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Whey Protein Nanofibrils: The Environment–Morphology–Functionality Relationship in Lyophilization, Rehydration, and Seeding

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ABSTRACT: Amyloid-like fibrils from β-lactoglobulin have potential as efficient thickening and gelling agents for food and biomedical applications, but the link between fibril morphology and bulk viscosity is poorly understood. We examined how lyophilization and rehydration affects the morphology and rheological properties of semiflexible (i.e., straight) and highly flexible (i.e., curly) fibrils, the latter made with 80 mM $CaCl_2$. Straight fibrils were fractured into short rods by lyophilization and rehydration, whereas curly fibrils sustained little damage. This was reflected in the viscosities of rehydrated fibril dispersions, which were much lower for straight fibrils than for curly fibrils. Lyophilized straight or curly fibrils seeded new fibril growth, but viscosity enhancement due to seeding was negligible. We believe that the increase in fibril concentration caused by seeding was counterbalanced by a decrease in fibril length, reducing the ability of fibrils to form physical entanglement networks.

KEYWORDS: *whey protein self-assembly, amyloid-like fibrils, seeding, calcium, ionic strength, rheology, transmission electron microscopy*

INTRODUCTION

Many proteins will self-assemble *in vitro* into long, thin nanofibrils under conditions that favor denaturation and/or mild hydrolysis but not random aggregation. Fine structure similarities with amyloid fibrils formed *in vivo* have led to *in vitro* nanofibrils being termed "amyloid-like". Because of their extreme aspect ratio, amyloid-like nanofibrils can form physical entanglement networks that enhance viscosity more efficiently, i.e. with less protein, than other kinds of protein aggregates. Efficient viscosity enhancement and gelation is of interest in food and biomedical industries, but other applications such as enzyme immobilization[1] or microencapsulation[2] are also envisaged.

For most proteins that form amyloid-like fibrils, adding fragments of preformed fibrils to a solution of unassembled building blocks, i.e., seeding, accelerates self-assembly in fibril-forming conditions. Seeding has been reported for amyloid-β protein,[3,4] β_2-microglobulin,[5] glucagon,[4,6] hen egg white lysozyme,[7] β-lactoglobulin,[8−11] and other proteins. Seeding seems to be protein-specific, in that one protein cannot seed fibrils of a different protein, and to a lesser extent species-specific, in that the ability of a protein to seed fibril formation by the same protein from another species is related to the degree of sequence homology between the two.[12]

In β-lactoglobulin, fibrils consisting of a peptide corresponding to the β-sheet A (βA) region (residues 17−26) will seed fibril formation in concentrated urea by the intact full-length protein.[8] Fibrils composed of the βG (102−108), βH (118−123), and βI (147−150) regions will seed fibrils of their own kind, but will not seed intact full-length β-lactoglobulin.[8] The self-assembly potential of the βA, βG, βH, and βI regions is confirmed by their appearance in β-lactoglobulin fibrils formed by heating at low pH and low ionic strength.[13]

For heat-induced β-lactoglobulin fibrils, seeding leads to an accelerated increase in Thioflavin T (ThT) fluorescence,[10] which is an indicator of the length concentration of fibril material. Bolder et al.[11] reported that adding 10−30% w/w seed material to β-lactoglobulin at pH 2 and 80 °C slightly improved rates of conversion to fibrils (i.e., protein retained in a 100 kDa filter) in stirred solutions, but had no effect on the apparent viscosity of fibril dispersions. However, they did not examine the effect of seeding without stirring. Hamada et al.[8] showed that fibrils composed of certain β-lactoglobulin peptide fragments could seed fibril formation by the full-length protein in urea. None of the aforementioned investigations have connected together the kinetic, morphological, and rheological effects of seeding, as we have sought to do here. The seeding ability of highly flexible fibrils made in the presence of salts has also not been examined.

Mudgal et al.[14] showed that, for highly flexible β-lactoglobulin fibrils formed at pH 3.35, there was a critical protein concentration of 6.9%, above which fibrils entangled into "microgels", and viscosity increased more sharply with protein concentration. Viscosity after lyophilization and rehydration was higher and more sensitive to protein concentration for fibril dispersions made with >6.9% protein, although the microstructure of rehydrated dispersions was not examined. The viscosity of flexible fibril dispersions formed at

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pH 3.35 with 8% β-lactoglobulin[14] is close to that of flexible fibril dispersions made with only 2% whey protein isolate at pH 2,[15] which suggests that the above findings for pH 3.35 fibrils may not be directly transferable to pH 2 fibrils.

It is generally recognized that the volume fraction of fibres and their aspect ratio play important roles in the rheology of their dispersions.[16] However, there is a scarcity of work linking the morphology of amyloid-like fibrils with the architecture and functionality (e.g., viscosity) of fibril dispersions, and we have sought to address this.[15,17,18] Notable findings have been a very poor correlation between ThT fluorescence and viscosity, and evidence that the bulk viscosity of fibril dispersions depends on fibril flexibility, length, and alignment rather than simply the volume or mass fraction of fibrils.

The flexibility of fibrils can be easily manipulated by adding NaCl[15,19−23] and/or $CaCl_2$[15,22,23] before heating at acid pH. Fibrils formed without added salts have contour length (L_c) of up to 5000−10 000 nm and persistence lengths (L_p) in the range 1846−4307 nm,[22,24] and so can be classified as "semiflexible" since L_p and L_c are of comparable magnitude.[25] The presence of ≥60 mM NaCl or ≥33 mM $CaCl_2$ during heating reduces L_c to a few hundred nanometers and L_p to 38−84 nm,[22] and the fact that $L_c \gg L_p$ qualifies these fibrils for the descriptor "flexible".[25]

Here we extend our previous work to examine how lyophilization affects the morphology of fibrils and their rheological properties, and how lyophilized fibrils can be used as seed material to accelerate fibril growth in heated whey protein isolate solutions. We compare how semiflexible and flexible fibrils are affected by lyophilization and seeding. In this study, fibrils were made using whey protein isolate (WPI), a commercial ion exchange product that comprises >90% protein,[26] of which up to 80% is β-lactoglobulin.

MATERIALS AND METHODS

Chemicals. Thioflavin T powder and HCl were obtained from Sigma-Aldrich (St. Louis, MO). AnalaR grade $CaCl_2$ was supplied by BDH (Poole, England), and solutions were made up in Milli-Q water. Fonterra Cooperative Ltd. (Auckland, New Zealand) supplied WPI (93.5% protein).

Sample Preparation. WPI was stirred into Milli-Q water, and then the solution was acidified to pH 2 with 6 M HCl and stirred overnight at 4 °C, after which pH was measured again and adjusted if necessary. Heating of WPI solutions at 80 ± 0.2 °C was carried out in a Lab Companion BS-11 water bath (Jeio Tech, Seoul, Korea).

To make seed material, 2% w/w WPI solutions containing 0.05% w/w sodium azide were heated in 50 mL tubes for 15 h at 80 °C either with 80 mM added $CaCl_2$ (curly fibrils) or without added salts (straight fibrils). Fibril dispersions thus created were dialyzed against water with 0.05% w/w azide inside Spectra/Por 1 membrane (Spectrum Laboratories, Rancho Dominguez, CA) with 6000−8000 Da molecular weight cutoff. To remove preservatives and hydrate the membrane, sections were rinsed and soaked in water for 1 h.

Dialysis was done at 4 °C for 3 days with daily replacement of water outside the membrane, which was continuously stirred at low speed. Dialyzed fibrils were frozen at −20 °C, lyophilized (Cuddon 0610 freeze-drier, Cuddon Ltd., Blenheim, New Zealand), and ground to a fine powder using a glass rod. For rehydration experiments, dried fibrils were added to water at pH 2 to make 2% w/w, 4% w/w, or 10% w/w and mixed at 4 °C overnight. For seeding experiments, seed material was added at 1 mg mL^{-1} or 5 mg mL^{-1} to 2% w/w WPI solutions at pH 2. Seeded WPI solutions were then heated in the water bath as described above.

Thioflavin T (ThT) Fluorescence Assay. The ThT working solution was prepared as described previously.[22] Excitation was at 440 nm, and emission was measured at 490 nm (RF-1501 spectrofluorimeter, Shimadzu, Kyoto, Japan). All measurements were corrected for the intrinsic ThT response of native β-lactoglobulin by subtracting the fluorescence of unheated protein solutions.

Negative Stain Transmission Electron Microscopy. Fibril samples were prepared for microscopy, applied to the Formvar/carbon grid, stained, and examined as described previously.[22] Contrast-stretching was applied to images using Adobe Photoshop Elements 2.0 (Adobe Systems Inc., San Jose, CA).

Rheometry. An AR-G2 rheometer (TA Instruments, New Castle, DE) fitted with a 60 mm diameter stainless steel cone with angle 4° and truncation length 112 μm was used to collect continuous rotational flow data at 20 °C. Sample handling and rheometric measurements were done as previously reported,[15] including preshearing at 200 s^{-1} for 2 min prior to measuring.

RESULTS

Part I: Rheological and Morphological Effects of Lyophilization. We have previously shown that WPI fibrils made with 80 mM $CaCl_2$ are highly flexible (curly), whereas those without added $CaCl_2$ were long and straight or semiflexible.[15] The effect of $CaCl_2$ on the viscosity of fibril dispersions was evident both immediately after creating fibrils by heating for 15 h, and also after lyophilizing and rehydrating (Figure 1). Adding 80 mM $CaCl_2$ increased final viscosity after

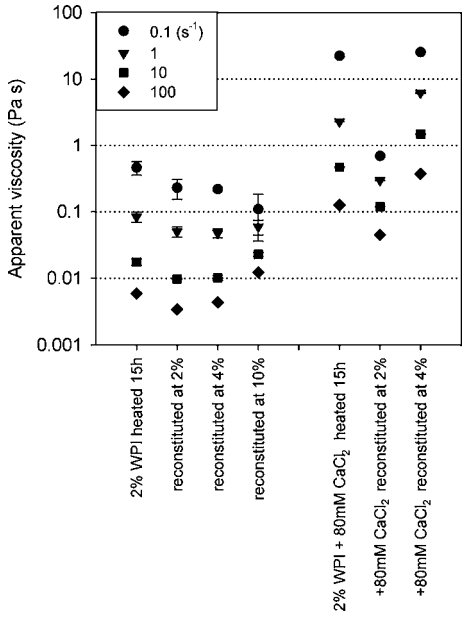

Figure 1. Viscosity of fibril dispersions before lyophilizing or after lyophilizing and rehydrating in water at 2%, 4%, or 10% w/w. Measurements were made after stirring overnight at 4 °C to ensure complete hydration. Fibrils made with 80 mM $CaCl_2$ could not be rehydrated at 10% w/w because a thick gel was formed, preventing even dispersion and hydration. Different symbols show apparent viscosity at different shear rates, and vertical bars are min and max of two replicates.

15 h by more than a decade. Lyophilizing and rehydrating decreased the viscosity of curly fibril dispersions much more than for straight fibril dispersions, which maintained their viscosity quite well. However, the viscosity of the rehydrated curly fibril dispersions at 2% w/w was still much higher than dispersions of straight fibrils at the same solids content, particularly at high shear rates. When straight fibrils were rehydrated at 4% w/w and 10% w/w, viscosity was slightly higher at high shear rates than when rehydrated at 2% w/w, but

viscosity at 0.1 s^{-1} decreased. In contrast, doubling the amount of dried curly fibrils from 2% w/w to 4% w/w increased viscosity at all shear rates by a decade or more. Rehydration at 10% w/w was not possible because a firm gel formed before all dried material could be adequately dispersed and hydrated. Rheological data were not collected in that case, because the dissolved/dispersed protein content was unknown. For all cases other than 10% w/w curly fibrils, dried material solubilized easily to produce a transparent and homogeneous dispersion.

In our earlier work,[15] we proposed that a small proportion of curly fibrils could bind long straight fibrils together into a larger network, synergistically increasing bulk viscosity. An alternative proposal is that mixtures of straight and curly fibrils could exhibit rheological synergy via segregative interactions analogous to those between different polysaccharide classes, such as kappa-carrageenan and konjac glucomannan.[27]

Here we mixed lyophilized straight and curly fibrils in a range of proportions to explore those possibilities. Dispersions of 100% straight fibrils were thixotropic (shear-thinning) between 0.04 and 100 s^{-1} (Figure 2). Slight dilatancy at very low and very high shear rates was probably a rheometer artifact, and disappeared with increasing proportions of curly fibrils. The power-law model (eq 1) was used to fit shear rate sweep data in the intermediate shear rate region where data were linear on log–log axes. In eq 1 η_a is apparent viscosity, K is the consistency coefficient (units Pa s^n), $\dot{\gamma}$ is the shear rate (s^{-1}), and n is the rate index (no units).

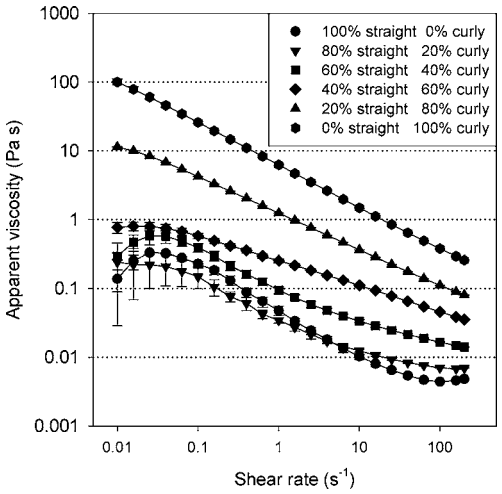

Figure 2. Shear rate sweep of lyophilized and rehydrated mixtures of straight and curly fibrils at total solids content of 4% w/w. Dispersions of straight fibrils (made without added salts) or curly fibrils (made with 80 mM $CaCl_2$) were dialyzed and lyophilized separately, and then mixed into water in the indicated mass proportions to make 4% w/w. Vertical bars are the min and max of two replicates.

$$\eta_a = K\dot{\gamma}^{n-1} \quad (1)$$

Values of K and n are plotted in Figure 3. The consistency coefficient, K, which corresponds to the value of the apparent viscosity at a shear rate of 1 s^{-1} in Figure 2, was unchanged when 20% curly fibrils were substituted for straight fibrils, but with >20% curly fibrils, K increased exponentially as the proportion of curly fibrils increased. The rate index, n, which is related to the slope of curves in Figure 2, increased from 0.32 ± 0.01 in dispersions of straight fibrils to approximately 0.6 with 20–60% curly fibrils, then decreased to 0.41 ± 0.02 for

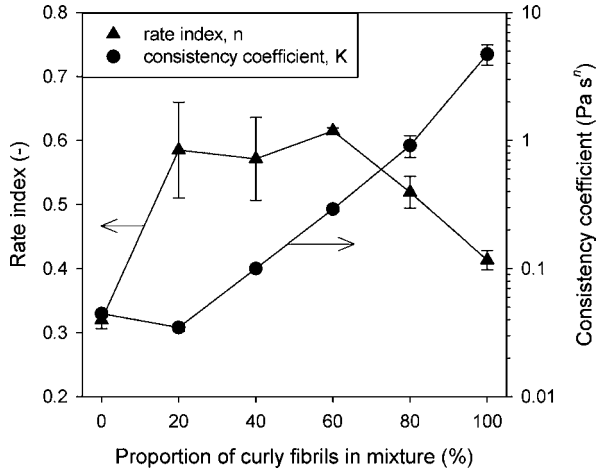

Figure 3. Power law parameters for shear rate sweeps (Figure 2) of mixed fibril dispersions (straight and curly in different proportions). Vertical bars are standard errors of 4–6 replicates.

dispersions of curly fibrils. Given that the values of n are all less than 1, all the dispersions were shear-thinning fluids.

TEM images of rehydrated straight fibril dispersions (Figure 4A) showed that fibrils which were previously up to several micrometers long[15] were broken up into fragments a few hundred nanometers long. By contrast, the appearance of fibrils made with 80 mM $CaCl_2$ changed little with lyophilization and rehydration (Figure 4C). In mixtures of straight and curly fibrils (Figure 4B), the curly fibrils appeared to bind together small flocs of straight fibrils.

Part II: Using Lyophilized Fibrils To Seed New Fibril Growth. Without seeding, ThT fluorescence increased little during 5 h heating, and seeding led to a sharp increase in fluorescence after 0.5 h (straight seeds) or a more steady increase beginning within the first 0.5 h (curly seeds) (Figure 5). Samples with 5 mg mL^{-1} seed material that were heated for >2 h (straight seeds) or >3 h (curly seeds) did not give reliable ThT fluorescence readings, which could have been due to formation of small gel particles in the sample. The seed material itself increased ThT fluorescence, and when this difference was accounted for, it was not clear whether adding 5 mg mL^{-1} of straight seeds had a greater effect than adding 1 mg mL^{-1}. However, 5 mg mL^{-1} of curly seeds clearly produced a steeper increase in ThT fluorescence than 1 mg mL^{-1} of the same seed material.

Despite the dramatic effect of seeding on ThT fluorescence, the effect on viscosity (Figure 6) was subtle. Seeding with straight fibrils produced a slightly greater final viscosity at high shear rates (Figure 6A), but no change to viscosity at low shear rates, and no effect on the kinetics of viscosity increase. The effect was clearer with curly fibril seeds (Figure 6B), which produced small but consistent increases in viscosity over the control, particularly with 5 mg mL^{-1} seeding.

TEM images were taken after 1 h heating with straight seeding and after 3 h heating with curly seeding, to correspond with the large differences in ThT fluorescence seen at these times (Figure 5). Samples seeded with 1 mg mL^{-1} straight seeds and heated for 1 h contained semiflexible fibrils, in many cases approximately 1 μm long but with very few longer than 2 μm. Some fibrils stained heavily, but others were lightly stained, as indicated in Figure 7A. The higher seeding level of 5 mg

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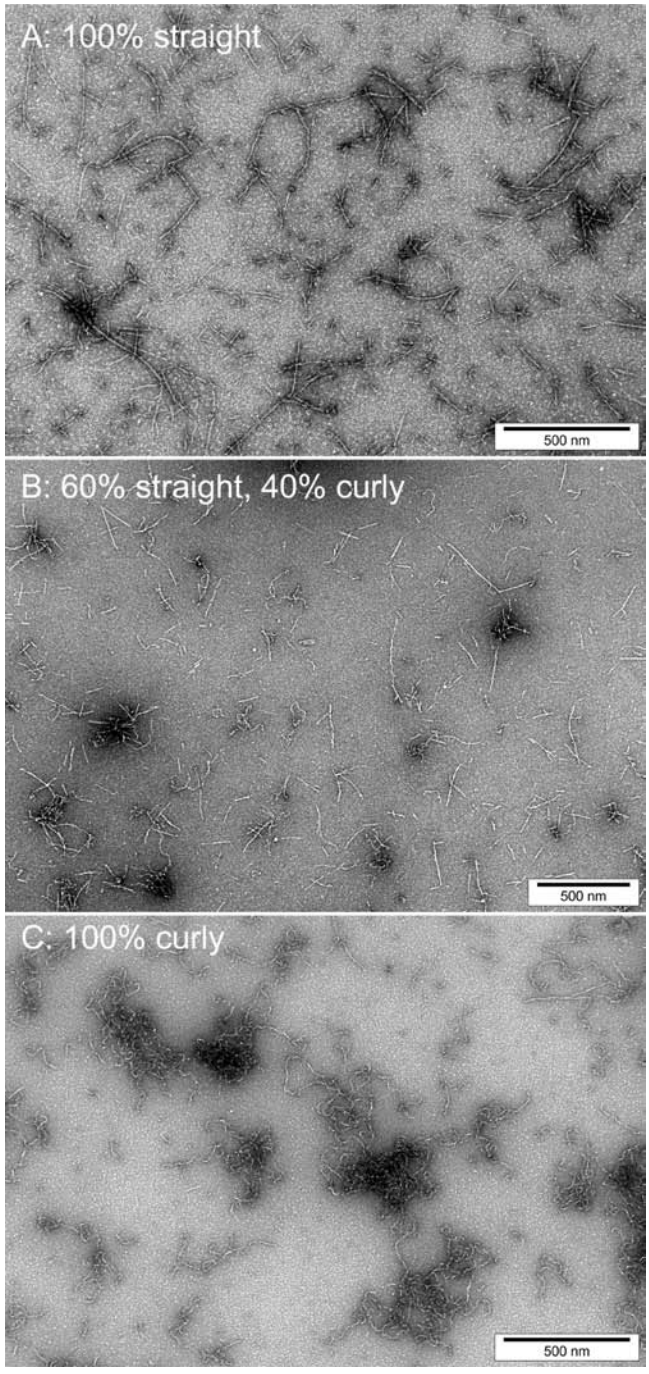

Figure 4. TEM images of 4% w/w fibril dispersions prepared by rehydrating lyophilized fibrils: (A) 100% straight; (B) 60% straight, 40% curly; (C) 100% curly.

mL^{-1} produced more numerous fibrils which were shorter but of otherwise identical morphology (Figure 7B).

Seeding with curly fibrils produced predominantly straight fibrils (Figure 8). Some curly fibrils were seen with both 1 and 5 mg mL^{-1} seeding (indicated by arrows in Figure 8), and these may be the remnants of seed material. More curly fibrils were seen in samples seeded at 5 mg mL^{-1}, but this may simply reflect the higher amount of seed material.

DISCUSSION

Part I: Rheological and Morphological Effects of Lyophilization. The solution environment around the protein ($CaCl_2$ concentration in this case) determined fibril morphology, and fibrils with different morphologies were affected differently by lyophilization and rehydration. Straight fibrils were extensively fragmented, and although curly fibrils were also fragmented somewhat, the original structure before drying was largely retained. Despite fragmentation of fibrils, lyophilization should have little effect on their internal structure, according to solid-state NMR studies with Alzheimer's β-amyloid peptide fibrils.[28,29]

When a biological solution is frozen, solutes and structures are concentrated into regions of unfrozen solution by ice crystals, which consist of pure water. Damage to biological structures can come about via four mechanisms, which relate to loss of membrane fluidity, freeze-concentration of solutes, dehydration of cells, and mechanical damage from ice crystals.[30] In dialyzed fibril dispersions, ionic strength is low, and no membrane or cellular structures are present, so the last mechanism is most likely here. Although rehydration involves very rapid imbibing of water by porous powder particles, the mechanical stresses involved are likely to be less than those experienced during freezing.

The stiffness of straight fibrils apparently made them vulnerable to breakage. Curly fibrils presumably flexed with the mechanical stresses induced by ice crystal formation during freezing. Bunches of curly fibrils may sequester hydration water within convoluted nanoscopic pores, in a way that bundles of straight fibrils cannot.

Straight fibrils in rehydrated dispersions looked like a collection of short rods, and this was reflected in the change in shear-thinning behavior with the percentage of added solids (Figure 1). With an increase in the amount of dried material from 2% w/w to 10% w/w, viscosity at high shear rates increased, most likely as a result of more frequent and energetic contacts between rods. Viscosity at low shear rates changed little, and this may be due to the preshearing (200 s^{-1} for 2 min) causing rods to align in the direction of flow prior to measurement. Even at 10% w/w solids, short rods would be unlikely to form a network, whereas both the original dispersion of long fibrils, and rehydrated dispersions of curly fibrils, could form networks, resulting in higher viscosities. Thus, the morphology of fibrils before lyophilization determined the impact of lyophilization and rehdyration on the structural integrity of fibrils, which in turn determined the impact on functionality, i.e., viscosity.

For β-lactoglobulin nanofibrils formed by heating at pH 3.35, initial protein concentration has a strong impact on viscosity after rehydrating.[14] Fibrils created at $\geq$7% w/w protein form entanglement regions or "microgels", and produce high-viscosity solutions after rehydrating, whereas lower protein concentrations in the heating step gives much lower viscosity after rehydration at the same level.[14] Lyophilized fibrils in our investigation were produced by heating 2% w/w solutions, and fibril performance after rehydration may be improved by forming fibrils at protein concentrations where extensive fibril entanglement can occur before lyophilization.

Synergistic enhancement of viscosity in mixtures of different carbohydrate polymers has been known for some time, and has been attributed to either the formation of junction zones[31] or segregative interactions[27] driven by thermodynamic incompatibility.[32] The two mechanisms may even coexist in binary combinations of polysaccharides of different classes.[33] Mixtures of straight and curly fibrils contain two populations of particles with very different shape and flexibility (2 orders of magnitude

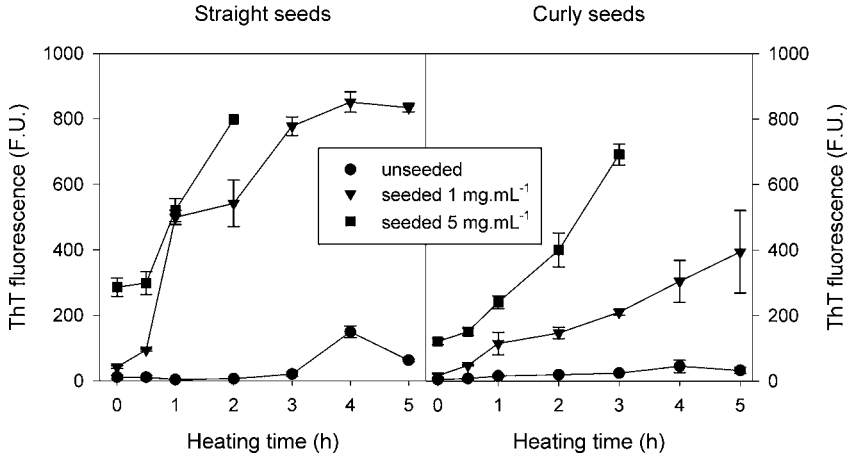

Figure 5. Thioflavin T fluorescence of 2% w/w WPI solutions at pH 2, heated at 80 °C with 0, 1, or 5 mg mL^{-1} added dry seed material from straight or curly fibrils.

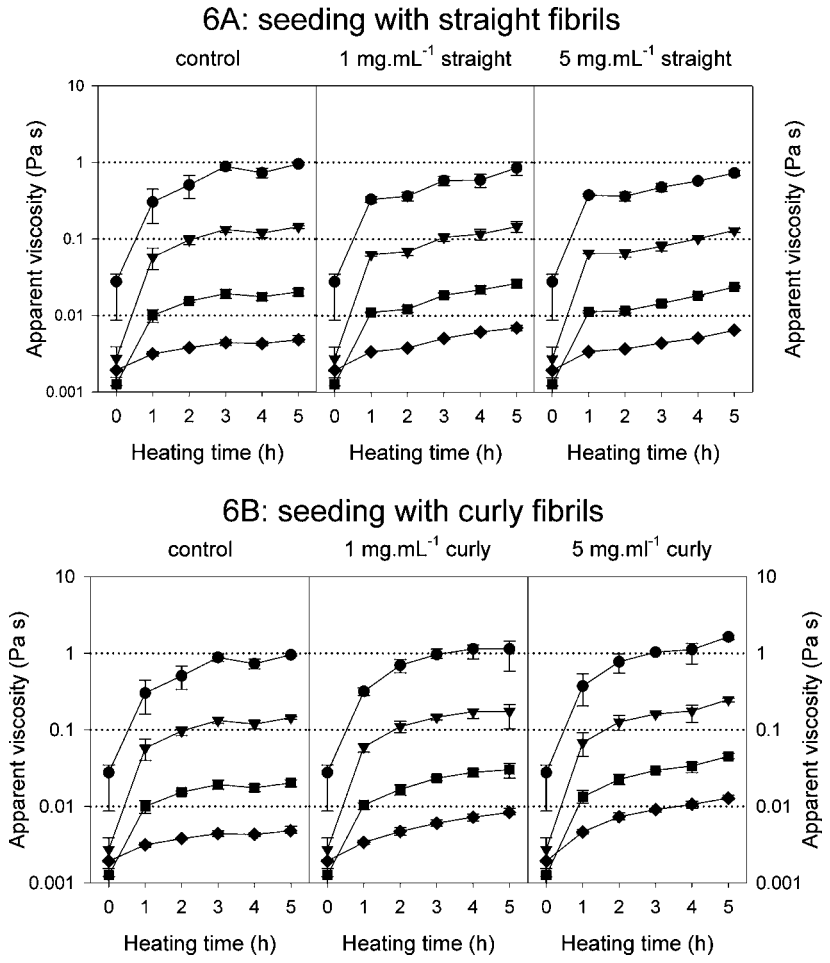

Figure 6. Effect of seeding type and level on the viscosity of 2% w/w WPI dispersions at pH 2 heated at 80 °C: (A) seeding with straight fibrils; (B) seeding with curly fibrils. Straight and curly seed materials were prepared as described in the text, and mixed into WPI solutions, which were then heated. Different symbols show apparent viscosity at different shear rates (see Figure 1 legend), and vertical bars are min and max of 2−5 replicates.

difference in L_p), and although both are much shorter and thicker than polysaccharide molecules, we wondered if a similar rheological synergy would occur with mixing curly and straight fibrils in different proportions. The two fibril types would be unlikely to form junction zones, as do synergistic polysaccharides, but segregative interactions could operate.

Indeed, with 20−60% curly fibrils, the rate index was higher than with 10%, 80%, or 100% curly fibrils (Figure 3). This means that mixtures in the intermediate range were more strongly shear-thinning than mixtures dominated by one type of fibril. However, the 100-fold increase in consistency coefficient (K) between 20% and 100% curly fibrils shows how viscosity increased consistently with increasing proportions of curly

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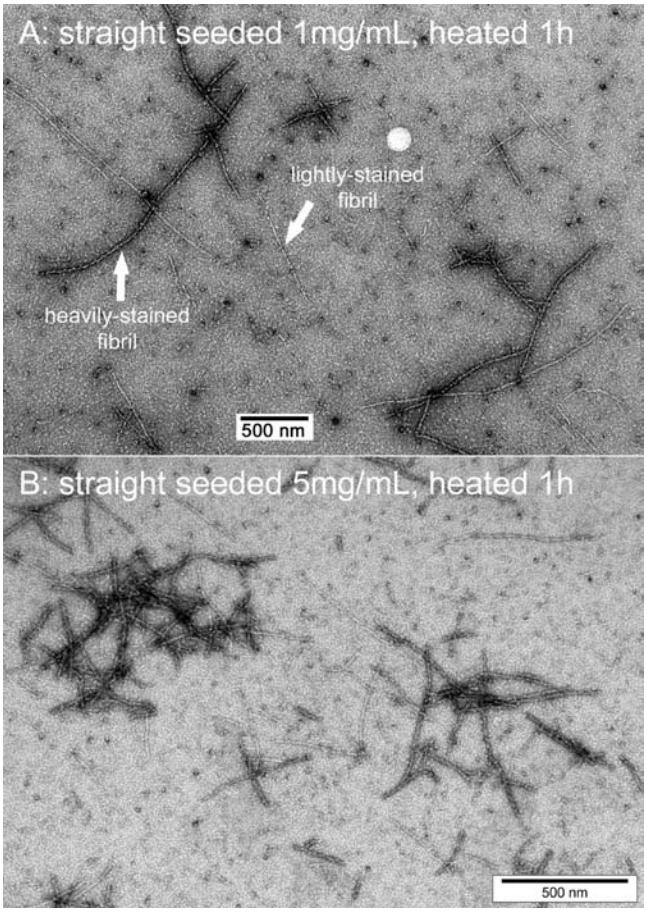

Figure 7. TEM images of fibrils formed by seeding with (A) 1 or (B) 5 mg mL^{-1} of straight fibril seeds. Sample was 2% w/w WPI at pH 2 heated for 1 h at 80 °C.

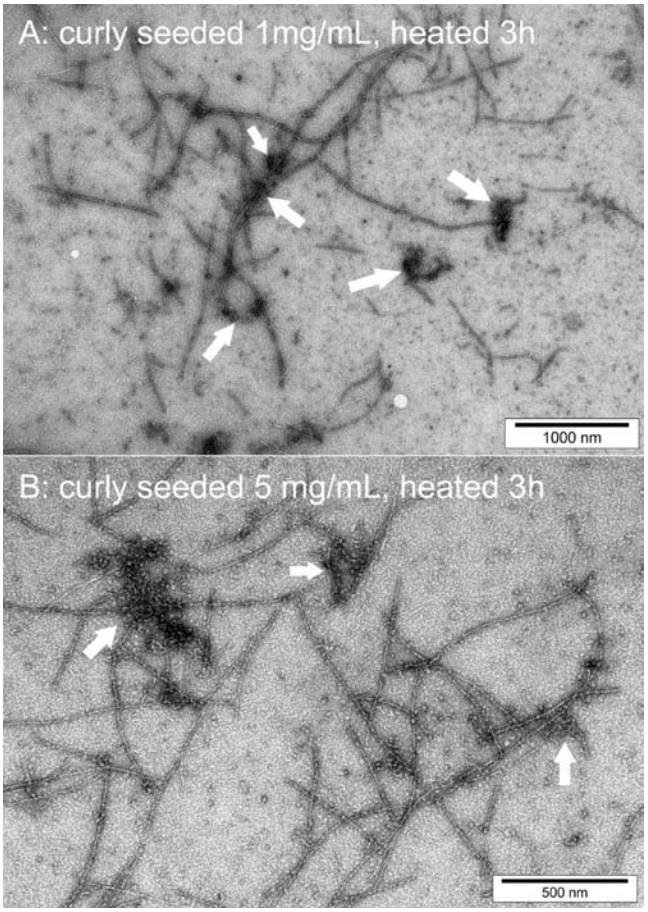

Figure 8. TEM images of fibrils formed by seeding with (A) 1 or (B) 5 mg mL^{-1} of curly fibril seeds. Sample was 2% w/w WPI at pH 2 heated for 3 h at 80 °C. White arrows indicate curly fibrils.

fibrils. Although intermediate mixtures of straight and curly fibrils performed differently from the components, no synergistic enhancement of overall viscosity was seen in these mixtures. This could be partly as a result of straight fibrils being fragmented during drying. Mixtures of straight and curly fibrils can be obtained without drying by heating whey proteins at low pH with low to moderate concentrations of NaCl or $CaCl_2$.[22]

Part II: Using Lyophilized Fibrils to Seed New Fibril Growth. ThT fluorescence measurements showed that seeding was effective at accelerating fibril formation (Figure 5), in agreement with the data of Hill et al.[10] However, viscosity enhancements due to seeding were negligible (Figure 6), indicating that the findings of Bolder et al.,[11] for β-lactoglobulin sheared during heating, also hold for quiescent conditions. For seed material, Bolder et al.[11] used aliquots of fibril dispersions, which would contain long, intact fibrils because no drying was involved. They proposed that seed fibrils may have undergone a termination step during growth over 10 h, rendering them unable to stimulate further growth, and a similar scenario was put forward for amyloid-β protein.[3] In our case, fibrils were fractured as a result of drying, which would create fresh growth sites from internal regions of fibrils, and would increase the number of fibril ends. ThT fluorescence results showed that seeding stimulated fibril growth, but this did not translate into higher viscosity. TEM images showed many short fibrils in seeded samples (Figure 7), and perhaps the effect of an increased amount of fibril material (higher ThT fluorescence) was counterbalanced by a decrease in the length of fibrils, rendering them less able to entangle in ways that enhance bulk viscosity. Thus, morphology (fibril length) determined macroscopic functionality (viscosity).

ThT fluorescence results suggested that curly fibril seeds were less effective at seeding fibril growth than straight seeds. This can be rationalized by considering that it is the ends of fibrils that provide sites for growth. The drying process fractured curly fibrils much less than straight fibrils, so the number of "active" fibril ends per unit mass of curly fibril seed material may be lower than in straight fibril seed material. Curly fibrils had a tendency to entangle with themselves and each other, and fibril ends could have easily become buried inside fibril clusters, rendering them sterically inaccessible to fibril building blocks.

Seeding with curly fibrils produced slightly higher viscosity than the control, particularly with 5 mg mL^{-1} seed material and particularly at high shear rates (Figure 6). TEM images of samples seeded with curly fibrils (Figure 8) showed a predominance of straight fibrils, which were much longer than those formed in the presence of straight seeds (Figure 7). Longer fibrils would be able to entangle better, and form larger, stronger networks. Another contributing factor may be the ability of curly fibrils to bind straight fibrils together, as seen in TEM images and predicted earlier for mixed fibril systems.[15] The proportions of straight and curly fibrils seen in images were roughly equal to the proportions of unheated whey protein (20

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mg mL^{-1}) and seed material (1 or 5 mg mL^{-1}), so the curly fibrils were probably seed material.

The fact that curly fibril seeds stimulated the growth of straight fibrils but not curly fibrils is intriguing. For β_2-microglobulin, straight fibril fragments will stimulate the growth of straight fibrils even when solution conditions favor curly fibrils; i.e., the templating effect of seeds outweighs effect of growth conditions (pH, ionic strength) and lower thermodynamic barriers to curly fibril growth.[5] However, in our investigation, curly fibril seeds apparently did not stimulate curly fibril growth under conditions that favored straight fibril growth; i.e., the growth conditions outweighed templating effects. This may relate to the lower number and accessibility of fibril ends in curly fibril seed material relative to straight fibril seeds, as discussed above. It remains to be seen whether straight fibril seeds can stimulate the growth of straight whey protein fibrils in conditions that favor curly fibril growth, as is the case for β_2-microglobulin.[5]

Previous work by our group[15,17,22,23] and others[19−21] established how the pH, ionic strength, and temperature at which β-lactoglobulin or WPI is heated determines the morphology of resulting fibrils. We provided links between fibril morphology and fibril dispersion viscosity in the context of different heating temperatures[17] and the impact of $CaCl_2$.[15] In the present investigation, these links were further explored in the context of a drying and rehydration process and the effects of seeding.

The majority of research to date on the biophysics of heat-induced amyloid-like fibril dispersions and gels has focused on the long, semiflexible fibrils that form at low ionic strength, with relatively little interest in the highly flexible fibrils that form at higher ionic strength. Perhaps this is because semiflexible fibrils are easier to measure microscopically and model mathematically than highly flexible fibrils, which appear more chaotic under the microscope. However, we have shown that dispersions of highly flexible "curly" fibrils have substantially higher viscosity than equivalent concentrations of straight fibrils, and withstand lyophilization and rehydration much better. In addition, curly fibrils assemble faster than straight fibrils.[21,22] Curly fibrils may therefore have more commercial potential as texturing and gelling agents than straight fibrils.

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Notes

The authors declare no competing financial interest.

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