# すり身表面の蛍光測定


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# Fluorometry on the Surface of Surimi

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Surface hydrophobicity of the solid state of Alaska pollack surimi was studied using a front-surface viewing system of fluorometer. Fluorescence intensity of the surimi (excitation wavelength: 365 nm, emission wavelength 470 nm) was measured in the presence of ANS (sodium 8-anilino-naphthalene-1-sulfonate) raising temperature from 0° to 80°C at a constant speed of 1.2°C/min. The intensity was increased above ca. 35°C and reached a maximum, but decreased rapidly above ca. 55°C. By the addition of 3% sodium chloride to the surimi, the intensity was increased at every temperature and the temperature of the maximum intensity (Tm) was lowered. However, by the addition of 10% sucrose, the intensity was decreased and Tm was raised. This feature of the surimi added with the sucrose was somewhat cancelled by further adding of 3% sodium chloride. For every case, the intensity was proportionately increased by cooling the once heated surimi.

In the previous paper,[1-3] we have reported that the muscular protein solutions enhance their fluorescence intensity measured under the presence of sodium 8-anilino-1-naphthalene-sulfonate (ANS) on heating at 40° or 80°C, the temperature of setting of the shio-surimi (salted-viscous fish flesh paste) and that of kamaboko (fish flesh gel) formation, respectively. However, the increment of the intensity was decreased with the progress of heating, presumably, due to a gradual increase in the turbidity of the sample solution. On the other hand, fluorometric measurements can be easily performed also on the turbid solution or solid sample using a front-surface viewing system of fluorometer[4,5] as understood from the fact that entire spectra is obtained from paper or thin layer chromatograph spots. In the present paper, we describe the temperature dependence of the fluorescence intensity of the surface of surimi (fish flesh mince) measured on such an apparatus.

## Materials and Methods

*Surimi*

Non-salted Alaska pollack frozen surimi (Taiyo Fisheries, SA grade) was homogenized with five volumes of 0.3% NaCl aqueous solution to remove additives such as sucrose or sodium pyrophosphate, and centrifuged at 8000 rpm for 10 min. After washed twice, the surimi was ground with 30% weight of 1% ANS (w/v) solution. Some of the surimi was further ground with 3% weight of NaCl, 10% weight of sucrose, or their mixture. The sample surimi was packed into a constructing cell for the determination of optical density (light pass length: 1 mm).

*Fluorescence Measurement*

A cell holder of fluorometer (Arakawa Kenkyu-sho, Separable minicolorimeter, VIS-Fl) was remodeled in order to determine the fluorescence intensity for the surface of solid sample under the controlled temperature. In Fig. 1, a drawing of the remodeled cell holder is shown, in which the excitation light irradiated the constructed cell

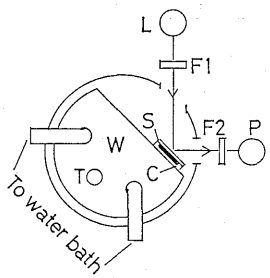


**Fig. 1.** A cell holder remodeled for the determination of fluorescence intensity of solid sample.
L: light source, S: sample, C: constructing cell, W: water jacket, T: thermometer, P: photomultiplier, F1: filter for excitation light, F2: filter for emission light.

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at an angle of 45° and the temperature of the cell holder was controlled by circulating water from an external water bath. The relative fluorescence intensity of the surimi was measured at an excitation wavelength of 365 nm and an emission wavelength of 470 nm after cooled to 0°C. Then, the temperature was raised to 80°C at a constant speed of 1.2°C/min. After the temperature reached 80°C, the cell holder was gradually cooled to a room temperature by circulating water. The fluorescence intensity was recorded successively during raising and lowering the temperature.

The sensitivity of the fluorometer was adjusted so as that the intensity of 0.004% fluorecein sodium solution became 50% at the emission wavelength of 510 nm (cell: 1 cm).[1)]

## Results

In Fig. 2, temperature-relative fluorescence intensity curves are shown for the Alaska pollack surimi with/without 3% sodium chloride. The intensity was slightly decreased with raising the temperature of the cell holder up to ca. 30°C irrespective of the presence and absence of sodium chloride. Above ca. 35°C, the intensity was increased, especially remarkable in the presence of sodium chloride, and immediately reached a maximum. In the presence of sodium chloride, the intensity was higher and the temperature at the maximum intensity (Tm) was lower by ca. 8°C than in its absence. For both cases, the intensity was rapidly decreased above ca. 55°C with raising the temperature. Next, on cooling the once heated surimi, the intensity was increased. From 80° to ca. 55°C, the temperature-intensity curve entirely coincided with that at the case of raising the temperature. Bellow ca. 55°C, the intensity was proportionately increased with lowering the temperature. The increment upon cooling was more remarkable in the presence of sodium chloride.

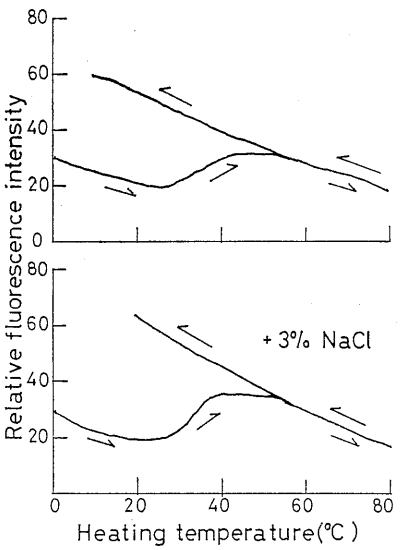


**Fig. 2.** Temperature dependence of relative fluorescence intensity of Alaska pollack frozen surimi.

Upper: washed surimi, Lower: the above surimi was ground with 3% NaCl.

Temperature was raised first at a constant speed of 1.2°C/min, then lowered to a room temperature by circulating water.

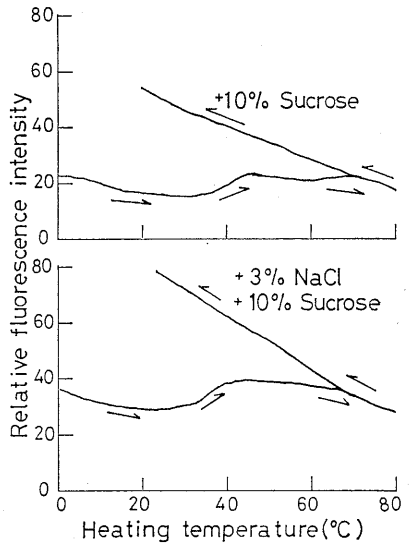


**Fig. 3.** Temperature dependence of relative fluorescence intensity of Alaska pollack frozen surimi.

Upper: Washed surimi was ground with 10% sucrose.

Lower: Washed surimi was ground with 3% NaCl and 10% sucrose.

In Fig. 3, the temperature-intensity curves are shown for the Alaska pollack surimi added with 10% sucrose and the surimi added with both of 3% sodium chloride and 10% sucrose. They were prepared respectively by adding sucrose or sodium chloride to the same washed surimi as in Fig. 2. The curves nearly resembled the above two ones, but by adding 10% sucrose the intensity was lowered at every temperature and Tm was raised. This feature in the surimi added with the sucrose was somewhat cancelled by further adding 3% sodium chloride, that is, the intensity was increased and Tm was lowered.

## Discussion

Fluorescence is known to be generated when "so-called" fluorescent probe, such as ANS, binds to the hydrophobic regions of the protein surface.[6,7] Because in our previous studies,[1-8] the fluorescence intensity of the heated actomyosin and myosin solutions was determined after cooled to a room temperature, their structure may be returned by cooling. The purpose of the present study is to clarify the behavior of the hydrophobic amino acid residues of the muscular proteins during the heat processing of the surimi. Furthermore, the fluorescence measurement was carried out here on the surface of the solid surimi, which is rather conformable to a practical process. The intensity was slightly lowered with raising temperature to ca. 30°C. The hydrophobic amino acid residues exposed on the surface of the muscular proteins may get into the interior and stabilize the molecular structure. In fact, some proteins are known more stable at room temperature than at 0°C.[8] Then, the intensity was increased at the temperature above ca. 35°C, where the ATP-ase activity of myosin would be lost. This has been already certified on the rabbit skeletal myosin solution and interpreted as an exposure of the hydrophobic residues buried in the interior of the native myosin molecule.[6] The agreement between the result on the myosin solution and that of ours suggests that the fluorometric measurement of the surimi can be performed also in the solid state.

By adding sodium chloride to the surimi, the intensity was increased. This is similar to our previous result in which the intensity of various fish actomyosin solutions was increased by the addition of potassium chloride.[9] The hydrophobic interactions would be strengthened on the protein surface by the addition of salt having water-structure making property, that is, the hydrophobic amino acid residues are assembled each other so as not to be in contact with water, and this is the reason why the setting of the shiro-surimi is induced by the salt. Strengthening the hydrophobic interactions by sodium chloride can be recognized also from the fact that Tm was lowered in its presence. The intensity was, however, rapidly decreased as the temperature overcomes ca. 55°C. This may be due to thermal quenching of fluorescence of ANS bound to the protein surface. However, it may also be due to weakening of the hydrophobic interactions at higher temperature,[7] where the hydrophobic hydration around the exposed hydrophobic residues is weakened by the heat-destabilization of hydrogen bond.

On cooling the surimi once heated to 80°C, the intensity was increased. This would be due to strengthening the interactions among the exposed hydrophobic residues, presumably, based on the recovery of the hydrophobic hydration. It is presumed also here that the hydrophobic interactions closely relate to the network structure not only within the setting gel but also within the kamaboko. On the other hand, by adding sucrose, the intensity of the surimi was lowered at every temperature and Tm was raised, which provides an evidence of stabilization of the muscular proteins. In fact, the denaturation temperature has been found to be raised by sucrose for some proteins.[10,11] Most recently, the effect of polyols containing sucrose,[11-16] and amino acids[17] on the heat stabilization of various proteins has vigorously been clarifying from thermodynamical standpoint. In the presence of sucrose also having the strong water-structure making property similarly to sodium chloride, the protein molecules are prefentially hydrated, meaning that the water around the protein molecules contains less sucrose than the bulk water, that is, sucrose molecules are near to fully excluded from the protein surface. This is due to that essentially hydrophilic sucrose molecules are antagonistic to the exposed hydrophobic residues. As a result, the hydrophobic residues tend to get into the interior of the protein molecules so as not to contact with the sucrose molecules and stabilize the molecular structure. Such the stabilization of the molecular structure was proven also here from the above mentioned fluorometric behavior of the surimi added with sucrose. Therefore, it is a matter of course that the setting of the surimi is retarded in the presence of sucrose. But, such the stabilization by sucrose was somewhat cancelled by further adding sodium chloride. The reason is still unknown, however, why sodium chloride also does not strengthen the intramolecular hydrophobic interactions, although the sodium chloride also has the water-structure making property and induces prefential hydration of the protein in a similar manner as sucrose.[18]

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