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Properties of fish protein–hydrophilic polymer hybrid gels

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Summary

Biogels and bio-hybrid gels were prepared from myofibril of fish and their properties were evaluated. The muscular protein used was extracted from fish meat. After washing and centrifugal separation, the muscular protein was mixed with 3 wt % of NaCl and the gels were prepared by heating the mixtures from 50 to 90 °C. The bio-hybrid gels were prepared from fish protein gel and poly(vinyl alcohol). Tensile tests revealed that the tensile strength, strain at break and Young's modulus of heated hybrid gels prepared at 50, 80 and 90 °C increased with increasing preparation temperature. This can be explained by the difference in crosslinking density. Also, the heated gels showed a good response to electric field in acid and alkaline solutions. Young modulus, tensile strength and elongation at break of the bio-hybrid gels increased with an increasing PVA content. Bending of hybrid gels in solutions of various pH under the electric stimulus was observed. The largest bending angle was 20-30° at pH = 1.2.

Introduction

Upon external stimulus, muscles transform chemical energy into mechanical energy efficiently and reversible contraction and relaxation take place. Myosin is the main component of muscle; it is a huge protein with the molecular weight of about 480,000 g/mol and length of 150 nm. All kinds of muscular myosin of animals change structurally by heating (denaturation), and hydrogel can be formed by heating after adding a salt. On the contrary, the conventional synthetic polymers like rubber, plastic, and fiber do not respond to the external stimulus reversibly. If the function that responds to an external stimulus is added to these synthetic polymers, it is possible that muscle-like polymers can be obtained [1].
The fishery in East Asia has stable marine resources, and many kinds of fish are present, mostly used as food. Fish paste, "kamaboko", is a typical food in East Asia.

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Processing and gelation of fish myofibrillar proteins were investigated by many fishery scientists. However, the properties of fish myofibrillar protein heated gels have not been investigated yet. In this study, fish myofibrillar protein was obtained from croakers, and used for the gel preparation. The heated gels and the hybrid gels with PVA were prepared. Mechanical and chemo-mechanical properties of the heated and hybrid gels were investigated.

Experimental

Preparation of heated fish protein gels

A fresh croaker was used as the sample fish. The head and internal organs of the sample fish were removed, and the fish was washed with cold water. The rest was sliced into two sheets, and the sheets were milled using the machine (hole 5 mm in diameter), which takes stamp type meat. Next, to the milled fish meat, a cold aqueous solution containing 1 wt. % sodium hydrogen phosphate (0.5 M solution of pH=7.5) and 0.001 wt % sodium azide were added. The solution was adjusted by cold distilled water, so that volume of fish meat: solution was approximately 1:10. After thorough mixing, the supernatant solution was removed and separated by centrifugation (500 *g*, 5 min). This process was performed twice. After stirring for 60 s with a mixer, 3 wt % NaCl was added to fish meat and stirring continued for another 60 s. The tendon was removed by the strainer, and centrifugation was continued to remove air bubbles. The obtained meat paste was placed in the plastic container (inner size 4.5×5.5 cm^2, height 5.5 cm). The gel was obtained by heating the fish meat paste for 1 hour from 50 to 90 °C. After heating, the sample was immediately cooled down with ice water [2].

Preparation of hybrid fish protein gels with poly(vinyl alcohol)

Rectangular sheet samples (35×3×3 mm^3) were cut from the fish protein heated gel sheet. Each sheet was immersed in 5 or 10 wt % poly(vinyl alcohol) (PVA) aqueous solution for 24 h. The number-average degree of polymerization of PVA was 500 and 1000. Alternatively, each protein sheet was dried at 80 °C before it was immersed in PVA solution. The sample code corresponds to PVA concentration and degree of polymerization, for example, 5-P1000, in another case - dried state(D) - PVA concentration - degree of polymerization; for example D-5-P1000.

Observation of heated protein gels and hybrid gels with optical microscope. Fish heated protein gels and hybrid gels were observed using optical microscope (Nikon Optiphot-2-POL, Japan).

TGA measurement
For TGA analysis, Thermoplus TG8120, Rigaku Denki, Japan, was used. Finely minced samples were weighed and inserted into the sample cells, the nitrogen flow was 30 ml min^{-1}. The temperature range was 25-500 °C, and heating rate was 20 °C min^{-1}.

Tensile test
Measurement was performed 3 times for each sample (20 mm initial length) and the value which showed maximum tensile strength was chosen. Three sides of the sliced

sample were measured with a sliding caliper, and the averaged magnitude was used for the calculation of the initial cross section area. The elongation rate was 5 mm min^{-1}.

Volume changes in acetone/water solutions
A piece of heated gel of size of 5×5×5 mm^3 was immersed in an acetone/water mixture at room temperature. Relative volume, V/V_0 of heated gel was pursued. Here, V_0 and V denote initial and swollen volumes, respectively. Three sides of the heated gel were measured with a slide caliper. Volume was calculated from the averaged magnitude.

Volume change with pH
The rectangular heated gels of size 7×4×4 mm^3 were cut from heated gel sheet. The sample was put into 0.1 M HCl, 0.1 M KCl, and 0.1 M KOH solutions and volume change of gel was measured at various immersion times.

Electric field response test
The rectangular pieces of heated gel of size 1×5×30 mm^3 was fixed to a stage and immersed in solution of pH = 1.1 - 13.2; also, the sample was placed between two platinum electrodes. Figure 1 shows definition of the bending angle for the electric field response test. The distance between the sample and each electrode was 2.5 cm. Imposed voltage and time were 10 V and 60 s, respectively.

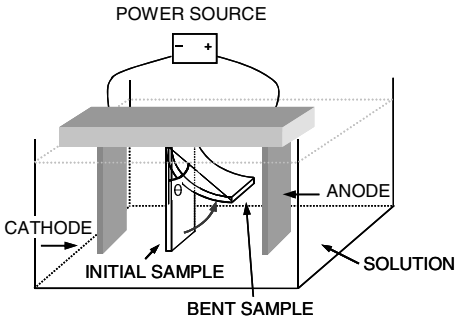

Figure 1. Definition of bending angle in electric-field-response test.

To evaluate the response to electric field quantitatively, the generated stress of heated gels by electric field was measured. Samples were fixed between two chucks, and a load cell was placed at one side to gain the force. The entire set-up except load cell was immersed in various solutions.

Results and discussion

Properties of heated fish protein gels

Figure 2 shows the stress-strain curves of the heated gels. Tensile strength, strain at break and Young's modulus are listed in Table 1. Tensile strength and strain at break of the heated gel prepared at 50 °C showed the highest values of the five gels. On the contrary, the Young's modulus of the heated gels decreased in the following order of heating temperature: 80 > 90 > 50 > 70 > 60 °C.
Generally, gel strength increases at low temperatures from 10 to 45 °C, and decreases at moderate temperatures from 40 to 50 °C. At high temperature, gel strength increases

Table 1. Mechanical properties of heated gels.

Preparation temperature [°C]	Tensile strength $\times 10^2$ [MPa]	Strain at break	Young's modulus $\times 10^2$ [MPa]
50	2.25	0.81	5.31
60	0.39	0.12	4.15
70	1.12	0.46	5.18
80	2.28	0.59	8.88
90	2.14	0.83	5.40

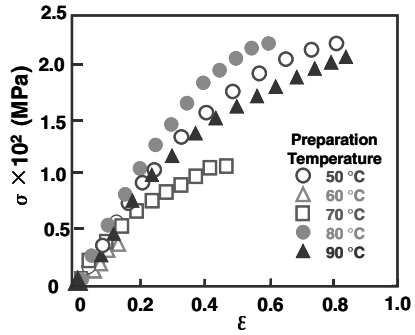

Figure 2. Stress-strain curves of heated gels prepared at various temperatures.

around 80 °C [4]. Gelation of myosin has been attributed to the aggregation of myosin head and/or twisting of the coiled-coil myosin tail by both physical crosslinkng due to hydrogen bonding, ionic bonding or hydrophobic bonding and chemical crosslinking due to formation of S-S crosslinks [5, 6]. Hydrogen bonding of myosin molecules breaks with increasing temperature. This results in conformational change on the myosin head and unwinding of coiled-coil tail. Inter-head S-S bondings and star-like multimers' are formed [7]. N. Seki et al. [8] reported that glutamic and lysine groups in a protein can be combined via T-glutaminase at around 45 °C. Furthermore, disulfide bond is formed between cysteine groups, and this trend gets stronger with increasing temperature above 60 °C [8, 9]. Therefore, it seems that the heated gels prepared at 50, 80, and 90 °C show up high gel strengths.

Figure 3 shows relative volumes of heated gels swollen in acetone/water mixtures. Swelling and deswelling are isotropic. Constant volumes were reached after 1 hour.

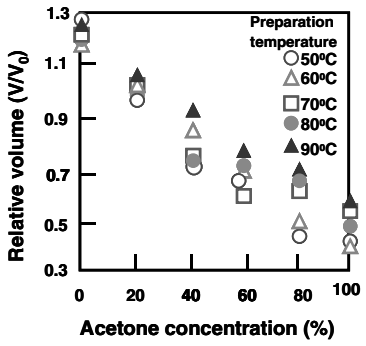

Figure 3. Relative volume of heated gels swollen in acetone/water mixtures.

For all samples, the relative volume decreased with increasing fraction of acetone. Moreover, the higher heating temperature, the smaller change in volume. The concentration of effective crosslinks in the heated gel is low when the gel is prepared at lower temperatures. Hence, the water molecule cannot be kept in the heated gel and diffuses out easily. On the contrary, since the heated gel prepared at higher temperatures has a large number of crosslinks, the water molecule is kept inside [3].
Table 2 shows the relative volume of the heated gels in solutions of various pH. The relative volume at pH 1.1 and 13.2 solutions showed larger value compared with that in pH 5.4. This result clearly indicates that the volume change is closely related to the electrostatic repulsions in the protein.

Table 2. Relative volume (V/V_0) of heated gels in solutions of various pH.

Sample	pH 1.1	pH 5.4	pH 13.2
50	2.00	1.19	3.48
60	1.81	1.23	3.29
70	1.87	1.12	3.02
80	1.61	1.20	3.20
90	1.93	1.24	3.48

Figure 4 shows bending angle of the heated gels in the electric field response test. The sample was bent to anode in acid solution and to cathode in neutral and alkaline solution. In acid solution, chloride ion attracted to the anode and the concentration chloride ion around the cathode decreases. Hence, it seems that amine cation (NH_3^+) at anode is neutralized more in comparison with the other side. This leads to decreasing gel volume on the anode side. Thus, it is likely that this volume change between two sides would be the reason for the heated gel to be bent to an anode in acid solution. Similarly, if potassium ions are attracted to cathode in alkaline solution, the reason for the opposite bending direction is analogous. In neutral solution, the isoelectric point of fish meat is expected around pH 5 and the positively charged protein gel is bent to the cathode side. The bending angles of the heated gels decreased in the following order: acid solution > alkaline solution > neutral solution. The smallest bending angle for the neutral solution implies that the bioelectric point of protein is around pH 5.

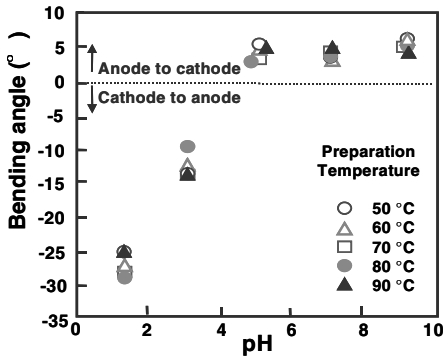

Figure 4. Bending angle of heated gels in electric field response test.

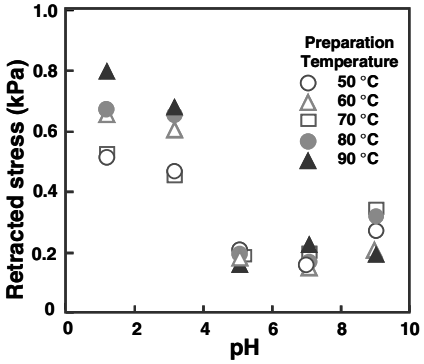

Figure 5. Retraction stress of heated gels in electric field response test.

The retraction stresses of protein heated gels were measured in selected aqueous solution of pH 1.1-9.3 under electric field. The dependence of retractive stress on pH is shown in Figure 5. This stress decreased from pH 1 to 5, and then it increased from pH 5 to 9. The maximum responsive stress was 0.5 – 0.8 kPa at pH 1.2 and 3. The minimum responsive stress was 0.2 - 0.4 kPa at pH 5 which was the isoelectric point of protein. It was found that the responsive stresses of heated gels were independent of preparation temperature.

Properties of hybrid fish protein heated gels

Optical micrographs showed that PVA penetrated the fish heated protein networks in the hybrid gels. In order to determine the PVA content in the hybrid heated gel, TGA measurement was carried out. The PVA content was calculated as difference between the weight loss (%) of the hybrid gel and that of the heated gel from 100 to 300 °C. The PVA content of the hybrid gels is shown in Table 3.

Table 3. PVA content and mechanical properties of hybrid gels.

Sample	PVA content [%]	Tensile strength $\times 10^2$ [MPa]	Strain at break	Young's modulus $\times 10^2$ [MPa]
Heated gel	0	3.63	0.72	8.76
5-P 500	8	5.71	0.95	11.20
10-P 500	17	6.58	1.78	8.82
D-heated gel	0	8.47	0.80	18.12
D-5-P 500	25	9.17	0.83	15.82
D-10-P 500	32	10.44	1.46	18.92
Heated gel	0	3.63	0.72	8.76
5-P 1000	7	4.18	1.38	6.04
10-P 1000	20	6.14	0.85	9.47
D-heated gel	0	8.47	0.80	18.12
D-5-P 1000	27	9.87	0.65	20.15
D-10-P 1000	35	11.98	0.65	26.49

The PVA content increased with increasing molecular weight of PVA and was higher in dried samples.

Figures 6 and 7 show the stress-strain curves of the hybrid gels prepared from wet heated gels and dried gels of P1000 series, respectively. Tensile strength, strain at break and Young modulus are listed in Table 3. These quantities increased with increasing PVA concentration. Tensile strength of the hybrid gels dried beforehand was larger by a factor of 2 – 3 compared with the hybrid gels. These results suggest that hybridization of PVA with fish heated protein gels is very effective in improving mechanical properties.

Figure 8 shows relationships between bending angle of the hybrid gels and pH in aqueous solution in the electric field response test. The sample was also bent to anode in acid solution and to opposite side in the case of neutral and alkaline solution. The bending behavior and bending angles showed up the same trends as heated gels. For the hybrid gels, the maximum bending angle was 20–30° at pH 1.2. PVA penetrated into fish protein heated gel did not affect the bending behavior.

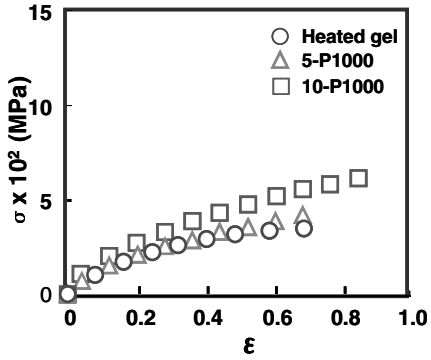

Figure 6. Stress-strain curves of hybrid gels prepared from wet heated gels.

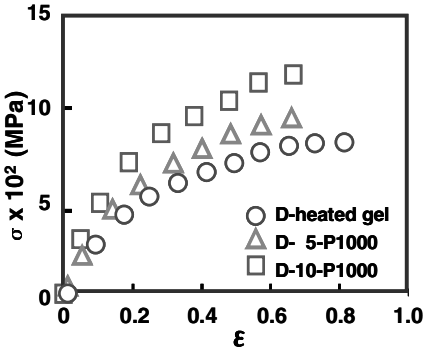

Figure 7. Stress-strain curves of hybrid gels prepared from heated gels dried beforehand.

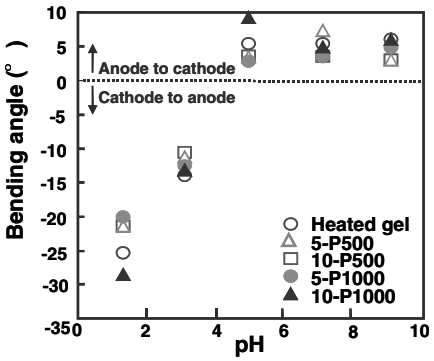

Figure 8. Bending angle of hybrid gels in electric field response test.

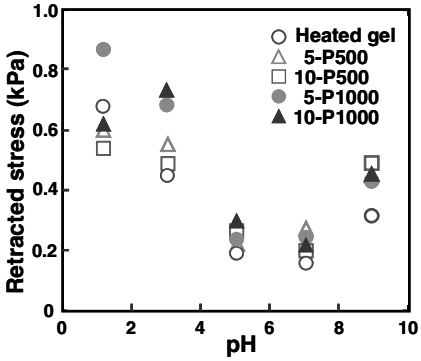

Figure 9. Retractive stress of hybrid gels in electric field response test.

The retractive stresses of hybrid gels were measured in selected aqueous solution of pH 1.1 - 9.3 under electric field. The dependence of retractive stress on pH is shown in Figure 8. Retractive stress of hybrid gels and protein heated gels decreased from pH 1 to 5, and then increased from pH 5 to 9. The minimum retractive stress was observed at pH 5 which was the isoelectric point of protein.

Conclusions

Heated gels were successfully prepared from unusual fish meat. From the relative volume of the heated gels swollen to equilibrium in acetone/water mixtures it follows that the crosslink density is quite dependent on the preparation temperature. Tensile tests revealed that the tensile strength, strain at break and Young's modulus of heated gels prepared at 50, 80 and 90 °C were higher compared with other gels. This can be explained well by the difference in the crosslink density. Also, the heated gels showed a good response to the electric field in acid and alkaline solutions.

The bio-hybrid gels were also prepared from fish protein gel and poly(vinyl alcohol). The Young's modulus, tensile strength, and elongation at break of the bio-hybrid gels

increased with increasing PVA content. The hybrid gels bent in solutions of various pH under the electric stimulus. These gels showed the largest bending angle (20-30°) and responsive stress at pH 1.2. This study suggests one of the most intriguing applications of unusual fish, for example, actuators, sensors, biodegradable materials, etc.

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