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Characterisation and thermo-reversible gelation of cod muscle protein isolates

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ABSTRACT

Cod (Gadus Morhua) muscle proteins were solubilized using alkaline treatment of the muscle. Solutions of similar protein composition were obtained between pH 10.5 and 12.0, however, pH > 11 was required for optimal yield. Addition of salt (up to 0.25 M NaCl) did not affect protein yield or composition. Light scattering showed that a significant fraction of the proteins was present as large self similar and flexible aggregates. When the pH was decreased below 10, gelation was observed below a critical temperature of about 25 °C, which could be reversed by heating. Slow irreversible aggregation was also observed leading to coarsening and syneresis of the gels or precipitation at higher temperatures. The rate of irreversible aggregation increased with decreasing pH and was fast below pH 8. Homogeneous thermo-reversible self supporting gels that were stable for a period of days could be prepared without heating at a narrow pH range between 8.5 and 9.5.

1. Introduction

With the decline in wild fish species abundance, better utilization is called for marine by-products and underutilized fish that is currently used for animal feed. The use of extreme pH to isolate proteins from marine catch and its by-products has been reported widely (Batista, 1999; Hultin & Kelleher, 2001; Kahn, Berk, Pariser, Goldblith, & Flink, 1974; Montecalvo, Constantinides, & Yang, 1984). This method has been suggested as an alternative to the more conventional surimi production, with several authors reporting better protein yields (Choi & Park, 2002; Kristinsson & Liang, 2006; Kristinsson, Theodore, Demir, & Ingadottir, 2005; Undeland, Kelleher, & Hultin, 2002) than is obtained in processing of surimi. It has also been reported that this method is more efficient than surimi processing in removal of both neutral and charged lipids (Hultin & Kelleher, 2001; Kristinsson & Liang, 2006; Kristinsson et al., 2005; Undeland et al., 2002) which is important for prevention of rancidity (Kristinsson et al., 2005; Lanier, 2000; Undeland, Hall, Wendin, Gangby, & Rutgersson, 2005; Undeland et al., 2002). Following usage in the literature (Kristinsson & Ingadottir, 2006; Kristinsson & Liang, 2006; Kristinsson, Theodore, & Ingadottir, 2007; Kristinsson et al., 2005; Thawornchinsombut & Park, 2007; Undeland et al., 2002) we will refer to solutions obtained with muscle tissue treatment at extreme pH as fish protein isolate (FPI).

Various studies have reported the gelation of fish muscle proteins without heating or added salt. Stefansson and Hultin (1994) observed gelation in one instance of fish proteins solubilized at very low ionic strength and neutral rather than extreme pH. Chawla, Venugopal, and Nair (1996), Lian, Lee, and Chung (2002) and Venugopal, Doke, and Nair (1994) have worked with heterogeneous fish protein suspensions, not dissimilar to those obtained in conventional surimi processing, and reported gelation in the presence of weak acids without addition of salts (and, in several experiments, without heating). On the other hand, exploring the possibilities of producing consistent, edible products from FPI obtained through extreme pH solubilisation has concentrated so far on gels prepared at high temperatures, usually combined with high salt concentrations (Choi & Park, 2002; Kim, Park, & Choi, 2003; Kristinsson & Liang, 2006; Kristinsson et al., 2005; Thawornchinsombut & Park, 2007; Undeland et al., 2002; Yongsawatdigul & Park, 2004), and often in the presence of different additives (Choi & Park, 2002; Kim et al., 2003; Kristinsson & Liang, 2006; Thawornchinsombut & Park, 2007; Undeland et al., 2002). It has not been reported, as far as we know, in the literature concerning FPI solubilized through extreme pH, that the resulting solution may show cold-setting gelation without addition of salt. Other workers have investigated gel production (Choi & Park, 2002; Kim et al., 2003; Kristinsson & Liang, 2006; Kristinsson et al., 2005; Thawornchinsombut & Park, 2007; Undeland et al., 2002; Yongsawatdigul & Park, 2004) starting from a heterogeneous suspension of FPI obtained by *iso*-electric precipitation. In contrast, the work presented here deals with FPI solutions at much lower protein concentrations (<25 g/L) and low ionic strength (<100 mM), and emphasizes the possibility of producing consistent FPI gels at low temperatures without added salt.

The aim of the work presented here was to optimize alkaline solubilisation of cod muscle protein; to characterize the FPI

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solutions after isolation; and to study the behaviour of the solutions after reducing the pH.

2. Materials and methods

2.1. Sample preparation

White muscle tissue from freshly caught cod (0–2 days from catch) was used. Repeated measurements showed that the area of harvesting and time of year had no effect on the observations made and results reported here. The muscle tissue was homogenized in 4–10 weight equivalents of distilled water, and the pH was adjusted to a value in the range 10.5–12.0. To check the effect of ionic strength on the protein yield, addition of 0.15–0.25 M NaCl to extraction solvent was tested, but only solutions obtained without added salt were used for gel production and the rheological study. Solutions were centrifuged for 10 min at 15,000*g* (Avanti Centrifuge J-20 XPI, Beckman Coulter, Fullerton, CA, USA), followed by filtration of the supernatant over two layers of gauze. The temperature was kept under 15 °C during the protein isolation. FPI solutions were stored at 4 °C and used for gel preparation within 24 h of extraction.

2.2. Determination of the protein concentration using UV absorption

The extinction coefficient of the protein isolates was determined as follows. The absorbance as a function of wavelength (λ) was measured at pH values ranging from 7 to 12. At all pH values, a broad peak with a maximum at $\lambda = 230$ nm was observed, indicating the presence of residual amounts of conjugated substances originating in the fish muscle. At pH values above 11.5 the protein absorption peak appears at $\lambda = 289$ nm as a shoulder on the broad peak. The absorbance at $\lambda = 289$ nm was measured at pH 12 and corrected for the effect of turbidity. The total protein content was obtained through elemental analysis of nitrogen, where we used a conversion factor of 6.0 to convert N to total protein amount. The extinction coefficient for the protein mixture at pH 12 and $\lambda = 289$ nm was found to be 0.71 ± 0.01 L g^{-1} cm^{-1}.

2.3. SDS–PAGE electrophoresis

SDS–PAGE electrophoresis was performed according to Laemmli (1970). The electrophoretic pattern of FPI was determined using polyacrylamide 10% gel slabs run on an electrophoresis unit (Bio-Rad, miniprotean II Cell) with a constant current of 20 mA per gel. The gels were scanned with a GelDoc 2000 scanner (Bio-Rad Laboratories Inc., Herts, UK) and analyzed using the software package GelCompar II, 2.01 (Applied Maths BVBA, Kortrijk, Belgium). Broad range protein standards were obtained from Biolabs (Biolabs, New England, US). All other reagents were obtained from Sigma–Aldrich.

2.4. Light scattering

Static and dynamic light scattering measurements were made using an ALV-5000 multiple tau digital correlator (ALV, Langen, Germany) and a JDS Uniphase He–Ne laser (model 1145P–3083, vertically polarized beam, wavelength 632.8 nm). The range of scattering wave vectors (q) covered in the experiment was $2.8 \times 10^{-3} - 2.6 \times 10^{-2}$ nm^{-1}. The scattering vector q is given as $q = (4\pi n_s/\lambda)\sin(\theta/2)$, where n_s is the refractive index of the sample and θ is the scattering angle. The temperature of solutions in light scattering experiments was controlled to within 0.2 °C using a thermostat water bath.

The relative excess scattering of particles, I_r, is related to their weight average molar mass, M_w, and their structure factor, $S(q)$ (Brown, 1996; Higgins & Benoit, 1994)

$$I_r = \frac{I_s - I_{sol}}{I_{tol}} = KCM_wS(q) \tag{1}$$

where C is the solute mass concentration and I_{tol} is the intensity of toluene that is used as a reference. I_s and I_{sol} refer to the intensity of scattering from the sample and from the neat solvent, respectively. K is an optical constant

$$K = \frac{4\pi^2 n_s^2}{\lambda^4 N_a}\cdot\left(\frac{\partial n}{\partial C}\right)^2\cdot\left(\frac{n_{tol}}{n_s}\right)^2\cdot\frac{1}{R_{tol}} \tag{2}$$

where I_a is Avogadro's number, $(\partial n/\partial C)$ is the refractive index increment, and R_{tol} is the Rayleigh ratio of toluene at 20 °C. We used $(\partial n/\partial C) = 0.19$ for the protein mixture and $R_{tol} = 1.35 \times 10^{-5}$ cm^{-1} at $\lambda = 633$ nm. $(n_{tol}/n_s)^2$ corrects for the difference in scattering volume of the solution and the toluene standard with refractive index n_{tol}.

At infinite dilution $S(q)$ depends on the particle size and shape and can be related to the z-average radius of gyration (R_{gz}) (Brown, 1996; Higgins & Benoit, 1994)

$$S(q) = (1 + (q\cdot R_{gz})^2/3)^{-1} \qquad qR_{gz} < 1, C \to 0 \tag{3}$$

If one applies Eqs. (1) and (3) to results obtained at finite concentrations, one obtains an apparent molar mass (M_a) that is inversely proportional to the osmotic compressibility and an apparent radius of gyration (R_{ga}) that is proportional to the correlation length of concentration fluctuations. At low concentrations the effect of interaction between the solute particles can be described in terms of the second virial coefficient (A_2) (Higgins & Benoit, 1994)

$$S(q) = (1 + 2A_2M_wC)^{-1} \qquad 2A_2M_wC < 1,\ q \to 0 \tag{4}$$

For large self similar structures, the structure factor has a power law dependence on q for $q\cdot R_g \gg 1$ (Nicolai, 2007)

$$S(q) \propto q^{-df} \qquad q\cdot R_g \gg 1 \tag{5}$$

With the technique of dynamic light scattering (DLS), the autocorrelation function of the scattered light intensity fluctuations is determined (Berne & Pecora, 1976; Brown, 1996). The normalized autocorrelation function (g2(t)) can be analyzed in terms of a distribution of exponential decays

$$g_2(t) - 1 = \left[\int A(\tau)\exp\left(-\frac{t}{\tau}\right)d\tau\right]^2 \tag{6}$$

where $A(\tau)$ is the amplitude of the exponential with relaxation time τ. In dilute solutions and for $q\cdot R_{gz} < 1$, the relaxation of intensity fluctuations is caused by centre of mass diffusion of the particles and τ depends on the diffusion coefficient of the particles (D)

$$\tau = (q^2D)^{-1} \qquad q\cdot R_{gz} < 1, C \to 0 \tag{7}$$

D is related to the hydrodynamic radius, R_h, through the Stokes–Einstein relation (Berne & Pecora, 1976; Brown, 1996; Higgins & Benoit, 1994)

$$D = kT/(6\pi\eta R_h) \tag{8}$$

with T the absolute temperature, k Boltzmann's constant and η the viscosity. For polydisperse solutions a distribution of relaxation times will be observed that corresponds to the distribution of hydrodynamic radii. Eqs. (7) and (8) are only valid if $q\cdot R_g < 1$, otherwise rotation and internal dynamics may play a role in the relaxation process. For fully flexible particles the apparent hydrodynamic radius (R_{ha}) decreases linearly with increasing q if $q\cdot R_g \gg 1$. Autocorrelation functions were analyzed in terms of

Eq. (6) using the CONTIN (Provencher, 1982) routine. The average relaxation rate ($\langle\Gamma\rangle = \langle\tau^{-1}\rangle$) was used to calculate the diffusion coefficient: $\langle D\rangle = \langle\Gamma\rangle/q^2$. The *z*-average hydrodynamic radius (R_{hz}) was obtained from the average diffusion coefficient using Eq. (8).

2.5. Rheology

Oscillatory shear measurements were done using a StressTech stress-controlled rheometer (Reologica, Lund, Sweden), equipped with a thermostat water bath and water jackets. The temperature was controlled to within 0.2 °C. All measurements were done in the linear response regime. Non-linear rheology of FPI gels will be reported elsewhere. The geometry used was a couette with inner and outer diameters of 25 and 27 nm, respectively. A thin layer of paraffin oil was added to prevent evaporation.

Gels used in the rheological study were prepared in the following manner. A solution of HCl (0.1–0.2 M) was added drop-wise at 20 °C to the alkaline protein solution while stirring. During pH adjustment some precipitation of proteins was observed, but the use of a relatively low HCl concentration and vigorous stirring minimized precipitation. The solution was stirred until the precipitate was completely dispersed, and loaded into the rheometer. Only alkaline solutions obtained without added salt were used for the rheological study, and we found their ionic strength to be close to 25 mM using a Sension 7 conductivity meter (Hach Lange, Dusseldorf, Germany). Since roughly 20–25 mM of NaCl are formed by the initial NaOH addition and the subsequent HCl addition, the final ionic strength of gels at pH 9.0 was close to 50 mM. The temperature of FPI solutions used for gel production never exceeded 20 °C. Cold-gelation properties of FPI preheated to higher temperatures before recooling will be reported elsewhere.

3. Results and discussion

3.1. Protein yield

For maximum yield the pH employed during protein isolation needed to be higher than 11. Solutions of the solubilized proteins exhibited slow hydrolysis post solubilisation. Observation of hydrolysis in FPI was previously ascribed to activity of several cathepsin-like proteases originating in the fish muscle (Choi & Park, 2002; Kim et al., 2003; Lanier, 2000; Thawornchinsombut & Park, 2007). The extent of this hydrolysis was monitored by measuring the pH of the protein solution and performing SDS–PAGE electrophoresis at different times post solubilisation. The electrophoretic pattern of the proteins showed extensive degradation had taken place when solutions were kept for several weeks at room temperature. Since protein degradation affects gelation properties, measures were taken to minimize its extent as much as possible. We have found that the rate of hydrolysis was moderate when the pH was 11.0–11.2, but increased with increasing pH and became extremely rapid above pH 12. For this reason, solubilisation was performed at pH 11.0–11.2; thus, solubilizing proteins at a pH slightly above 11 ensures both a maximal protein yield and a minimal hydrolysis of the resulting protein solutions. The rate of hydrolysis was much slower when FPI were kept at 4 °C compared to room temperature. We have found that the extent of hydrolysis was negligible and did not affect rheological measurements provided solutions (at pH 11.0–11.2) were kept at 4 °C for periods not exceeding 24 h before gel preparation. This protocol was followed for all gels produced in this study.

No effect on the yield was found when the isolation was done with salt free water or with 0.15–0.25 M NaCl. Employing a muscle tissue concentration of about 15 wt% vs. 85 wt% extraction solvent was found to be a good compromise between high yield and high protein concentration. Assuming 18 wt% protein content in the cod muscle (Foegeding, Lanier, & Hultin, 1996; Stefansson & Hultin, 1994), we obtained yields of 60 ± 10%. Protein yield was calculated according to the following equation

$$Y = \frac{A \times V_p}{\varepsilon \times M_s \times 0.18} \times 100\% \quad (9)$$

where Y is the yield, A is the absorbance at pH 12, V_p is the volume of the FPI solution obtained, ε is the extinction coefficient quoted above (0.71 L g^{-1} cm^{-1}) and M_s is the initial mass of cod muscle used.

3.2. Composition of FPI

The protein composition of FPI was determined using SDS–PAGE electrophoresis and was found to be independent of the pH (10.5–12) and ionic strength (0–0.25 M NaCl) of the solvent used for the extraction. A typical electrophoretic pattern of the proteins is presented in Supplementary data.

The electrographs showed that myosin (heavy chain ~210 kDa, light chains 17–23 kDa), actin (43 kDa) and tropomyosin (38 kDa) were the most dominant proteins. Bands at higher molar mass than myosin heavy chain are tentatively assigned to titin and nebulin (Hu, Kimura, & Maruyama, 1986; Stefansson & Hultin, 1994). Other weak bands may correspond to other structural proteins and proteolytic segments of the main myofibrillar proteins. The ill-separated bands between 160 and 185 kDa are thought to be proteolytic segments of myosin heavy chain (Undeland et al., 2002). The presence of these bands was reported earlier for FPI obtained through acidic and alkaline solubilisation (Kristinsson & Liang, 2006; Undeland et al., 2002), and for washed fish muscle post treatment with weak organic acids (Chawla et al., 1996). We could not identify the band at 28 kDa which was also found by Undeland et al. (2002) after alkaline treatment of fish muscle. The intensity of the band seems too strong for the band to be a product of proteolysis.

Several authors have attributed the solubility of muscle proteins at extreme pH to electrostatic repulsion between charges on the protein molecules at pH far from the *iso*-electric point (Choi & Park, 2002; Kim et al., 2003; Kristinsson & Ingadottir, 2006; Kristinsson & Liang, 2006; Kristinsson et al., 2005; Yongsawatdigul & Park, 2004). However, electrostatic interaction can be screened by adding salt and we found no effect of adding salt on the yield. This finding is in agreement with that of Thawornchinsombut and Park (2007) and Kahn et al. (1974) who did not observe a significant effect of adding NaCl on protein solubility at extreme pH for Pacific Whiting and squid, respectively. These observations question the importance of electrostatic interaction on the solubility at extreme pH.

The ionic strength of fish muscle tissue is close to 0.15 M, with the monovalent ions chloride, potassium and sodium being dominant (Kilimann & Heilmeyer, 1977; Stefansson & Hultin, 1994; Wu, Atallah, & Hultin, 1991). This was confirmed by elemental analysis of FPI. For cod, the typical fat content in muscle is 0.3 wt% (Foegeding et al., 1996). However, extraction of the proteins from muscle tissue using extreme pH removed a considerable amount of the total lipid content through creaming and through precipitation of the membrane-bound lipids, both of which are removed in the centrifugation step (Hultin & Kelleher, 2001). The fat content of FPI was measured following the method reported by Bligh and Dyer (1959) and found to be negligible (<0.005 wt%).

3.3. Characterisation of FPI in solution

Freshly extracted FPI at pH 11, with protein concentrations (C) between 0.5 and 8 g/L, were analyzed using static and dynamic

light scattering. The light scattering intensity had a power law dependence on q over the entire accessible range, see Fig. 1a. This means that the system contained particles that were larger than 1μm with a self-similar structure characterized by a fractal dimension of 1.8–1.9, cf. Eq. (5). The same results were obtained at different FPI concentrations. Self similar aggregates were also shown to be formed in pure cod myosin solutions after heating (Brenner, Johannsson, & Nicolai, 2009). Aggregates with very similar overall structure are also formed when globular proteins are heated in aqueous solution (Nicolai, 2007).

Sedimentation of these large aggregates under gravity could be observed visually within one day after preparation. We found that on average about 10–15% of the proteins precipitated within the week after preparation. About the same fraction of proteins was removed when the solutions were centrifuged at 50,000g for 90 min and filtered through 0.45 μm pore-size filters. The intensity scattered by filtered solutions was much reduced especially at small q-values and had a weaker q-dependence, see Fig. 1b.

The apparent molar mass and radius of gyration could be calculated as a function of the concentration. The radius of gyration varied little with the protein concentration in the range used in the experiment and was found to be about 150 nm which is much larger than that of the individual protein components. Filtration through 0.2 μm pore-size filters was not possible, which shows that a significant fraction of the proteins was aggregated and blocked the pores of the filters. It is likely that the size distribution of the aggregates is very broad and that centrifugation and filtration simply removed the fraction of largest aggregates. We have not been able to establish if and how much protein is present as individual molecules. KC/I_r was found to increase linearly with the concentration, see Fig. 2, which shows that interaction between the protein aggregates is repulsive. The weight average molar mass and second virial coefficient could be determined from a linear least squares fit, cf. Eq. (4): $M_w = 7.8{*}10^6$ g/mol, and $A_2 = 5.0{*}10^{-9}$ mol L g^{-2}.

Intensity autocorrelation functions could be described by a single relaxation time distribution. The apparent hydrodynamic radius decreased linearly with increasing q over the whole q-range, see Fig. 3, implying that the very large aggregates were flexible. After centrifugation and filtration the q-dependence was weaker which allowed us to estimate R_h as about 180 nm.

We measured the light scattering intensity of FPI solutions at pH 11 both before and after filtration as a function of the temperature between 5 °C and 80 °C and found it to be insensitive to temperature in this pH and concentration range (0.5–8 g/L). The bonds between the aggregates were strong in the sense that they resisted

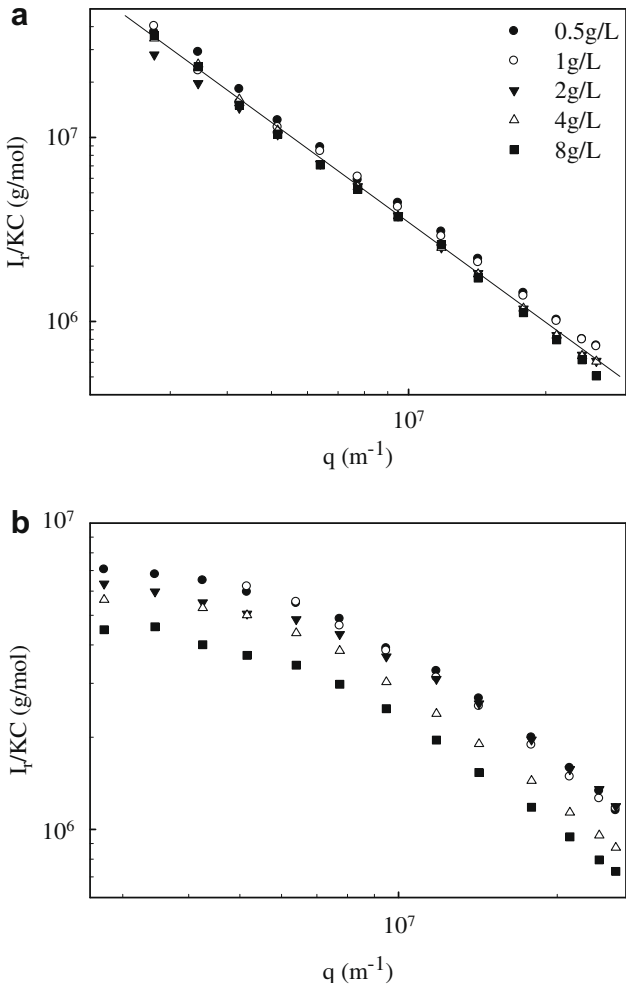

Fig. 1. Dependence of I_r/KC on the scattering wave vector of freshly prepared FPI solutions at pH 11 for different concentrations indicated in the figure. Fig. 2a shows the results before centrifugation and filtration, while Fig. 2b shows the results after centrifugation and filtration. The solid line in Fig. 2a has a slope of −1.8.

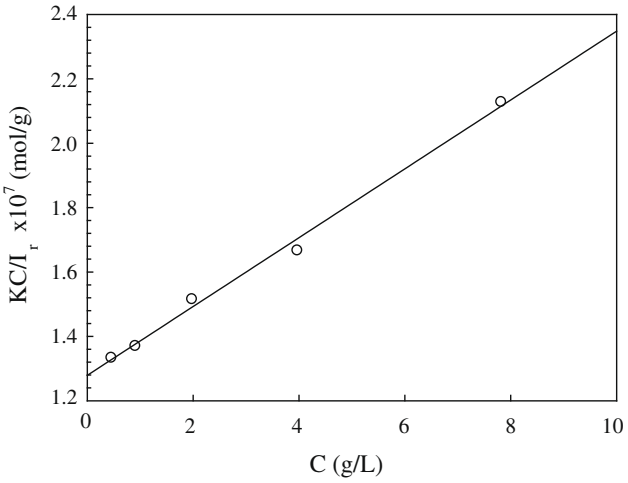

Fig. 2. Concentration dependence of KC/I_r (extrapolated to $q = 0$) for filtered FPI solutions at pH 11. The solid line represents a linear least-squares fit.

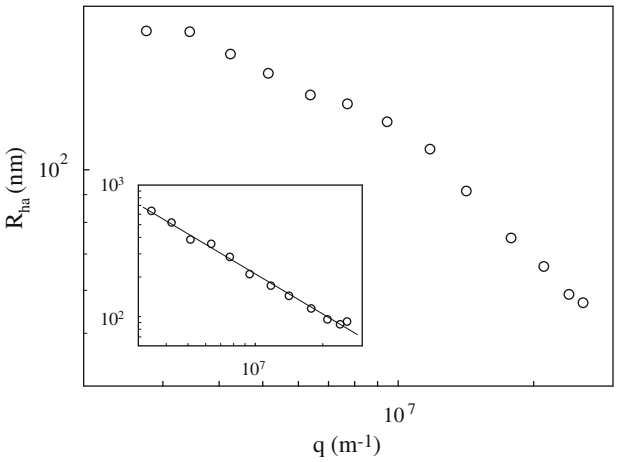

Fig. 3. Dependence of the apparent hydrodynamic radius on q of a filtered FPI solution at pH 11.0 and $C = 1$ g/L. The inset shows the results obtained without filtration. The solid line has slope −1.

heating and dilution. However, SDS–PAGE electrophoresis showed that there were no covalent bonds.

3.4. Reversible gelation

The systems stopped flowing when tilted if the pH was decreased below 9.5 at room temperature after a waiting time on the order of minutes in the range of pH 8.5–9.5. Down to pH 8.5, gels were formed that remained visually homogeneous for a period of at least one week. Below pH 8.5, large scale heterogeneity and syneresis appeared after a time that decreased with decreasing pH. Below pH 8 this occurred very quickly after setting the pH and no homogeneous gels were formed down to about pH 4.2. Between pH 4.2 and 3.8 gels were formed, but again heterogeneity appeared after some time. Finally, below pH 3.5 homogeneous solutions were obtained that resembled those at pH 11 showing some precipitation with time.

Fig. 4 shows the storage (G') and loss (G'') moduli of a FPI solution (pH 9.0, $C = 20$ g/L) at a shearing frequency $f = 0.01$ Hz during heating from 5 °C to 60 °C and subsequent cooling back to 5 °C. At high temperatures the system is a liquid with $G'' > G'$. During cooling, both G' and G'' increase steeply below about 30 °C and G' crosses G'' at about 25 °C. The same cross-over temperature was found for repeated measurements at pH 8.5 and 9.0 at this concentration, but the gelling point shifted to lower temperatures at lower protein concentrations.

Apart from the reversible cross-over from $G'' > G'$ at high temperatures (liquid) to $G' > G''$ at low temperatures (gel) and the strong increase of both moduli below 30 °C, another strong indicator of the reversible gelation is the dependence of G' and G'' on the shearing frequency f at high and low temperatures. At high temperatures we found that $G'' \propto f$ and $G \propto f^2$, as expected for viscous liquids, see Fig. 2 in Supplementary data. At 5 °C both G' and G'' were almost independent of the frequency, see Fig. 5, showing that the system had gelled. Gelation induced by cooling could be reversed by heating that showed melting of the gel at approximately the same temperature, see Fig. 4. Gel formation and melting was observed in repeated cooling and heating cycles. We speculate that hydrogen bonds are formed when the system is cooled, but more work is needed to elucidate the reversible gelation mechanism.

Besides thermo-reversible bonds, slow irreversible aggregation leading to precipitation from solution (or gel disintegration) was observed at all pH values. The rate of irreversible cross-linking increased with decreasing pH down to pH 5.5. Rheological measurements of self supporting gels were thus limited to pH 8.5–9.5, since only in this range gels were stable at a time scale of days.

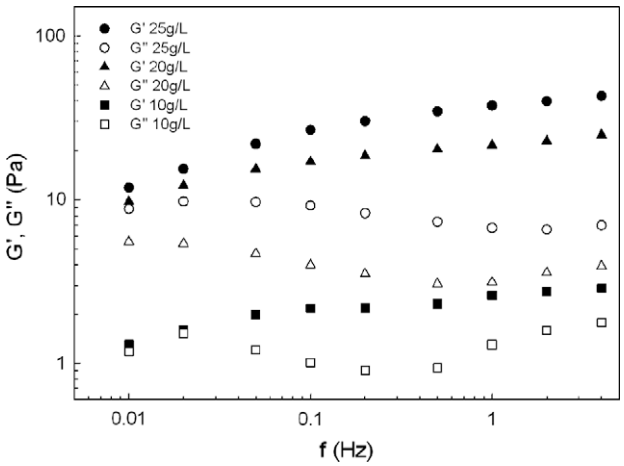

Fig. 5. Frequency dependence of G' and G'' of FPI gels (pH 9.0, 5 °C) at different concentrations indicated in the figure.

Fig. 5 shows the frequency dependence of G' and G'' of gels formed by direct cooling from 20 °C to 5 °C and pH 9.0 for different protein concentrations. Similar results were obtained at pH 8.5. G' was constant at high frequencies, but decreased slightly at lower frequencies. G" was lower than G' over the whole frequency domain and showed a weak upturn at low frequencies indicating a dissipation process at low frequencies.

Both storage and loss moduli of FPI cooled directly from 20 °C to 5 °C and 1 Hz increased strongly with decreasing pH in the pH range 10.0–9.0. G' was typically two orders of magnitude higher at pH 9.0 than at pH 10.0 for protein concentrations between 15 and 25 g/L, but exhibited only a weak dependence on the pH between pH 9.0 and 8.5. We typically observed very low storage moduli for gels at pH 10.0; such weak gels were observed to break and flow when tilted. Above pH 10.0 the system is clearly a liquid with $G'' \propto f$. The same conclusion can be drawn from the fact that G' is larger than G'' at 1 Hz only at pH < 10.0, see Fig. 3 in Supplementary data.

The storage and loss moduli at 1 Hz are plotted as a function of the protein concentration in Fig. 6 for pH 9.0 at 5 °C. With decreasing concentration the gels become weaker and flow when tilted for

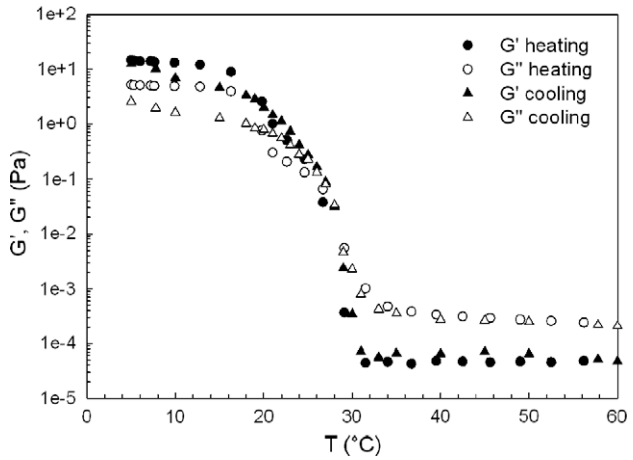

Fig. 4. Temperature dependence of G' and G'' at 0.01 Hz during heating and cooling of a FPI solution at pH 9.0 and $C = 20$ g/L. Filled symbols: G', open symbols: G''.

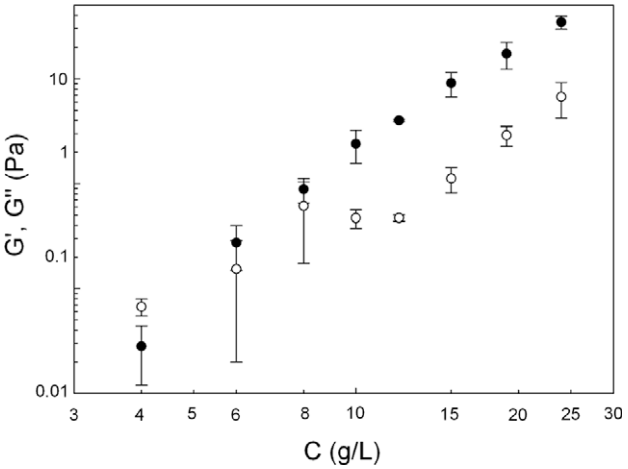

Fig. 6. Concentration dependence of G' (filled symbols) and G'' (open symbols) at 1 Hz of FPI gels (pH 9.0, 5 °C). The data represent averages of 4 trials and the error bars represent the standard deviation.

concentrations below 10 g/L. For pH 9.0, at $C = 5$ g/L and lower, G' at 1 Hz was less than G'' indicating that at such low concentration the system can no longer be considered a gel. At higher concentrations, G' increases strongly with increasing concentration and reaches about 35 Pa at 25 g/L. At pH 8.5 we found the same concentration dependence as at pH 9.0, but G' was roughly 50% higher than at pH 9.0 over the entire concentration range, see Fig. 4 in Supplementary data.

We only mention here that heating the systems above 30 °C influences the cold-setting gelation. The influence was small below pH 9.5 at the concentrations and frequencies studied here. However, it was important at higher pH at which self supporting gels did not form without preheating. When concentrated FPI solutions ($C = 25$ g/L) were heated above 30 °C and cooled down again, gelation was observed below a critical temperature of about 25 °C at pH values up to the pH at which FPI was isolated (11.0–11.2). These gels also exhibited reversible cold-setting, and melted when reheated again above 25 °C. A detailed study of the effect of preheating on cold-setting gelation of FPI will be reported elsewhere.

4. Summary

Cod muscle proteins could be solubilized to a large extent (about 60%) in alkaline solutions at pH > 11. Solutions of similar protein composition were obtained between pH 10.5–12.0, however, pH > 11 was required for optimal yield. Addition of salt (up to 0.25 M NaCl) did not affect protein yield or composition. A significant fraction of proteins was present in the solutions as large aggregates that slowly precipitated. These aggregates had a self similar structure with a fractal dimension close to two. When the pH was decreased below about 10, additional bonds were formed upon cooling leading to gelation below about 25 °C. The gels melted again at approximately the same temperature when the system was reheated. Irreversible aggregation also occurred at a rate that was very slow down to pH 8.5, but became increasingly faster with decreasing pH and was very fast below pH 8 and almost instantaneous below pH 7. Irreversible aggregation led to coarsening of the gel and syneresis at low temperatures or precipitation at high temperatures.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.foodchem.2008.11.046.

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