9).

Microstructure

The microstructure of heat-set gels prepared in water at pH 10 and pH 5, observed by TEM, are given in Figs. 1 and 2. Similar types of network structures of protein gels have been reported (1). Observations by SEM of gels at pH 10, pH 5 and in urea (8 mol/l) are shown in Figs. 3–5. As is evident from the TEM pictures (Figs. 1, 2), the homogeneity of the protein distribution is an important parameter. At pH 10 a homogeneous

![Fig. 2. TEM micrograph of ovalbumin gel (20 g/100 g), pH 5. Dark area represents protein.](image)

![Fig. 3. SEM micrographs of ovalbumin gel (20 g/100 g), pH 10. a: freeze-drying, b: critical point drying.](image)

![Fig. 4. SEM micrographs of ovalbumin gel (20 g/100 g), pH 5. a: freeze-drying, b: critical point drying.](image)

![Fig. 5. SEM micrograph of ovalbumin/urea (8 mol/l) gel (20 g/100 g). Freeze drying.](image)
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distribution of protein filaments is found, whereas at pH 5 an inhomogeneous distribution is observed.

The SEM pictures, obtained via freeze-drying, show a granular structure of pH 5 gels (Fig. 4a), whereas a cellular type of structure is observed at pH 10 (Fig. 3a) and in the urea gel (Fig. 5). Comparing the TEM results with those of the SEM it is most likely that the cellular type of structure is induced by ice crystal formation during the freezing step in the sample preparation (2, 7, 11). The granular structure observed at pH 5 is indicative of an inhomogeneous distribution of protein filaments. Apparently ice crystals also cause the thin filaments visible in the TEM micrograph (Fig. 2) to be concentrated onto the dense protein regions, so that they are no longer visible in the SEM micrograph. The cellular structure at pH 10 and in urea is considered to indicate a homogeneous distribution. This view is further confirmed by comparing the results of freeze-drying (Figs. 3a and 4a) to those of critical point drying (Figs. 3b and 4b). At pH 5 the same type of granular structure is observed in both cases, whereas at pH 10 critical point drying leads to an amorphous, homogeneous, non cellular structure. Gelation of ovalbumin in water at pH 5 apparently proceeds via the formation of relatively large aggregates, which form a network. Gelation at pH 10 and in urea proceeds via interaction of thin protein filaments.

When a gel made in urea was immersed for 24 h in water which was changed several times to ensure complete exchange of urea by water, the microstructure of the resulting gel was similar to that of the original gel. However, when the experiment was carried out in the opposite way, i.e., if a water pH 5 gel was swollen in urea solution (8 mol/l), the microstructure of the swollen gel was that of a urea gel. Thus, an ovalbumin/urea gel retains its homogeneous (cellular after freeze-drying) microstructure when urea is exchanged by water, but the microstructure of an ovalbumin/water pH 5 gel changes from granular to homogeneous when the water in the gel is exchanged by urea.

**Rheology**

Typical results of tensile measurements are shown in Fig. 6. The ultimate properties of the gels, as expressed by the breaking stress (τ₀) and the deformation at break (λ₀) are given in Table 1. The breaking stress for the pH 10 gels is considerably larger (about 30 times) than that for the pH 5 gels. The deformation at break differs by a factor of two.

The rheological properties of the pH 10 gels are very similar to those of the gels made in urea (8,9) and both differ considerably from those of the pH 5 gels. The difference in ultimate properties between a urea (6 mol/l) gel and a pH 5 gel is demonstrated in Fig. 7, showing the shear stress-strain behaviour: the deformability and the stress at breaking of the gel gel is much higher than that of the pH 5 gel. A more detailed discussion of the rheological aspects of the heat induced gelation of proteins is given in Ref. 9.

**NMR measurements**

Fig. 8 shows the 1H-NMR spectra of ovalbumin/D₂O solutions. The integrated intensity of the NMR signal from 3.0 to 0.5 ppm of a fully unfolded ovalbumin molecule (i.e., in urea solution of 8 mol/l at 60°C) was taken as 100%. At room temperature, the intensity is about 30–50%, indicating a low mobility of the hydrophobic (aliphatic) groups of the protein. Up to 70°C, the behaviour of the pH 5 and pH 10 solutions is the same: intensity and resolution increase. From 70 to 90°C, the pH 10 solution shows further increase of intensity and resolution, whereas the pH 5 solution shows constant 1 and increase of resolution. At pH 10, a 10 g/l ovalbumin gel unfolds fully at increasing temperature. At pH 5, this gel unfolds partly up to 70°C; at higher temperature, the constancy of I indicates that the partly unfolded protein aggregates.

**Glutaraldehyde as a fixative**

During sample preparation for electron microscopy all ovalbumin gels were fixed in an aqueous solution of glutaraldehyde. In particular for the ovalbumin/urea and the ovalbumin/water pH 10 gels, the nature of the solvent during fixation was different from that in which the protein was dissolved and heat-set. As the nature of the solvent considerably influences the gel properties, it may be questioned whether these circumstances glutaraldehyde fixes the gel structure in its original state. Therefore the effect of glutaraldehyde on the microstructure and the swelling properties of the gels was investigated.

Aqueous gels at pH 5 were subsequently immersed in different media (Table 2). The degree of swelling (S) is defined as the ratio of the weight at a certain time and the original weight

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**Table 1. Comparison of fracture properties of pH 5 and pH 10 ovalbumin gels (20 g/100 g); λ₀: deformation at break; τ₀ breaking stress (average values ± standard deviation).**

<table>
<thead>
<tr>
<th>Cross head speed (cm/min)</th>
<th>pH 5</th>
<th>pH 10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>λ₀</td>
<td>τ₀/10⁵Pa</td>
</tr>
<tr>
<td>0.1</td>
<td>±0.12</td>
<td>±0.98</td>
</tr>
<tr>
<td>1</td>
<td>±0.06</td>
<td>±0.41</td>
</tr>
<tr>
<td>10</td>
<td>±0.04</td>
<td>±0.37</td>
</tr>
<tr>
<td>100</td>
<td>±0.07</td>
<td>±0.58</td>
</tr>
</tbody>
</table>

*Since the measured values of λ₀ were very similar and the number of successful experiments was rather low, an average value for the three crosshead speeds is given.

**Table 2. Microstructure (Scanning EM, freeze-drying) and degree of swelling (S) of ovalbumin pH 5 gel after immersion in various media. Urea concentration 8 mol/l; GA: glutaraldehyde.**

<table>
<thead>
<tr>
<th>Medium</th>
<th>Microstructure</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water + 3% GA</td>
<td>granular structure typical of pH 5 gel</td>
<td>1.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>1.1</td>
</tr>
<tr>
<td>Urea for 20 h followed by 3% GA/urea for 1 h</td>
<td>cellular structure typical of pH 5 gel</td>
<td>5.5</td>
</tr>
<tr>
<td>3% GA/water followed by urea for 20 h and by 3% GA/urea for 1 h</td>
<td>granular structure typical of pH 5 gel</td>
<td>1.0</td>
</tr>
<tr>
<td>3% GA/urea for 20 h</td>
<td>granular structure typical of pH 5 gel</td>
<td>1.2</td>
</tr>
</tbody>
</table>
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Fig. 6. Tensile curves of ovalbumin gels (20 g/100 g) at pH 5 and pH 10. Cross-head speed 10 cm/min; 20°C.

Fig. 8. 200 MHz ¹H NMR spectra of ovalbumin gels in D₂O (1 g/100 g) at pH 5 and pH 10 as a function of temperature. 1: percentage observable aliphatic protons.

of the gel. Only when the gel is first immersed in aqueous urea (8 mol/l), is the original microstructure completely changed, which is accompanied by considerable swelling. However, when the gel is directly immersed in a mixture of aqueous glutaraldehyde (30 g/l) and urea (8 mol/l), the microstructure of the original pH 5 gel is retained and swelling is very limited. These results indicate that glutaraldehyde has a rapid and effective fixative capacity which largely counteracts the action of urea as a swelling agent. Apparently, glutaraldehyde is effective in fixing the structure of the original gel.

Discussion

As is generally assumed, protein gelation involves the thermal denaturation of protein molecules followed by aggregation into a network (1, 4, 5, 6, 8, 9). The nature of the gelation represents a rather delicate balance between chain-solvent and chain-chain forces (3). When there is much chain-chain interaction, a coarse network is obtained. Due to this localized structuring and the resulting large pores, such a gel has a high opacity and shows syneresis (contraction accompanied by water exudation). When there are no strong interactions, but only the possibility of isolated points of contact between the protein molecules, a fine network and a clear gel with no syneresis is obtained (3). Therefore the forces between the denatured protein molecules determine the final gel structure and gel properties. The types of forces between the protein chains are those normally encountered in protein chemistry: electrostatic, hydrogen bond, hydrophobic and covalent (S-S) interactions. In denaturing agents such as concentrated solutions of urea, proteins can be regarded as unfolded, flexible molecules, having random coil conformation, without residual non-covalent structure (10, 13). The mechanical properties of ovalbumin/urea gels approximate those of an ideal rubber (8). In a recent, mainly rheological, study (12) it is shown that textural characteristics of egg white can be significantly altered by means of chemical modification.

In the present work both the microstructure and the rheological properties of the ovalbumin/urea and the pH 10 gels appeared to be quite similar, whereas the pH 5 gels showed quite different microstructures and rheological properties. Furthermore, the gels heat-set at pH 5 were inhomogeneous, opaque and showed water exudation, whereas the urea and pH 10 gels were homogeneous, transparent and did not show syneresis. The NMR measurements (Fig. 8) show that when an ovalbumin solution is heated at pH 10, complete unfolding takes place prior to network formation, whereas at pH 5 some unfolding occurs followed by aggregation of the only partly unfolded protein. So both for the pH 10 and the urea gel, network formation occurs via flexible unfolded protein chains, whereas at pH 5 this formation occurs via aggregates forming a network. This is not
surprising: at the low pH, near the isoelectric point of ovalbumin (pH 4.5), the protein conformation is strongly stabilized by hydrogen bonds and hydrophobic interactions and gelation will take place between these globular entities. At the high pH, the protein molecule is negatively charged, which leads to electrostatic repulsions and at elevated temperatures to flexible unfolded protein chains, without much interaction. Indeed the microstructure observed is in good harmony with this picture: when there are strong inter and intramolecular interactions (pH 5), a granular inhomogeneous microstructure (Figs. 2 and 4) is observed, whereas at high pH and in urea, with small interaction forces between the largely unfolded protein molecules, a homogeneous, uniform distribution of protein over the gel volume is found (Figs. 1, 3 and 5).

The ultimate rheological properties (76, Table 1) of the gels are well in accordance with this type of microstructure and with the character of the forces between the protein molecules. The structure which is composed of flexible, unfolded not strongly interacting protein chains (pH 10, urea) can be extended further without breaking, than the aggregated structure composed of compact, strongly interacting protein molecules (pH 5). The regions of low protein concentration in the inhomogeneous gels (pH 5), will act as weak points, resulting in a low breaking stress. A summary of the properties of the different ovalbumin gels is given in Table 3.

Conclusions

To fully understand the behaviour of proteinaceous gels, information on all levels of structural organisation is needed (3). Microstructural information can give insight into the attractive and repulsive molecular forces in the structure forming process and in macroscopic properties such as mechanical behaviour and water binding. These elements are necessary ingredients in the optimal use of proteins and modified proteins in food and food processing.

References


Discussion with Reviewers

V.E. Colombo: Can you explain how the use of urea can give insight into the structure formation and how this may lead to proposals for product or process improvement?

H.A. Morris: How can the information of this paper lead to proposals for product or process improvement?

Authors: It is true that for food products as such, urea cannot be applied. Urea was used as a model to distinguish between covalent and non-covalent interaction and it was found that a similar distinction could be made when using high pH aqueous solutions. We feel that it is relevant to have information on the effect of solvents and pH on molecular interactions since it is shown in this paper that the nature of the molecular interactions has an effect on the mechanical properties of the gels. This knowledge can now be applied in food technology for introducing in food products the desired rheological, and thus tactile and organoleptic properties.

V.E. Colombo: Could you comment on your statement that the homogeneity of the protein distribution would be an important parameter?

Authors: When in a gel the proteins are distributed evenly over the volume, each cross section will have the same average protein concentration. With an inhomogeneous distribution there will be areas with more and with less than the average protein concentration. Assuming that the breaking stress is related to the weakest points, it will be clear that the more inhomogeneous the protein distribution is, the larger the number of weak points will be. The highest strength is thus obtained with a completely homogeneous distribution.

V.E. Colombo: You attribute cellular types of gel structures to ice crystals originating during the freezing step by means of nitrogen slash. Could you comment on whether any improvements could be achieved by using freezing agents with higher freezing rates, such as propane or various kinds of Freon?

Authors: The freezing rate of a sample is not only determined by the properties of the coolant but also by the sample size. To
Microstructure and Rheology of Ovalbumin Gels

Table 3. Summary of properties of ovalbumin gels in different media

<table>
<thead>
<tr>
<th></th>
<th>pH 5</th>
<th>pH 10</th>
<th>urea (8 mol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microstructure</strong></td>
<td>inhomogeneous, granular;</td>
<td>homogeneous, uniform;</td>
<td>homogeneous, uniform;</td>
</tr>
<tr>
<td></td>
<td>network of aggregated</td>
<td>network of protein filaments</td>
<td>network of protein filaments</td>
</tr>
<tr>
<td><strong>Transparency</strong></td>
<td>opaque</td>
<td>transparent</td>
<td>transparent</td>
</tr>
<tr>
<td><strong>Rheology</strong></td>
<td>low breaking stress</td>
<td>high breaking stress</td>
<td>high breaking stress</td>
</tr>
<tr>
<td><strong>Conformation (NMR)</strong></td>
<td>rigid globular protein chain</td>
<td>flexible unfolded protein chain</td>
<td>flexible unfolded protein chain</td>
</tr>
<tr>
<td><strong>Protein-Protein interaction, (hydrophobic, hydrogen bond)</strong></td>
<td>strong</td>
<td>weak</td>
<td>weak</td>
</tr>
<tr>
<td><strong>Protein-water interaction</strong></td>
<td>weak</td>
<td>strong</td>
<td>strong</td>
</tr>
<tr>
<td><strong>Water release</strong></td>
<td>yes</td>
<td>no</td>
<td>no</td>
</tr>
</tbody>
</table>

avoid the present ice crystal artefacts, very thin (about 10 μm) samples would be required. As the thickness of our samples was 3 mm the application of better coolants such as propane or Freon would be of no use. If it would have been possible to prepare thinner samples then the use of the best coolants should be recommended.

V.E. Colombo: Is there any objective reason why you relate to the NMR signal of a fully unfolded ovalbumin molecule at 60°C, whereas ovalbumin/D2O solutions have been otherwise measured at 70–90°C?

Authors: In urea it was found that “T” (Fig. 8) reached its maximum value at 60°C; obviously some residual three dimensional structure remained even in 8 M urea at room temperature, but this was completely lost at 60°C and higher.

V.E. Colombo: You mentioned that you have a Balzers freeze-etching apparatus at hand. Have you ever tried to prepare ovalbumin gels according to the freeze-etching method? This could possibly help to understand better your findings of cellular and globular structures.

Authors: We have not looked at these samples in relation to freeze-etching. It would certainly have been worthwhile to do this.

A.M. Hermansson: Why was such a high protein concentration as 20% used in the structure evaluation? A lower protein concentration and a higher magnification with TEM might have revealed more details about the network structure of the gel made at pH 10.

Authors: Gels have been prepared of protein concentrations of 10% up to 50%. The 10% gels were so weak (too little mechanical strength) that they could not be characterized in tensile experiments. With 20% this was possible. Consequently all other measurements were made at this concentration.

A.M. Hermansson: The breaking stress (τ_b) was influenced by the crosshead speed for the gel made at pH 10 but not for the gel made at pH 5. Was that due to relaxation during tension of the gel at pH 10 or are there any other explanations for this phenomenon?

Authors: We agree that Table 1 indicates that for pH 10 gels the breaking stress increases with increasing crosshead speed. We feel however that this also takes place, albeit to a lesser extent, for the pH 5 gels. As is indicated by the standard deviations given in Table 1, the reproducibility of the measurements is rather poor and the number of successful experiments is too low to draw firm conclusions about the effect of crosshead speed on breaking stress. But, as said before, we agree there is a trend (for both pH 5 and pH 10 gels) that τ_b increases with increasing crosshead speed. This behaviour is common for viscoelastic materials, in which stress relaxation during extension can take place.

A.M. Hermansson: It is concluded that flexible unfolded protein molecules are present in urea and at pH 10. Have the authors any ideas on how these molecules interact to form chains in a continuous network structure?

Authors: It has not been our intention to suggest that the flexible unfolded protein molecules present in urea and at pH 10 (at high temperatures) form chains in a continuous network. When we speak about chains, we mean protein chains. How the flexible chains are structured in the final gel network cannot be concluded from our rheological and microstructural analysis.

S.S.H. Rizvi: I agree with the conclusion that cellular structure observed in freeze-dried gels at pH 10 (Fig. 3a) and urea heated gels (Fig. 5) is an artefact induced by ice crystal formation during sample preparation. However, it has also been observed (Ziegler GR. (1982). The heat-induced polymerization and gelation of beef natural actomyosin. M.S. Thesis, Clemson University; and Woodward SA, Cotterill OI. (1985). Preparation of cooked egg white, egg yolk and whole egg gels for scanning electron microscopy. J. Food Sci. 50(6): 1624) that gels fixed with glutaraldehyde alone shrank by as much as 50% during critical point drying. Can the interpretations made and conclusions drawn about protein interactions be supported, given the fact that major artefacts are introduced by either preparation technique?

Authors: We agree with the criticism on both the freeze drying and critical point drying techniques. Therefore it should always be recommended to use other independent preparation techniques. We therefore applied, apart from the SEM techniques, a thin sectioning TEM preparation technique. We feel
as we have discussed in our paper, that considering the possible artefacts, there exists a good agreement between the different preparation techniques. Certainly in as far as we have concluded that an aggregated structure is present at pH 5 and a non-aggregated structure at pH 10 and in urea solution. Consequently we are of the opinion that our conclusions about the character of the protein interactions are justified.