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# Molecular cooking: physical transformations in Chinese 'century' eggs

**Erika Eiser,*[abc] Caroline S. Miles,[d] Nienke Geerts,[e] Peter Verschuren[c] and Cait E. MacPhee[d]**

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Over two thousand years ago the Chinese developed a method to preserve eggs such that they remain edible for many months. The room temperature, physico-chemical preservation process that is used to prepare 'century' eggs transforms the egg white into a yellow, transparent gel with optical and mechanical properties that are very different to those of the familiar white protein aggregate that forms upon boiling a raw egg. Here we show that boiled egg white gels can be further transformed into an elastic and transparent gel using the Chinese preservation method. We demonstrate that the resulting protein gel is made of fine-stranded globular assemblies of partially denatured protein, and resembles the aggregates formed by colloidal particles interacting through long-range electrostatic repulsion combined with short-range attraction. These gels are not only highly deformable but are also very stable, maintaining their structure even when boiled. We suggest that the mechanism responsible for gelation in century eggs illustrates a non-specific aggregation pathway available to globular proteins.

## Introduction

Many proteins can aggregate to form volume-spanning structures. This phenomenon is of substantial practical importance to the food industry, where it underlies food texture and material properties. It is also of significance in biomolecular science where protein gelation often competes with crystallisation. Three different mechanisms appear to underlie protein aggregation, depending on the net charge carried by the protein, the presence or absence of ions in the medium, and the degree of unfolding of the protein chain induced by thermal or chemical denaturation. The first mechanism has gained attention due to its apparent role in a number of debilitating human diseases, including Alzheimer's disease and adult-onset diabetes. In this case, partial protein unfolding (typically at acid pH, but also in the presence of other chemical denaturants or as a result of a mutation) causes the self-assembly of misfolded proteins into multimolecular filamentous aggregates known as 'amyloid fibrils'. These fibrils are characterised by the extensive formation of 'β-sheet' secondary structure, regardless of the initial native structure of the aggregating protein. Because the β-sheet conformation involves the formation of hydrogen bonds between groups in the ubiquitous backbone of the polypeptide chain, it is thought that amyloid fibril assembly is a property common to proteins.[1] Entanglement of these fibrils results in the formation of a transparent or translucent gel.

The second mechanism of protein gelation is familiar to anyone who has boiled an egg. Following heating of globular protein solutions individual chains partially denature, exposing hydrophobic groups otherwise buried within the interior of the folded protein. These hydrophobic sticky patches cause the individual molecules to aggregate non-specifically. At pH values close to the isoelectric point of the protein (where it carries comparatively little net charge) or in the presence of salt to screen electrostatic interactions, the proteins form large, isotropic, particulate aggregates that give the final gel a turbid character.[2] Unlike in the amyloid fibril case, however, the constituent proteins remain substantially globular, although some formation of intermolecular $\beta$-sheets has been reported.[3] Such particulate globular protein gels are widespread,[2] largely considered irreversible,[4] and may represent a second pathway to protein aggregation.[5] Characteristically, the diameter of individual aggregates ranges from a few 100 nm up to a few microns, depending on the treatment of the protein, its concentration, and the strength of the non-specific interactions between molecules.

A third mechanism of protein gelation leads to the formation of transparent, elastic gels made up of filamentous aggregates of partially denatured protein. However, in contrast to amyloid fibril gels, the constituent proteins retain much of their native structure. Such 'fine-stranded' gels are typically formed following thermal denaturation of globular proteins at pH values away from the isoelectric point and at low salt concentrations. At these extreme pH values the protein molecules carry a high net charge and therefore interact mainly *via* repulsive Coulomb forces.[2,6] Under such conditions, partial unfolding of the protein chains promotes aggregation *via* the exposure of hydrophobic patches, however, the high electrostatic repulsion leads to limited contact between individual proteins,[7] resulting in proteinaceous strings-of-beads rather than particulate aggregates.

Here we report the molecular transformation of hard-boiled eggs from turbid and brittle particulate aggregates to a transparent and elastic gel by pickling in a strong alkaline solution at room temperature, conditions similar to those used to form the

[a]*University of Cambridge, Department of Physics, Cavendish Laboratory, Madingley Road, Cambridge, UK, CB3 0HE*
[b]*BP Institute, Madingley Road, Cambridge, UK, CB3 0ZE. E-mail: ee247@cam.ac.uk; Fax: +44 1223 337423; Tel: +44 1223 337007*
[c]*Van 't Hoff Institute for Molecular Sciences, Universiteit van Amsterdam, Nieuwe Achtergracht 166, 1018WV Amsterdam, The Netherlands*
[d]*SUPA, School of Physics and Astronomy, The University of Edinburgh, James Clerk Maxwell Building, The King's Buildings, Mayfield Road, Edinburgh, UK, EH9 3JZ*
[e]*FOM Institute AMOLF, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands*

traditional Chinese delicacy pidan ('century' egg in English). We also demonstrate that this transparent elastic gel is formed by the egg white protein ovalbumin aggregating into nanometre thick protein filaments. Considering the wealth of experimental information available describing fine-stranded globular gels, and because the formation of these filaments involves non-specific interactions, we suggest that the protein self-assembly in pidan represents a further generic mechanism for aggregation of globular proteins.

## Materials and methods

### Preparation of alkaline chicken eggs

Chicken eggs were purchased from a supermarket and processed when fresh; colour, size, type of chicken or feeding did not alter the outcome of the gelling experiments. The pickling solution was, for all gels presented here, a 0.9 M NaOH–0.5 M NaCl solution, except for one egg (Fig. 1d) that was pickled in a 0.9 M KOH–0.5 M KCl solution. We used hydroxide pellets (≥98%) and chloride salts (≥99.5%) from Sigma-Aldrich, and deionized water. The whole eggs (with shell) were pickled in Pyrex beakers covered with cling film. After 14 to 18 d the eggs were removed from the beaker, washed thoroughly under running water, stored dry, and peeled only when used for experiments.

### Preparation of ovalbumin gels

Ovalbumin (grade II and grade V) was purchased from Sigma. 60–100 mg $ml^{-1}$ of grade II protein was prepared in Eppendorf tubes in ultra-pure water ($\rho = 18$ MΩ $cm^{-1}$) and carefully mixed. Under these conditions the protein had limited solubility and solutions were turbid. The pH was adjusted by the addition of NaOH from a 1 M stock solution. All pH values were measured using an InLab® Viscous pH electrode for use at high pH values (Mettler Toledo). On addition of alkali, the cloudy solutions immediately cleared and then gelled. Samples were considered gelled if they did not flow when the tube was inverted. Grade V protein showed similar gelation characteristics and pH sensitivity; protein samples were, however, clear when resuspended in $H_2O$.

### Transmission electron microscopy

3 μl of undiluted sample was deposited onto the carbon-coated FORMVAR film of a copper 400-mesh TEM grid (TAAB Laboratories Equipment Ltd, UK). The grid was immediately rinsed with three successive 10 μl washes with ultra-pure water ($\rho = 18$ MΩcm$^{-1}$), each removed with filter paper, before being stained with 10 μl (2%, w/v) uranyl acetate solution. Grids were allowed to air-dry before being stored. The grids were imaged using a Philips (FEI) CM120 transmission electron microscope.

### Circular dichroism spectra

Circular dichroism spectra were acquired on a Jasco J-810 spectrophotometer at 0.1 nm intervals, at a scanning speed of 50 nm $min^{-1}$. Spectra shown here are the averages of three scans. Solution samples: ovalbumin was prepared as a 10 mg $ml^{-1}$ stock solution in 100 mM phosphate buffer pH 7.4 or in 0.17 M NaOH. Under these conditions the protein was soluble. Samples were prepared for spectroscopy by diluting the protein to 15 μg $ml^{-1}$ in 10 mM phosphate buffer pH 7.4 in a 1 cm pathlength cuvette. Gelled sample: ovalbumin was prepared as an 80 mg $ml^{-1}$ stock solution in water and the pH adjusted to 12.6 by the addition of NaOH. A drop was immediately placed in a 0.01 mm pathlength demountable CD cuvette. Data acquisition parameters were the same as for the ovalbumin solutions. The stock solution formed a firm gel within 15 s, and the sample in the spectrophotometer was found to have gelled following dismantling of the cuvette.

### Fluorescence spectroscopy

Steady-state fluorescence emission spectra were acquired using a Varian Cary Eclipse fluorescence spectrophotometer averaging 10 scans for each spectrum shown. The final protein concentration in each sample was 10 μg $ml^{-1}$. Ovalbumin was prepared as 10 mg $ml^{-1}$ stock solutions in either 100 mM phosphate buffer pH 7.4, or 0.17 M NaOH. Samples were diluted into either 100 mM phosphate buffer pH 7.4, or 0.17 M NaOH for measurement in a 1 cm pathlength cuvette. Denatured protein was prepared by incubating a 60 mg $ml^{-1}$ sample of ovalbumin, pH 12.6, at room temperature for 8 weeks. This stock was diluted into 100 mM phosphate buffer pH 7.4 to a final concentration of 10 μg $ml^{-1}$ for measurement in a 1 cm pathlength cuvette.

### Rheology

All rheology measurements were done on an Anton-Paar MCR501 stress controlled rheometer, using a plate-plate geometry. The upper plate had a diameter of 50 mm, while the gap height varied between 1 and 2 mm depending on the thickness of the sample. Care was taken to cut parallel slices from the gelled and hard-boiled egg to fit into the plate-plate gap. All measurements were performed at 22 °C.

## Results and discussion

Traditionally, pidan are made from duck eggs that are wrapped in an alkaline paste of lime (CaO), clay, salt, ash from (pine)-wood and strong tea.[8] Protected by rice hulls these eggs are then stored in a dry place at temperatures between 5 and 25 °C, typically for three months to a hundred days. The main protein constituting the egg white of avian eggs is ovalbumin. Thus, we used mainly chicken eggs and a modern preparation method[8,9] to prepare pidan gels from raw and hard-boiled eggs as well as from solutions of purified ovalbumin. This modern method reduces the necessary ingredients to the use of a high pH sodium hydroxide–salt solution.

First we explored the stability of the turbid, particulate gel formed in hard-boiled chicken eggs by incubating them in their hard protective shell for up to 26 days in a solution containing 0.9 M NaOH–0.5 M NaCl.[8,9] Despite the widespread belief that the formation of particulate aggregates is effectively irreversible, we observed a gradual transformation of the white of the hard-boiled egg into a transparent elastic gel (Fig. 1A). The egg yolk remained substantially unchanged. To explore this transformation further, we studied fresh whole chicken eggs in parallel. Fig. 1B shows an example of a fresh egg that gelled

 

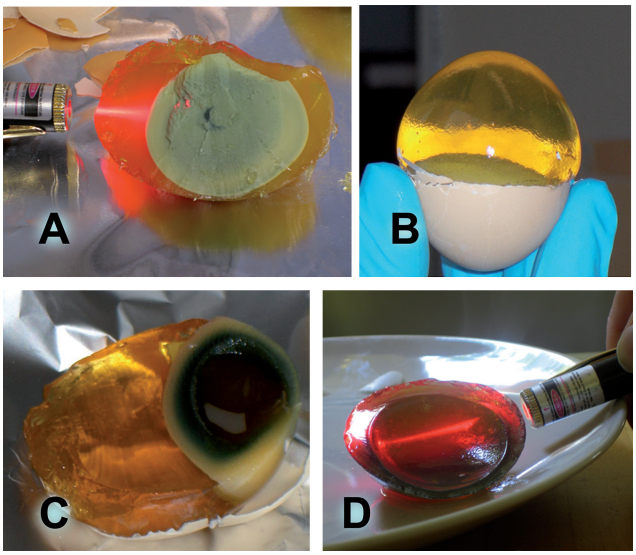


**Fig. 1** Photographs of variously prepared egg white gels. (A) A hard-boiled fresh egg pickled for 23 d in a 0.9 M NaOH–0.5 M NaCl solution. The laser light ($\lambda \approx 660$ nm) shows that the initial white egg gel becomes transparent. (B) A fresh chicken egg incubated in a similar NaOH–NaCl solution for 15 d. (C) Another fresh chicken egg incubated for 15 d in a 0.9 M KOH–0.5 M KCl solution. (D) A slice of a fresh chicken-egg gel prepared in the same way as before remains transparent after boiling for 10 min in $H_2O$.

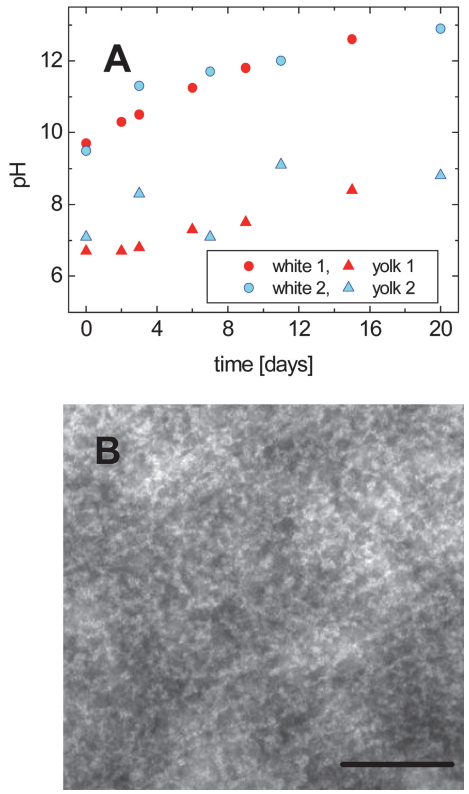


**Fig. 2** Evolution of the pH in chicken eggs over time and representation of the resulting protein gel structure. (A) Time evolution of the pH of egg white and yolk in two independently prepared batches of eggs pickled in 0.9 M NaOH–0.5 M NaCl solutions (pH 13.8). (B) Transmission electron micrograph of an ovalbumin gel prepared at pH 12.6. Scale bar: 200 nm.

under alkaline conditions similar to those used to pickle the hard-boiled egg. Fresh eggs typically gelled within 15 d at room temperature, whereas hard-boiled eggs exposed to alkaline pH formed a similar transparent gel only after 23 to 26 d. Control experiments showed that raw eggs incubated in pure 0.9 M NaOH solution also gelled, while those kept in 0.5 M NaCl remained unchanged, demonstrating that it is the high pH that is responsible for egg gelation at room temperature. In Fig. 1C we further show that the type of monovalent cation used does not play a role: eggs incubated in 0.9 M KOH–0.5 M KCl solutions also gelled by day 15. Perhaps the most striking observation we made, however, was that century eggs are resistant to heat denaturation, remaining transparent and mechanically inert even after boiling the peeled egg white gel for 10 min in pure water (Fig. 1D).

To explore this gelation process in greater detail, we incubated a large number of fresh eggs in two containers under alkaline conditions and extracted an egg every few days from both batches. Fig. 2A shows the pH of the egg white as a function of time. We found that full gelation only occurs when the pH within the egg exceeds 12, after approximately 12–14 d.[10] Eggs pickled for more than 25 d fluidised, suggesting chemical degradation of the egg white proteins over time. The pH of gelled eggs removed from the pickling solution at day 14 decreased progressively to reach a value of 10.9 after a further 14 d; in commercial duck-egg pidan we find a pH $\sim$ 10.

The glycoprotein ovalbumin comprises approximately 54% of the total protein content of egg white,[11] and is thought to be responsible for the gelation properties of heat-treated eggs[12] and in pidan as we show below. It belongs to the serpin (serine protease inhibitor) family of proteins but has no known inhibitory activity, nor is it known to form the linear self-assembled aggregates known as 'serpin polymers' characteristic of some members of the serpin family.[13] The molecular weight of ovalbumin is close to 45 kDa, including carbohydrate moieties, a single intramolecular disulfide bond and four free cysteines.[14,15] While the gelation behaviour of heat-denatured ovalbumin has been extensively documented[16] less is known about the type of gelation in pidan. Hence, to explore the gelation properties of ovalbumin at room temperature and alkaline pH we prepared 100 mg ml$^{-1}$ ovalbumin solutions in pure water and varied the pH by titrating with 1 M NaOH. For pH values up to about 12.4 the ovalbumin solutions stayed fluid. Only in a narrow pH range between 12.5 and 13.6 did we observe formation of the transparent gel typical of century eggs. We estimate from the protein sequence that at pH 12.5 approximately 15% of all the amino acid residues within ovalbumin carry a negative charge, and the net charge per protein is approximately 60. Transmission electron microscopy (Fig. 2B) and shear-induced birefringence suggest that the gel is filamentous in nature, however it does not bind the dyes congo red or thioflavin T, both hallmarks of amyloid fibril formation. Exposing ovalbumin solutions directly to alkaline pH greatly speeds up gelation: gels formed within a few seconds, compared to 15 d within the egg shell. Fresh egg white also gelled rapidly upon direct addition of NaOH such that a pH of 12.5 was reached.[17] Unlike the century eggs, however, gels made from pure ovalbumin solutions fluidised over a period

of 24 h. We suggest that fluidisation of the gels is the result of chemical degradation of the protein(s) following prolonged exposure to these extreme pH values, as has also been observed when century eggs remain exposed to a pH > 12 after they have gelled. As in commercial pidan our pickled eggs remained in a gelled state for months when removed from the caustic environment after 14–18 d.

To determine how the structure of native ovalbumin changes when exposed to high pH values we performed tryptophan fluorescence and circular dichroism (CD) spectroscopy. Both confirm the presence of residual structure at the pH where pidan gelation occurs. Native ovalbumin suspended in 0.1 M phosphate buffer (pH 7.4) has a fluorescence emission maximum of 337 nm when excited at 285 nm (Fig. 3A).[18] Ovalbumin freshly prepared in NaOH at low concentrations, where gelation is not observed, has a fluorescence emission maximum at 345 nm. In contrast, a denatured ovalbumin sample exposed to pH 12.6 for 8 weeks has an emission maximum of 351 nm, similar to the urea-unfolded protein with an emission maximum at 352 nm[19] and consistent with a fully unfolded protein in which the three tryptophan residues are exposed to the solvent. CD spectroscopy also demonstrates substantial residual secondary structure in the ovalbumin gel (Fig. 3B). Ovalbumin suspended in 0.1 M phosphate buffer shows a CD spectrum with a minimum at 222 nm, a shoulder at 208 nm and a maximum at 192 nm, all consistent with the native secondary structure comprising ~30% $\alpha$-helix and ~30% $\beta$-sheet content (see also ref. 15). An ovalbumin sample that formed a gel at alkaline pH shows a large shift in the CD spectrum with the minimum at 220 nm decreasing in magnitude, the appearance of a large minimum at 205 nm, and a decrease in the intensity of the maximum with a shift to lower wavelengths, all consistent with either a loss of $\beta$-sheet conformation or an increase in random coil structure. The CD spectrum of the gelled material is, however, inconsistent with either complete unfolding of the protein, or the formation of the β-sheet structure characteristic of amyloid fibrils. Our findings are in agreement with theories by Clark and others[7,8] that gelation of globular proteins involves aggregation of partially structured molecules rather than fully unfolded random polymer coils.

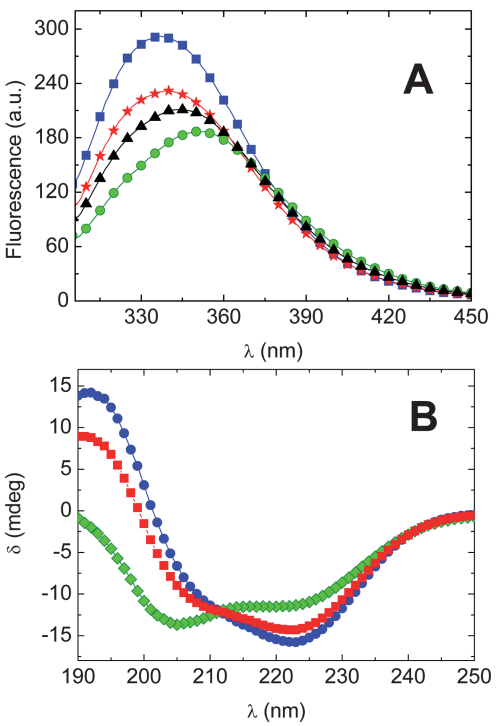


**Fig. 3** Fluorescence and circular dichroism (CD) spectra, indicating the structural changes in ovalbumin under the influence of strongly alkaline solutions. (A) Fluorescence emission from 10 μg ml$^{-1}$ ovalbumin solutions: native ovalbumin pH 7.4 (squares); ovalbumin briefly exposed to pH 12.6, then diluted to pH 7.4 (stars); ovalbumin at pH 12.6 (triangles); ovalbumin denatured following prolonged exposure to pH 12.6, diluted to pH 7.4 (circles). (B) Circular dichroism spectra of native (circles), refolded (squares), and gelled ovalbumin (60 mg ml$^{-1}$; diamonds).

Our tryptophan fluorescence and CD data also demonstrate a rapid and irreversible structural change in ovalbumin on even brief exposure to alkaline pH (Fig. 3). An ovalbumin sample incubated at pH 12.6 for 2 min prior to dilution to pH 7.4 shows a small but significant red shift of 3 nm to 340 nm, and a 20% decrease in fluorescence intensity (Fig. 3A). Similarly, a considerable change in the CD spectrum is observed when pure ovalbumin solutions are exposed to NaOH at pH 12.6 and then immediately diluted to pH 7.4 (Fig. 3B). The structural change is triggered rapidly (<2 min) and appears irreversible even at low protein concentrations where we do not observe gelation. Irreversible structural changes in ovalbumin have previously been observed as a result of prolonged exposure to alkaline conditions (pH 10) at elevated temperatures (typically >50 °C); the resulting thermostable structural conformer is termed 'S-ovalbumin'.[20,21] In contrast to the structure we observe following exposure to pH 12.6 at room temperature, however, S-ovalbumin has a substantially unchanged secondary structure.[20,21]

We next explored the mechanical properties of century egg white, using classical rheology on raw, gelled and hard-boiled eggs. Fig. 4 shows the elastic and loss moduli, $G'(\omega)$ and $G''(\omega)$, that we measured for the three differently treated samples. As many cooks can confirm, raw egg white has some elasticity over the whole frequency range measured. We find that it has an elastic modulus $G' \approx 1$ Pa at 1 Hz. The loss modulus $G''$ is only a factor of 3 smaller than $G'$, reflecting the viscoelastic nature of raw egg white. Gelled egg white from eggs that have been pickled in a 0.9 M NaOH–0.5 M NaCl solution for 15 d displays an elastic modulus $G' \approx 60$ Pa, which is more than an order of magnitude larger. The elastic modulus varies only within a few percent over the entire frequency range measured, and the loss modulus is approximately a factor of 20 smaller. Such a large difference means that the aggregated protein strands form a cross-linked network with very few unbound chain ends that can freely fluctuate and thus contribute to the viscous dissipation expressed in $G''$. Our results are consistent with previous studies of fine-stranded gels formed as a result of thermal (rather than chemical) denaturation: *e.g.* $G'$ and $G''$ are independent of frequency and $G'$ is substantially greater than $G''$.[22] Assuming that our gel is indeed a fine-stranded protein network whose elasticity is entropic in origin we can estimate the characteristic mesh size $\xi$ of the system through $G' \approx G \approx k_B T/\xi^3$, where $k_B$ is Boltzmann's constant and $T$ the temperature. We find $\xi$ to be 30–50 nm, which explains the transparency of the gel. Hard-boiled

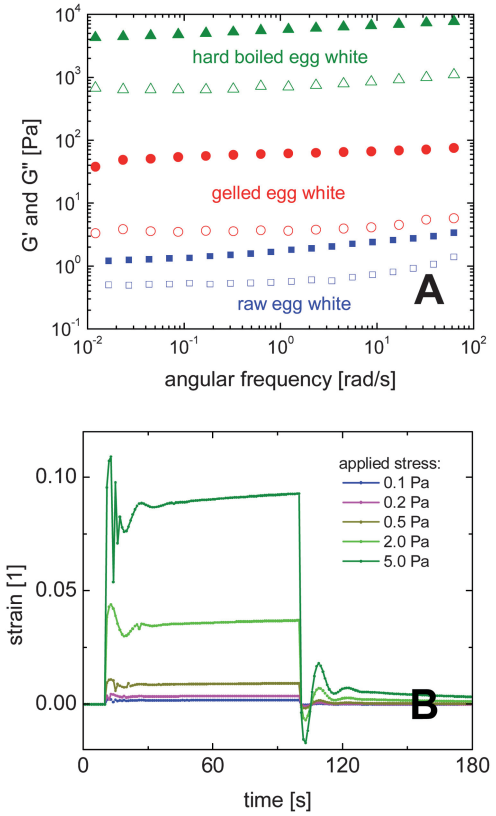


**Fig. 4** Mechanical responses of raw, gelled and hard-boiled eggs to oscillatory deformations, and the linear response of pidan to defined stresses. (A) Elastic and loss moduli, $G'(\omega)$ and $G''(\omega)$ (solid and open symbols, respectively) of raw egg white, egg white gelled in NaOH–NaCl solution for 15 d, and of egg white from eggs boiled for 10 min at 80 °C. All measurements were performed with a strain amplitude of $\gamma = 0.5\%$. (B) Creep measurements on the gelled egg, applying step-stresses for 90 s, which were subsequently relaxed to zero. Here no normal compression ($\sigma_{zz} = 0$) was applied. Both oscillatory and creep measurements were done in a plate-plate geometry.

egg white on the other hand has an even higher elastic modulus, $G' \approx 6000$ Pa at 1 Hz, but like the raw egg its loss part is only a factor of 3–4 lower. Our data for $G'$ and $G''$ of boiled eggs are in good agreement with studies of strong gels of thermally denatured ovalbumin samples[23] and of gels of dry-sprayed egg samples[24] which showed an elastic modulus in the order of $10^4$ Pa. Moreover, these gels exhibit 'creep' behaviour, not fully recovering their original shape after a small applied shear stress is removed, and at larger stresses the gels fracture. In contrast, century egg white shows a remarkable degree of elastic recovery, even following deformations of up to 10% (Fig. 4), reminiscent of swollen rubbers.[25] This elastic behaviour under shear is another indication of the network-like structure of the gel. The oscillations that we observe in the creep measurements on gelled egg white illustrate its highly elastic nature.

Previous experiments by other groups, reviewed by Doi,[16] focussed predominantly on transparent, fine-stranded, heat-set ovalbumin gels formed by a two-step heating method. Doi pointed out[16] that the formation of 'strings-of-beads' would require two attachment sites located on opposite sides of the aggregating protein. Such a situation is found in some members of the serpin family of proteins. They undergo a large conformational change, exchanging regions of the polypeptide chain to form intermolecular interactions ('domain-swapping') leading to the assembly of long polymeric aggregates.[13] Ovalbumin does not naturally undergo this structural rearrangement, however mutation of a single key arginine residue gives rise to a protein capable of the same conformational change observed in polymerising serpins.[26,27] It is also worth noting that the p$K_a$ of arginine (12.5) corresponds to the pH at which we observe the switch to gelation in ovalbumin solutions. However, the formation of fine-stranded globular protein gels as a result of either thermal[28] or chemical[29,30] denaturation by proteins that are not members of the serpin superfamily suggests that the mechanism underlying ovalbumin aggregation is unlikely to be serpin polymerisation.

Here it is interesting to refer to work by Doi[16] who heat-treated ovalbumin under conditions of low salt, predisposing the protein towards the fine-stranded conformer. This resulted in a viscous liquid comprising aggregates of limited size. On reheating this liquid in the presence of salt—conditions that would normally yield a particulate and turbid network—the protein instead formed a transparent fine-stranded gel. Thus, aggregates formed following partial denaturation under low salt conditions were stable to thermal denaturation under conditions that would otherwise lead to a particulate gel. Similarly, work by Su and Lin[31] and Handa *et al.*[32] showed that alkaline pretreatment of whole eggs (pH 12) or egg white (pH 11), respectively, results in a transparent gel when the protein is subsequently heated. These results suggest that fine-stranded heat resistant protein gels can be obtained by a stepwise exposure of fresh protein solutions to low salt concentration or alkaline pH between 10 and 12 and subsequent heating below the complete denaturation temperature. In contrast, our results demonstrate that the egg white gel of century eggs obtained from pH 12.6 exposure (in the absence of salt) at room temperature is a more direct and faster method to obtain gels that are stable to thermal denaturation. These combined results suggest that the conformer that makes up the fine-stranded gel is a stable structure that is resistant to heat-denaturation. Additional contributions to network stability may arise from the formation of covalent interactions between protein molecules, for example non-native disulfide bonds or cross-links between lysinoalanine residues, that have been previously observed in alkaline-treated duck eggs.[33]

Gels of similar structure and mechanical properties that are well understood are those made of colloidal particles. They may provide additional clues as to the nature of the stable aggregates observed in egg white gels. Campbell *et al.*[34] have studied colloidal aggregation under conditions where the interaction between particles is governed by a short-range attractive force and a long-range Coulombic repulsion. In the ovalbumin system, the equivalent interactions may be a short-range attraction arising from hydrophobic patches exposed on the partially denatured protein, and long-range electrostatic repulsion caused by the high net charge on the protein at alkaline pH. In the colloidal system, such an interaction potential results in the formation of dense strands of particles that, at high enough particle densities, form a dynamically arrested volume-spanning gel. Individual strands comprise ordered tetrahedral colloidal clusters, with evidence to suggest self-assembly of the colloids

into "Bernal spirals". Strikingly, Doi and coworkers have previously postulated the formation of tetrahedral clusters from a light-scattering investigation of solutions of ovalbumin thermally denatured under low salt conditions.[35] We speculate that the transparent alkaline gel formed in Chinese century eggs and in ovalbumin solutions comprises a network of locally ordered non-specific aggregates, and that this order yields a stable and elastic network. This is supported by both the regular appearance of the network-like structure we observed in electron microscopy and the low elastic shear modulus $G'$ of pidan gels.

## Conclusions

Summarising our results it is becoming increasingly clear that a few simple rules govern non-specific protein self assembly and gelation. The protein folding state, net charge, and ionic strength of the surrounding medium dictate whether proteins (mis)assemble into amyloid-like fibrils, spherical particulate aggregates, or the stable and elastic filamentous aggregates of globular proteins observed in Chinese century eggs. The nature of the aggregates in turn determines the physical properties of the resulting macroscopic gel, which can be tuned to be transparent and elastic, or turbid and brittle. Such simple rules offer promise for our understanding of protein misfolding diseases, and also provide opportunities for new macroscopic and nanostructured materials.

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