Suppressive Effect of Phosphatidylcholine on the Thermal Gelation of Alaska Pollack Surimi†

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Addition of phospholipid (PL) significantly lowered the breaking force and deformation of the thermal gel of Alaska pollack surimi by suppressing the setting, while triglyceride (TG) did not affect the gelation profile. Phosphatidylcholine (PC) was responsible for the inhibitory effect of PL on setting. Neither choline nor phosphorylcholine had such an effect. PL addition did not inhibit the cross-linking reaction of the myosin heavy chain occurring in the setting process at all. It was, therefore, concluded that the amphipathic property of PC was required to inhibit the setting and that noncovalent bonds necessary for network formation in the set gel might be interfered by PL in a liposome-like form.

Keywords: *Gelation; phosphatidylcholine; pollack; surimi; transglutaminase*

INTRODUCTION

Fish lipid is characterized by its high content (about 35%) of polyunsaturated fatty acids such as eicosapentaenoic acid (20:5) or docosahexaenoic acid (22:6) by adaptation to low environmental temperatures. Studies on the functionality of these fatty acids have proved that they have health promotion and preventive effects especially related to cardiovascular diseases (Leaf and Weber, 1988; Kinsella et al., 1990). Despite the functionalities of fish lipid, intake of these fatty acids through consuming fish seems to be limited because of its unique smell and taste, especially for Western people. Under the circumstances, it seems it is necessary to find a more acceptable and easier way for intake of these fatty acids. One of the possible ways might be to incorporate purified fish lipid free of the unfavorable smell into seafood products. Surimi seems a favorable material for this purpose because a thermal elastic gel such as kamaboko or crab meat analogue made from surimi is now popular worldwide.

An unfavorable property of polyunsaturated fatty acids is that they are easily oxidized by absorbing oxygen during storage, generating harmful oxidation products (Cho et al., 1987; Fritsche and Johnston, 1988). However, it has been recently reported that purified fatty acids are quite resistant to oxidation in an aqueous medium (Miyashita et al., 1995). This suggests that lipid abundant in polyunsaturated fatty acids incorporated into surimi may also be kept stable. This might be another merit of the method.

Thermal gelation of meat, including surimi, is regarded as a controlled denaturation process of myosin; heat induced unfolding is followed by a stable matrix formation (Ishioroshi et al., 1979). In addition to this, in kamaboko processing, a step termed "suwari" or "setting", a procedure to place the salted surimi at a relatively low temperature prior to heating at a high temperature, is included for improving the gel strength of the final product. Potential action of transglutaminase (TGase, EC 2.3.2.13) catalyzing a cross-linking reaction of the myosin heavy chain (HC) in the setting process has been proposed (Seki et al., 1990; Kimura et al., 1991).

In this study, we elucidated the effect of lipid in a form of either triglyceride (TG) or phospholipid (PL) on the thermal gelation of Alaska pollack surimi.

METHODS AND MATERIALS

Materials. Purified sardine oil consisting mainly of TG was purchased from Nippon Oil and Fats Co., Ltd. (Ibaragi, Japan). PL prepared from antarctic krill consisting of 90% phosphatidylcholine (PC) and 8% phosphatidylethanolamine (PE) and a small amount of cholesterol was donated by Nissui Co., Ltd. (Tokyo, Japan). PL from carp eggs was also a donation by Toyama Food Research Institute (Toyama, Japan). PC was isolated from the krill PL by column chromatography packed routinely with silica gel. Briefly, crude PL dissolved in chloroform was applied to the column (2.5 cm × 45 cm) of Wako gel 200, and the components were eluted by increasing the proportion of methanol to chloroform in the elution solvent. PC was mainly eluted with a solvent consisting of a methanol/chloroform ratio of 3/1. Crude lecithin (or PL) from chicken egg yolk and soybean and choline chloride were purchased from Wako Pure Chemical Industry Ltd. (Osaka, Japan). Phosphorylcholine chloride calcium salt was purchased from Sigma (St. Louis, MO). Choline chloride and phosphorylcholine chloride were both used after neutralization with either NaOH or HCl.

Frozen surimi (FA grade) of Alaska pollack (*Theragra chalcogramma*) was donated from Nissui. The surimi contained 4% sucrose, 4% sorbitol, and 0.25% sodium polyphosphate. Its water content and protein content were 75.3 and 17%, respectively.

Preparation of Thermal Gel. Frozen surimi stored at −40 °C was removed to a cold room (+4 °C) prior to use. Diced surimi was chopped in a food processor (Speed Cutter MK−K7, Matsushita Electric Industrial Co., Ltd. Osaka, Japan). To the surimi, 25% cold water was added together with 2.5%

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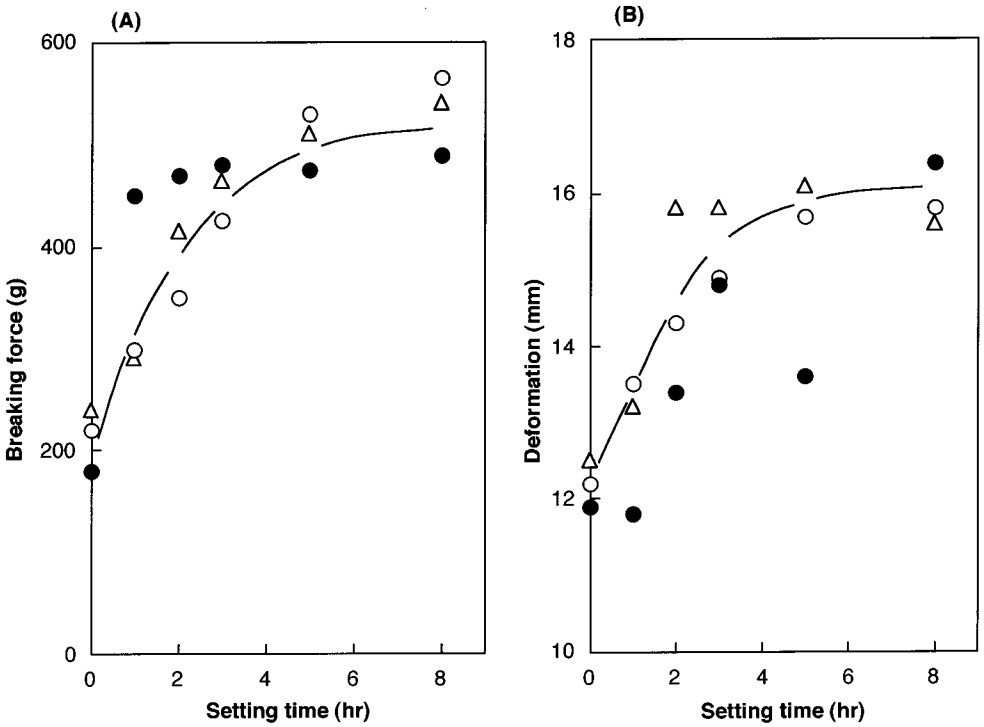

Figure 1. Effect of sardine TG on the thermal gelation of Alaska pollack surimi: ●, gel without TG; ○, gel with 10% TG; △, gel with 15% TG. (A) Breaking force; (B) deformation.

NaCl. The mixture was further chopped 8 times for 30 s with 30 s intervals to make a homogeneous salted meat paste. At this stage, lipid was added. The fluid TG was mixed well with the salted surimi, while hard paste-like PL was not. It was essential to dissolve PL in ethanol in advance for its homogeneous mixing. The final ethanol content was adjusted to 2.5%, which did not affect the gelling profile. The control gel always contained 2.5% ethanol. Prepared salted paste was subsequently stuffed into an aluminum pipe (17 mm diameter and 20 mm length), and wrapped with poly(vinylidene chloride) film (Riken Vinyl Industry Ltd., Tokyo, Japan). The samples were transferred to the water bath controlled at 25 °C for setting up to 8 h. The set gel was further cooked at 90 °C for 20 min to produce kamaboko or thermal gel.

Rheological Measurement of Kamaboko Gel. Before the puncture test, the thermal gel was taken from the ice-cold water bath and left for 2−3 h at room temperature (about 25 °C). Then, both ends of the kamaboko gel were cut evenly, and the gel was removed from the container carefully. Breaking force (g) and deformation (cm) were both recorded on a Rheometer RE-3305 (Yamaden, Tokyo, Japan) using a 3 mm diameter cylindrical plunger with a loading speed of 0.5 mm/s.

Solubilization of the Set Gel and Sodium Dodecyl Sulfate−Polyacrylamide Gel Electrophoresis (SDS−PAGE). To determine the cross-linking of myosin HC, the set gel was first boiled for 2 min in a medium of 8 M urea, 2% SDS, 2% 2-mercaptoethanol, and 20 mM Tris-HCl (pH 8.0) to terminate the cross-linking reaction and then dissolved into the solution by constant stirring overnight at room temperature (Numakura et al., 1985). The solution was clarified by centrifugation at 3000*g* for 15 min, and the clear supernatant was saved as a solubilized protein solution. Practically no proteins were sedimented by the centrifugation. When the TG containing gel was solubilized, the protein solution was turbid due to emulsified TG. TG was well-removed from the protein solution as a floating lipid layer by centrifugation. In this case, the clear middle layer of the centrifugal tube was carefully saved as the protein solution.

To resolve cross-linked myosin HC bands on mini slab gels (8 × 9 cm), SDS−PAGE was carried out with 3−0.5% polyacrylamide−agarose gel containing 0.1% SDS (Varelas et al., 1991). After electrophoresis, protein bands were stained with Coomassie brilliant blue R-250. Acrylamide HG (Wako) and Agarose GP-36 (Nakalai Tesque, Kyoto, Japan), both of electrophoresis grade, were found suitable for preparing the above fragile gels.

The experiment was repeatedly performed.

RESULTS AND DISCUSSION

Thermal Gelation of Surimi Which Contained TG. Japanese researchers generally used stainless steel cylindrical pipes 30 mm in diameter and 30 mm in height as containers for the thermal gel, but this size was too large to use in our experiment because of a limited amount of lipid available, especially PL. Instead, small size aluminum pipes were used as containers in this paper. Consequently, we used 3 mm diameter plungers not 5 mm plungers.

For investigating the effect of lipid on the gel formation, homogeneous mixing is the first problem to be solved. An attempt to mix TG with the unsalted surimi was unsuccessful, but it was found to be quite easy to mix TG homogeneously after salting the surimi.

Parts A and B of Figure 1 demonstrated that the breaking force and deformation (or strain) for the control gel both increased with duration at 25 °C; namely, the setting effect was well-recognizable. Setting for 6−8 h increased the breaking force from 200 to 500 g, roughly a 250% increase. Though the data were scattered, the deformation similarly increased from 12 to 16 mm by the setting procedures. As demonstrated in Figure 1A,B, these increases in the breaking force and deformation by setting procedures were unaffected by the addition of sardine oil at 10 or 15%. The data indicated that TG had no effect on the setting effect, or TG could be added to the thermal gel without affecting the rheological properties of the final product. The maximal amount of TG the gel could hold was found to be roughly 15% when the conventional mixing method was employed.

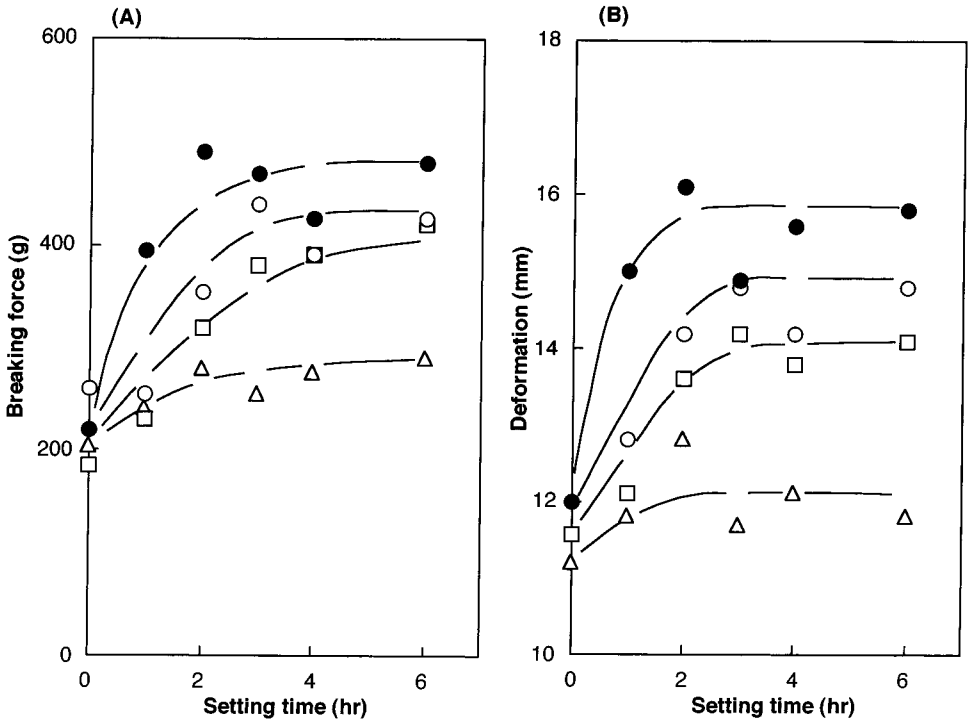

Figure 2. Effect of krill PL on the thermal gelation of Alaska pollack surimi: ●, gels without PL but 2.5% ethanol; ○, gel with 1% PL; □, gel with 2.5%; △, gel with 5% PL. (A) Breaking force; (B) deformation.

Thermal Gelation of Surimi with PL. The next step was to examine whether PL, an amphipathic lipid, affected the thermal gelation of surimi. It was found to be practically impossible to disperse PL homogeneously in the salted surimi paste by direct mixing because of the hard paste-like conditions of PL. This problem was overcome by dissolving PL in ethanol. By investigating the effect of ethanol on the gel formation, we confirmed that the ethanol content up to 5% was negligible in the gelation of surimi. Nevertheless, we always added the corresponding amount of ethanol used for solubilization of PL to the control gel. The effects of PL on the properties of the thermal gel are presented in Figure 2. In contrast to the case of TG, an increase in the breaking force was strongly suppressed upon addition of PL in a dose-dependent manner. For instance, setting for 6 h very slightly increased the breaking force from 200 to 270 g for a gel containing 5% PL. It should be noted that PL addition only slightly decreased the breaking force for the directly heated gel at 90 °C without the setting process. An increase in the deformation by setting was also decreased upon addition of PL. It was demonstrated that PL lowered the two rheological parameters of the thermal gel by reducing the setting effect.

The effect of PL on setting was further studied by varying the PL concentration. Salted surimi containing various amounts of PL was incubated at 25 °C for 4 h, sufficient for developing the full breaking force. Breaking forces obtained are shown in Figure 3. Incubating the salted surimi for 4 h increased the breaking force by about 2.3 times. By increasing the addition of PL, this high breaking force for the set gel gradually decreased and finally approached a level similar to that for the gel without setting. Approximately 5% PL was sufficient for a full decrease in the breaking force under these conditions. It is again noteworthy that the breaking force for the gel directly heated at 90 °C without the setting process was nearly the same, irrespective of PL content. It was, therefore, concluded that PL reduced the gel strength of the thermal gel by suppressing the setting effect rather than by inhibiting heat-induced gel formation.

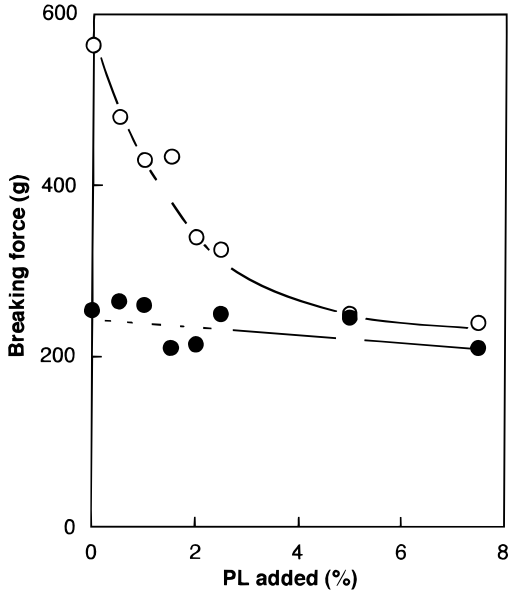

Figure 3. Concentration-dependent inhibitory effect of krill PL on setting: ●, gels without setting; ○, gels set for 4 h.

Thermal Gelation of Surimi with PL from Various Sources. We next studied whether the PL from other sources equally affected the setting process. We tested crude PL (lecithin) from chicken egg yolk, soybean, and carp egg as well as krill. As shown in Figure 4, all PL tested similarly suppressed the breaking force increment regardless of its source. As sardine TG unaffected the setting (Figure 1), then we tested the effect of TG from another source. Triolein, the TG we used, did not affect the setting, the same result as that with sardine oil (data not shown).

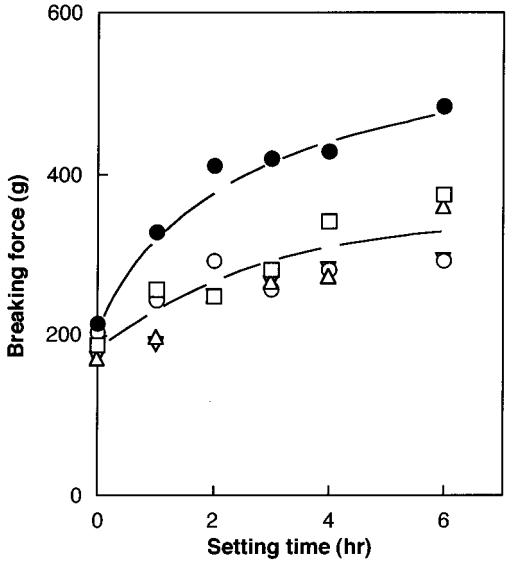

Figure 4. Effect of PL from various sources on the setting: ●, gel without PL but 2.5% ethanol; ○, gel with PL from krill; □, gel with PL from chicken egg yolk; △, gel with PL from soybean; ▽, gel with PL from carp egg. PL added were all 5%.

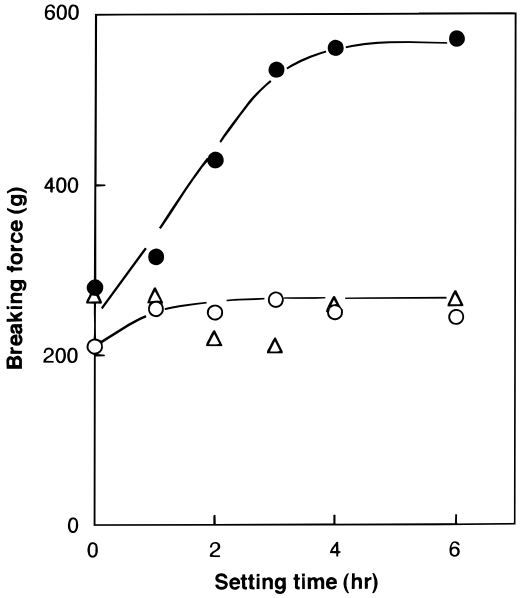

Figure 5. Effect of PC isolated from antarctic krill PL on the gelling profile: ●, gel without lipid; □, gel with 5% PL; △, gel with 5% PC.

Since all PL suppressed the setting and since all TG did not, the structural difference of PL from TG, namely, polar or nonpolar, seemed to determine the effect. Although it is known that the fatty acids composition characterizes the physical properties of PL such as the melting point or fluidity, these seemed to have nothing to do with the inhibitory effect of PL.

Suppressive Effect of Phosphatidylcholine on Setting. The component of PL responsible for the inhibitory effect was investigated next. Because PC is a major component in krill PL, PC was isolated and its effect on the setting was tested (Figure 5). PC at 5% suppressed the increase of the breaking force nearly as completely as parent PL. It is, therefore, concluded that PC is the component that reduces the setting effect. Involvement of PE is still possible, but we could not test its effect because of the insufficient amount available.

Effect of Choline and Phosphorylcholine on Setting. The structural difference of PC from TG was the replacement of one fatty acid at the γ position by phosphorylcholine giving polar properties to PC. We then studied whether choline or phosphorylcholine itself inhibited the setting. When we determined the amount of components to be added, we took the molar basis rather than the weight basis. Assuming the molecular weight of PC as 780 (diolein), 5% PC corresponded to 0.9% choline chloride (molecular weight, 140) and 1.65% phosphorylcholine chloride (molecular weight, 258) as the molar basis. Thus, choline chloride up to 1.25 and phosphorylcholine chloride up to 2% were added for testing the effect. By increasing these added compounds, the breaking force for the gel decreased gradually. However, this decrease was suggested to be due to the increased ionic strength caused by the addition of the compounds to the surimi, not due to the suppressive effect on setting. As the calculated molarity of these compounds added at the highest concentration was roughly 80 mM, we measured the breaking force for the gel without the compounds but with an additional 80 mM of NaCl. As shown in Figure 6, an additional 80 mM NaCl reduced the breaking force with an extent similar to that by choline chloride or phosphorylcholine. It was, therefore, concluded that the suppressive effect requires the whole PC structure, not a phosphorylcholine moiety.

Cross-Linking Reaction of Myosin HC in the Set Gel with PL. The cross-linking reaction of myosin HC catalyzed by TGase is a prominent change detectable in the protein composition during the setting process. Thus formed cross-linked myosin HC is believed to be attributed to the network structure of the set gel (Numakura et al., 1985; Seki et al., 1990; Kimura et al., 1991). Since the setting effect was drastically suppressed upon addition of PL, we examined whether the cross-linking reaction of HC was inhibited by the addition of PL. The cross-linking reaction was monitored by SDS−PAGE on a mini slab gel consisting of 3% poly(acrylamide) and 0.5% agarose. The SDS−PAGE pattern of the salted surimi (Figure 7A, 0 h setting) indicated that the SDS−PAGE system clearly resolved the high molecular weight band of connectin (or titin) (Maruyama, 1986) with a size of 2 000 000 appearing near the top of the gel. Moreover, the SDS−PAGE also separated the low molecular weight band of myosin light chains or troponin subunits with a size of roughly 20 000. The gel system seemed suitable for the analysis of the changes in protein composition of surimi during the setting process, because the generated high molecular weight bands by cross-linking of myosin HC and degraded low molecular weight fragments derived from HC by protease action could both be analyzed on a single slab gel plate. As Figure 7 demonstrates, the most dominant change in the pattern occurring in the set gel was a decrease in the HC content accompanying a generation of cross-linked HCs denoted HC2, HC3, HC4, and HC5 (Numakura et al., 1985). Degradation of myosin HC might be negligible under these conditions because detectable degraded fragments were not in the pattern. The disappearing profile of the HC band and the appearing profile of cross-linked products for the set gel with PL were indistinguishable from those for the control gel. Moreover, protein stacked on the top of the gel was equally detected for both set gels when incubated for 4 h. This might be highly cross-linked HC unable to penetrate into the polyacrylamide gel network. These results demonstrated that the addition of PL did

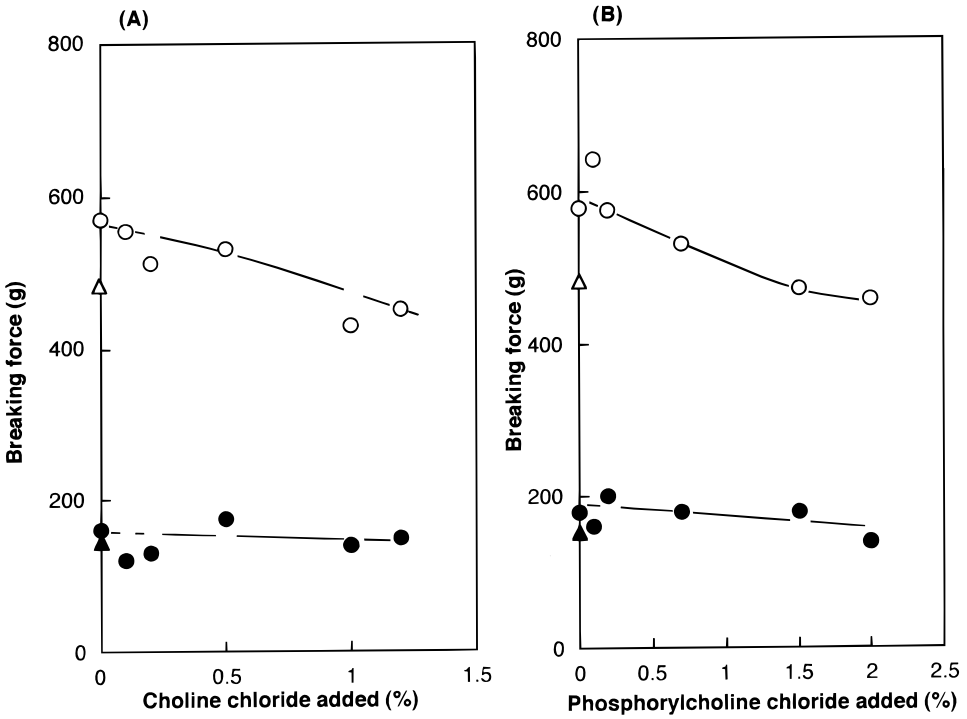

Figure 6. Effect of choline chloride and phosphorylcholine calcium salt on setting: ●, gel without setting; ○, gel set for 4 h; ▲, control gel with additional 80 mM NaCl without setting; △, control gel with additional 80 mM NaCl set for 4 h. (A) Choline chloride; (B) phosphorylcholine chloride.

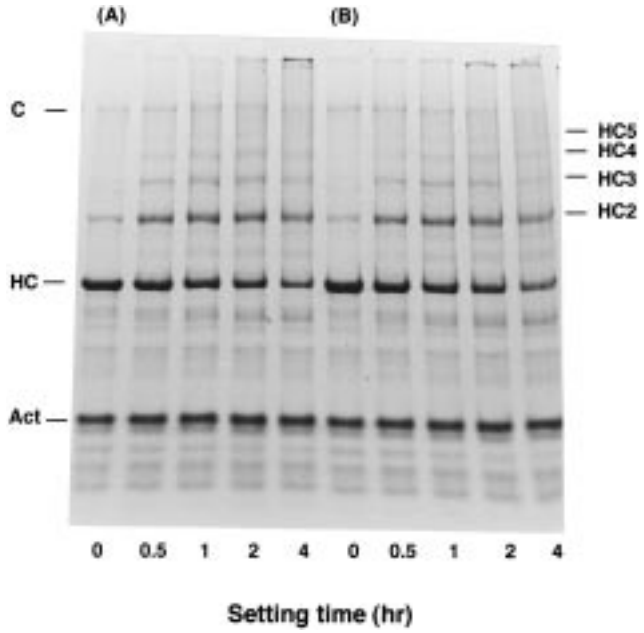

Figure 7. Change in the SDS−PAGE pattern of salted surimi during the setting: (A) set gels without PL; (B) set gels with 5% krill PL. HC, HC_2, HC_3, HC_4, and HC_5, are myosin heavy chain monomer, dimer, trimer, tetramer, and pentamer, respectively. Act and C are actin and connectin bands, respectively.

not inhibit the cross-linking reaction at all. Assuming that the cross-linking of HC is catalyzed by TGase, PL seemed not to inhibit the enzyme activity. Since a SDS−PAGE analysis of the set gel only provided the information on the covalent bond formation, namely, the cross-linking of HC, and as various types of noncovalent bonds are suggested to be involved in the elastic gel formation, PL seemed to prevent the noncovalent interaction of the myosin molecule necessary for gel formation, not a covalent bond formation.

Regarding the state of the lipids and compounds tested in the set gel, nonpolar TG would exist as a small oil droplet, or it forms emulsion in the surimi, while amphipathic PC would be finely dispersed in the surimi as liposome-like vesicles. Water-soluble choline chloride and phosphorylcholine would be evenly distributed in the gel without forming such a specific structure. Since only PC suppressed the setting among them, it suggests that liposome-like vesicles made of PC dispersed in the salted paste might interfere with the noncovalent bond formation necessary for the gel network formation.

Indeed, myosin HC cross-linking is one major factor in determination of the setting effect. We demonstrated in this paper that the cross-linking reaction of HC did not always correlate with the setting effect or gel strength. We here emphasize the importance of noncovalent interaction in the set gel containing PL. The importance of the noncovalent bond in the setting mechanism has been proposed by Niwa et al. (1995).

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