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The effects of sodium bicarbonate on conformational changes of natural actomyosin from Pacific white shrimp (*Litopenaeus vannamei*)

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ABSTRACT

Changes in natural actomyosin (NAM) from Pacific white shrimp (*Litopenaeus vannamei*) treated with sodium bicarbonate ($NaHCO_3$) at different concentrations (0–1 M) in the absence or the presence of 2.5% NaCl were studied. Turbidity of NAM solutions decreased with coincidental increase in solubility as the concentration of $NaHCO_3$ increased. Surface hydrophobicity (S_oANS) and total sulfhydryl content of NAM also increased when $NaHCO_3$ concentration increased. Greater decreases in Ca^{2+}- and Mg^{2+}-ATPase activity were found in all NAM as $NaHCO_3$ concentration increased, suggesting the denaturation of myosin head and the dissociation of actomyosin complex. The zeta potential (ζ) analysis suggested that the surface of NAM became more negatively charged (−12.12 to −26.98) as $NaHCO_3$ concentration increased. Those changes were more intense in the presence of 2.5% NaCl. Transmission electron microscopy showed that the structure of actomyosin was more dissociated and lost the filamental structure when $NaHCO_3$ at higher levels was used.

1. Introduction

Functional properties of muscle protein are closely associated with the integrity of proteins. Denaturation and degradation of fish muscle proteins mainly contribute to the loss of those functionalities (Montecchia, Roura, Roldan, Perez-borla, & Crupkin, 1997). Protein–protein interactions termed association, aggregation and polymerisation, are dependent upon many factors such as temperature, pH, etc. (Zayas, 1997). Since protein–protein interactions lead to changes in the secondary and tertiary structures of the protein molecules, these changes could affect fat and water-binding affinities of these molecules (He, Azuma, & Yang, 2010). The ability of muscle to absorb the added water during processing and capacity of retaining the water after cooking and freezing are the important factors governing the quality of seafood and seafood products (Ogawa, Tamiya, & Tsuchiya, 1994). Textural changes of meat or seafoods are due to protein denaturation and aggregation and are associated with water holding capacity (WHC) (Xiong, Xiong, Blanchard, Wang, & Tidwell, 2007).

To increase water holding capacity of meat or seafoods, phosphate compounds have been intensively used (Rattanasatheirn, Benjakul, Visessanguan, & Kijroongrojana, 2008). Due to the strict regulation of using phosphates in seafoods, especially shrimp, other additives with the similar properties in increasing the yield have been paid increasing attention.

Non-phosphate additives, particularly sodium bicarbonate, have been reported to be effective in improving the water-holding capacity, colour, and organoleptic properties of fresh meats, beef, pork and poultry (Kauffman, Greaser, Pospiech, & Russell, 2000). Bicarbonate has been also used to minimise the problem of pale, soft and exudative pork (Wynveen et al., 2001) and to mask the typical aroma and flavour in sow meat (Sindelar et al., 2003). Furthermore, salts, especially sodium chloride, have been often used to modify the ionic strength of muscle. Salt can slightly stabilise or destabilise the proteins, depending on the nature of the specific charge distribution within the protein (Record, Zhang, & Anderson, 1998). NaCl at a level of 2.5% was used in combination with 0.875% sodium acid pyrophosphate (SAPP) and 2.625% tetrasodium pyrophosphate (TSPP) to increase the yield of Pacific white shrimp (Rattanasatheirn et al., 2008). Recently, Chantarasuwan, Benjakul, and Visessanguan (accepted for publication) reported the increase in water uptake by 11.7% when white shrimp were soaked in 2.0% $NaHCO_3$ for 4 h. However, no information regarding the role of sodium bicarbonate in muscle proteins of Pacific white shrimp (*Litopenaeus vannamei*), an economically important species of Thailand, has been reported. The objectives of this study were to investigate the changes in biochemical properties and

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microstructure of natural actomyosin of Pacific white shrimp as affected by sodium bicarbonate at different concentrations in combination with and without 2.5% NaCl.

2. Materials and methods

2.1. Chemicals

Adenosine-5′-triphosphate (ATP), 8-anilino-1-naphthalenesulphonic acid (ANS), guanidine thiocyanate, sodium hydrogen sulphite, guanidine thiocyanate and Tris-maleate were procured from Sigma–Aldrich, Inc. (St. Louis, MO, USA). Potassium chloride, sodium chloride, calcium chloride, trichloroacetic acid, potassium dihydrogen phosphate and ammonium molybdate were purchased from Merck (Darmstadt, Germany). 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) was obtained from Wako Pure Chemical Industries (Tokyo, Japan). Bovine serum albumin (BSA) was purchased from Fluka (Buchs, Switzerland). Sodium bicarbonate ($NaHCO_3$) was obtained from Asahi chemical industry company Ltd. (Tokyo, Japan).

2.2. Sample preparation

Pacific white shrimp (*L. vannamei*) with the weight of 20–22.5 g and the length of 12.5–13.5 cm were obtained from a farm in Songkhla province, Thailand. The shrimp were placed in ice with an ice/shrimp ratio of 2:1 (w/w) and transported to the Department of Food Technology, Prince of Songkla University, Hat Yai, Songkhla within approximately 1 h. Upon the arrival, shrimp were washed with clean water and beheaded, peeled, deveined and the meat was collected. The meat was then finely chopped and stored in ice until use.

2.3. Preparation of natural actomyosin (NAM)

NAM was prepared according to the method of Benjakul, Visessanguan, Ishizaki, and Tanaka (2001) with a slight modification. Shrimp mince (50 g) was homogenised in 10 volumes of chilled 0.6 M KCl, pH 7.0 at a ratio of 1:10 (w/v) at a speed of 10,000 rpm using a homogeniser (IKA, Labortechnik, Selangor, Malaysia). To avoid over heating, the sample was placed in ice and homogenised for 20 s, followed by a 20 s rest interval for a total extraction time of 4 min. The extract was centrifuged at 5000*g* for 30 min at 4 °C using a refrigerated centrifuge (Avanti® J-E, Beckman Coulter, Inc., Palo Alto, CA, USA). Three volumes of chilled deionised water were added to precipitate NAM. The NAM was collected by centrifuging at 5000*g* for 20 min at 4 °C. The NAM pellet was stored in ice until use.

2.4. Effects of sodium bicarbonate in the presence or absence of 2.5% NaCl on the changes of NAM from Pacific white shrimp

NAM pellet was suspended in chilled sodium bicarbonate solution with different ionic strength (0, 0.2, 0.4, 0.6, 0.8 and 1.0 M) containing 0 and 2.5% NaCl (pH 8.5). The mixtures were stirred gently for 10 min in ice. Thereafter the mixtures were allowed to stand at 4 °C for 30 min prior to analysis. Samples were taken for analyses. The concentration of NAM solution was adjusted to the concentration of 4.5 mg protein/ml.

2.5. Analyses

2.5.1. Measurement of turbidity and solubility

NAM solutions (4.5 mg protein/ml) with different treatments were placed in a cuvette (path length of 1 cm). Turbidity was determined by measuring the absorbance at 660 nm against the blanks using a UV1601 UV–vis spectrophotometer (Shimadzu, Tokyo, Japan) (Sano, Ohno, Otsuka-Fuchino, Matsumoto, & Tsuchiya, 1994). To determine the solubility, NAM solutions with different treatments were subjected to centrifugation at 20,000*g* for 30 min. The obtained supernatants were determined for soluble protein content using the Biuret assay (Robinson & Hogden, 1940). To determine total protein in the pellet, the exact amount of pellet was completely solubilised using 0.5 M NaOH. Solubility was expressed as that found in the supernatant relative to that obtained in the pellet.

2.5.2. Determination of surface hydrophobicity

Surface hydrophobicity was measured according to the method of Benjakul et al. (2001) using 8-anilo-1-naphthalenesulfonic acid (ANS) as a probe. Treated NAM solutions were diluted to 0.125, 0.25, 0.5 and 1 mg/ml using the same buffer. To 2.0 ml of diluted NAM solution, 10 ml of 10 mM ANS dissolved in 50 mM potassium phosphate buffer (pH 7.0) was added and the mixtures were mixed thoroughly. Sample blanks of each protein concentration were prepared in the same manner, except the same volume of 50 mM potassium phosphate buffer (pH 7.0) was used instead of ANS solution. Fluorescence intensity was measured using a RF-1501 spectrofluorometer (Shimadzu, Kyoto, Japan) at the excitation and emission wavelength of 374 and 485 nm, respectively. Surface hydrophobicity was calculated from the initial slope of the plot of fluorescence intensity against protein concentrations and was referred to as 'S_0ANS'.

2.5.3. Determination of total sulfhydryl and disulphide bond contents

Total sulfhydryl (SH) content was measured according to the method of Ellman (1959) as modified by Benjakul, Seymour, Morrissey, and An (1997) with a slight modification. To 1 ml of NAM solutions (4.5 mg protein/ml), 9 ml of 0.2 M Tris–HCl buffer (pH 8.0) containing 8 M urea, 2% sodium dodecyl sulphate (SDS) and 10 mM EDTA were added. After incubation with 0.4 ml of 0.1% DTNB in 0.2 M Tris–HCl buffer, (pH 8.0) at 40 °C for 25 min, the absorbance at 412 nm was measured using a spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan). Reagent blank was prepared by replacing the sample with 50 mM potassium phosphate buffer (pH 7.0) containing 0.5 M NaCl. For the sample blank, the reaction was run in the same manner except that 0.2 M Tris–HCl buffer (pH 8.0) was used instead of DTNB solution. The total SH content was calculated using a molar extinction coefficient of 13,600 M^{-1} cm^{-1}.

Disulphide bond content in samples was determined using the 2-nitro-5-thiosulphobenzoate (NTSB) assay as described by Thannhauser, Konishi, and Scheraga (1987). To 2 ml of NAM solution (4.5 mg protein/ml), 3 ml of freshly prepared NTSB assay solution were added. The mixtures were incubated in the dark at room temperature (25–27 °C) for 25 min. Absorbance was then measured at 412 nm. The disulphide bond content was calculated using a molar extinction coefficient of 13,900 M^{-1} cm^{-1}.

2.5.4. Assay of Ca^{2+}-ATPase and Mg^{2+}-ATPase activities

Ca^{2+}-ATPase and Mg^{2+}-ATPase activities were determined as described by Benjakul et al. (1997). To 1 ml of NAM solutions (4.5 mg protein/ml), 0.6 ml of 0.5 M Tris-maleate, pH 7.0, was added. $CaCl_2$ or $MgCl_2$ solutions were added to the system, with the total volume of 9.5 ml, to obtain final concentrations of 10 mM and 2 mM for Ca^{2+}-ATPase and Mg^{2+}-ATPase activity assays, respectively. To each assay solution, 0.5 ml of 20 mM ATP was added to initiate the reaction. The reaction was conducted for 10 min at 25 °C and stopped by addition of 5 ml chilled 15% (w/v) trichloroacetic acid. The reaction mixture was subjected to centrifugation at 6500*g* for 5 min. The inorganic phosphate liberated in the supernatant was measured by the method of Fiske and Subbarow (1925). Specific activity was expressed as μmoles inorganic phosphate (Pi) released/mg protein/min. A blank was performed by adding the chilled trichloroacetic acid prior to the addition of ATP.

2.5.5. Transmission electron microscopy

NAM solutions (4.5 mg protein/ml) were diluted to 0.2 mg protein/ml with the corresponding solutions. A drop of sample was fixed for 5 min on a carbon-coated grid, negatively stained with 4% uranyl acetate for 5 min and washed with distilled water until the grid was cleaned. The specimens were visualised using a JEOL JEM-2010 transmission electron microscope (JEOL Ltd., Tokyo, Japan) (25,000×) at an accelerating voltage of 160 kV.

2.5.6. Measurement of zeta potential

The NAM solutions (4.5 mg protein/ml) with different treatments were stirred gently for 10 min in ice. Thereafter the mixtures were allowed to stand at 4 °C for 30 min prior to analysis. The zeta (ζ) potential of NAM solutions was measured using a Zeta-PALs analyser (Brookhaven Instruments Co., Holtsville, NY, USA) at room temperature.

2.5.7. Protein determination

Protein content was measured using the Biuret method (Robinson & Hogden, 1940). Bovine serum albumin was used as a standard.

2.6. Statistical analysis

All experiments were run in triplicate and completely randomized design was used throughout the study. Data were subjected to analysis of variance (ANOVA) and mean comparisons were carried out by using Duncan's Multiple Range Test. For pair comparison, *T*-test was used (Steel & Torrie, 1980). Statistical analysis was performed using the statistical Package for Social Sciences (SPSS for windows: SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Turbidity and solubility of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Changes in turbidity and solubility of NAM from Pacific white shrimp treated with $NaHCO_3$ at different concentrations (0, 0.2, 0.4, 0.6, 0.8 and 1 M) in the presence or absence of 2.5% NaCl are shown in Fig. 1. Turbidity of NAM solutions decreased when the concentrations of $NaHCO_3$ increased up to 0.6 M ($P < 0.05$) (Fig. 1A). No further changes in turbidity were noticeable when $NaHCO_3$ increased up to 1 M ($P > 0.05$). In the presence of 2.5% NaCl, the sharp decrease in turbidity was obtained when $NaHCO_3$ concentration was 0.2 M. No changes in turbidity were found thereafter ($P > 0.05$). In the presence of $NaHCO_3$ at the concentration range of 0–0.6 M, turbidity was lower when 2.5% NaCl was incorporated, in comparison with samples without NaCl ($P < 0.05$). Thus, NaCl might exhibit the synergistic effect on dissociation of NAM in conjunction with $NaHCO_3$ at the sufficient concentration. Higher ionic strength of sample containing 2.5% NaCl might lower the ionic interaction of NAM more effectively. Protein solubility is a complex function of the physiochemical nature of the proteins, which

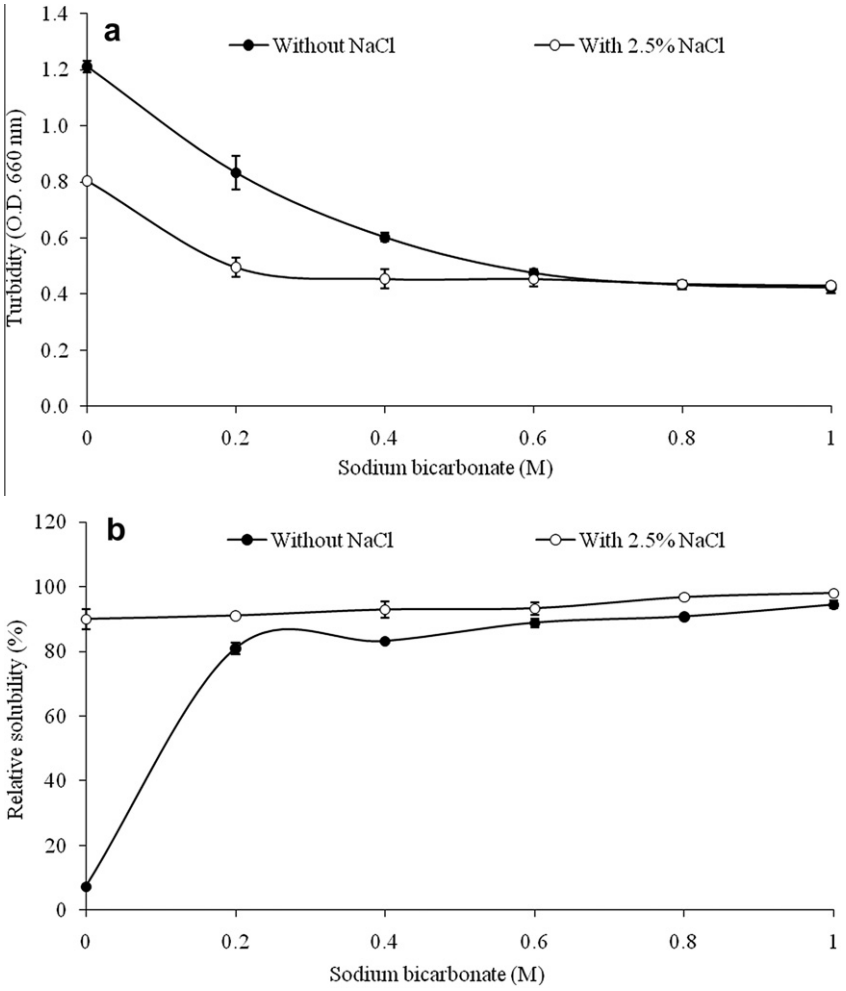

Fig. 1. Turbidity (A) and solubility (B) of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation ($n = 3$).

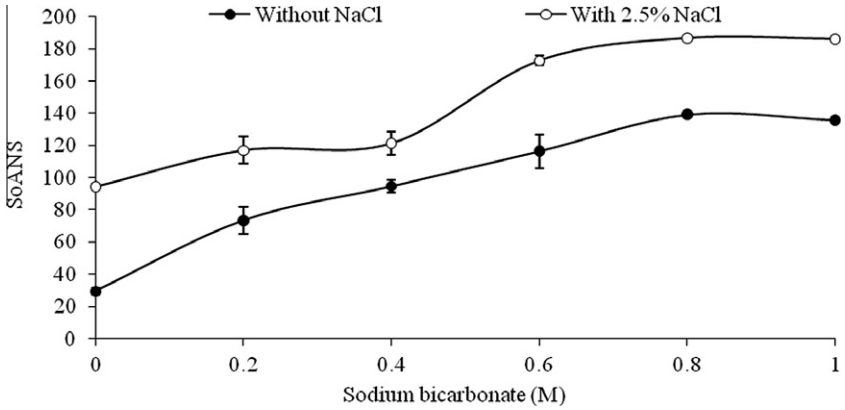

Fig. 2. Changes in surface hydryphobicity of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation ($n = 3$).

depends on pH, temperature and the concentration of the salt used. It also depends on whether the salt is Kosomtropic (stabilizes water structure) or Chaotropic (disrupts water structure). High ionic strength was shown to decrease actin–myosin interactions in the relaxed and activated muscle (Zayas, 1997). Additionally Wu and Smith (1987) reported that the increasing ionic strength or increasing incubation time decreased the turbidity of the bovine longissimus myofibrillar proteins and increased the solubility.

Furthermore, the chlorides clearly had a major effect on muscle protein solubility and on water holding properties (Kauffman et al., 2000). $NaHCO_3$ at concentration greater than 0.2 M had the less impact on solubility in the presence of 2.5% NaCl. Protein solubility depends on protein structure, pH, concentration of salt, temperature, duration of extraction and other intrinsic factors (Zayas, 1997). The degree of protein solubility in an aqueous medium is the result of electrostatic and hydrophobic interactions between protein molecules, and proteins are extracted when electrostatic repulsion between proteins is greater than hydrophobic interactions (Zayas, 1997). Wu and Smith, (1987) reported a marked decrease in the turbidity and the increase in solubility of bovine longissimus myofibrillar protein treated with KCl or NaCl having 0.10 to 0.35 M ionic strength. At $NaHCO_3$ concentration greater than 0.2 M, 2.5% NaCl had no synergistic effect on lowering turbidity of NAM ($P > 0.05$). Thus NaCl might not be required when $NaHCO_3$ concentration was higher than 0.2 M.

For solubility (Fig. 1B), high solubility of NAM was obtained in the presence of 2.5% NaCl with the range of 89.9–98.0%. Slight increase in solubility was noticeable as the concentration of $NaHCO_3$ increased ($P < 0.05$). In the absence of 2.5% NaCl, the solubility of 94.4% was obtained when $NaHCO_3$ at a level of 0.2 M was used as solubility medium. At the concentration above 0.2 M, the gradual increase in solubility was obtained up to 1.0 M. NaCl has been added to solubilise myofibrilar proteins, including NAM (He et al., 2010). However, NAM could be more solubilised by only $NaHCO_3$ when the sufficient concentrations were implemented.

3.2. Surface hydrophobicity, total sulfhydryl group and disulphide bond contents of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Changes in surface hydrophobicity (S_oANS) of NAM from Pacific white shrimp suspended in $NaHCO_3$ at different concentrations in the presence or absence of 2.5% NaCl are depicted in Fig. 2. S_oANS of NAM continuously increased when the concentrations of $NaHCO_3$ increased up to 0.8 M ($P < 0.05$). Thereafter, S_oANS was constant when $NaHCO_3$ concentration was above 0.8 M. The increase in S_oANS indicated the structural changes of NAM by increasing $NaHCO_3$ concentrations. Upon treatment with $NaHCO_3$, the aromatic hydrophobic amino acid residues, i.e. phenylalanine and tryptophan, might be exposed to a greater extent. ANS, an effective fluorescent probe, has been found to bind at non-polar regions of protein (Wicker, Lanier, Hamann, & Akahane, 1986). The increase in ANS binding of NAM was more likely due to the exposed hydrophobic domains. This was in agreement with the greater solubility of NAM in the presence of $NaHCO_3$ at the higher concentrations (Fig. 1A). At the same concentration of $NaHCO_3$, the higher S_oANS was observed in NAM suspended in medium containing 2.5% NaCl, compared with that without 2.5% NaCl ($P < 0.05$). The exposure of hydrophobic domains could be synergistically induced by NaCl. Increasing ionic strength decreases the sphere of each charge on the proteins, thereby weakening the structural integrity of myofibrils (Raymond & Zubay, 1983). As the protein moves away from the pI, the ionisable groups in proteins become increasingly charged up to a point where the charge repulsion causes the

Table 1
Changes in total sulfhydryl group and disulphide bond contents of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl.

Sodium bicarbonate (M)	Total SH group content (mole/10^5 g protein)		Disulphide bond content (mole/10^5 g protein)	
	Without NaCl	With 2.5% NaCl	Without NaCl	With 2.5% NaCl
0	3.102 ± 0.013^{fB}	3.278 ± 0.015^{eA}	0.334 ± 0.004^{aA}	0.329 ± 0.003^{aA}
0.2	3.156 ± 0.009^{eB}	3.294 ± 0.001^{deA}	0.331 ± 0.007^{aA}	0.324 ± 0.002^{abA}
0.4	3.231 ± 0.012^{dB}	3.307 ± 0.005^{dA}	0.329 ± 0.003^{aA}	0.320 ± 0.005^{bB}
0.6	3.351 ± 0.033^{cA}	3.370 ± 0.005^{cA}	0.332 ± 0.003^{aA}	0.322 ± 0.005^{abB}
0.8	3.405 ± 0.004^{bB}	3.501 ± 0.017^{bA}	0.330 ± 0.006^{aA}	0.325 ± 0.004^{abA}
1.0	3.473 ± 0.012^{aB}	3.535 ± 0.017^{aA}	0.305 ± 0.006^{bA}	0.310 ± 0.006^{cA}

Means ± SD ($n = 3$).
The different letters in the same column indicate significant differences ($p < 0.05$). Different capital letters in the same row within the same parameter tested indicate the significant differences ($p < 0.05$).

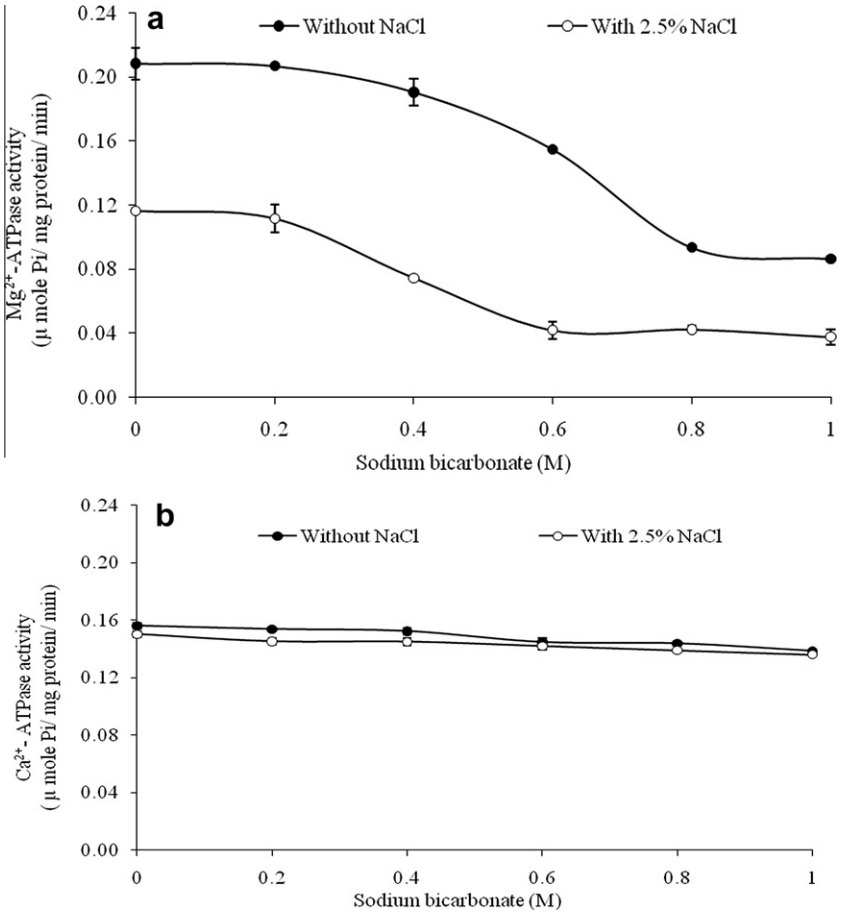

Fig. 3. Ca^{2+}-ATPase activity (A) and Mg^{2+}-ATPase activity (B) of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl. Bars represent standard deviation ($n = 3$).

protein molecule to unfold. Along with NaCl, which is able to solubilise the myofibrillar proteins, the solubilised protein molecules with the modified charge caused by alkaline pH of $NaHCO_3$ were more likely unfolded as evidenced by the increased surface hydrophobicity.

Total sulfhydryl (SH) group and disulphide bond contents of NAM from Pacific white shrimp treated with $NaHCO_3$ at various concentrations in combination with and without 2.5% NaCl are shown in Table 1. Slight increase in total SH group content of NAM was observed as $NaHCO_3$ increased up to 1.0 M ($P < 0.05$), regardless of NaCl incorporation. No changes in disulphide bond content of NAM samples were obtained when $NaHCO_3$ concentration increased up to 0.8 M ($P > 0.05$). However, a slight decrease in disulphide content bond was noticeable when $NaHCO_3$ at 1 M was used. Furthermore, NaCl had no impact on disulphide bond formation in NAM. At very high $NaHCO_3$ concentration, disulphide bond might be destroyed to some degree under alkaline condition. Chan, Gill, Thompson, and Singer (1995) reported that myosin contained 42 SH groups. Two types of SH groups on the myosin head portion, named SH1 and SH2, have been reported to be involved in ATPase activity of myosin; another SH group (SHa) localised in the light meromyosin contributes to oxidation (Benjakul et al., 1997).

Apart from induction of the exposure of hydrophobic domains, $NaHCO_3$ at high concentration also enhanced the unfolding of NAM molecules, in which SH groups were more exposed. The breakdown of disulphide bond might also cause the looser muscle structure, leading to the higher water holding capacity of muscle treated with $NaHCO_3$. Chantarasuwan et al. (accepted for publication) reported that Pacific white shrimp treated with 2.0% $NaHCO_3$ had the increase in water uptake.

3.3. Ca^{2+}-ATPase and Mg^{2+}-ATPase activities of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Remaining Ca^{2+}-ATPase activity of NAM treated with $NaHCO_3$ at various concentrations in the presence or absence of 2.5% NaCl is shown in Fig. 3A. After incubation at 4 °C for 30 min, Ca^{2+}-ATPase activity of the NAM treated with $NaHCO_3$ decreased slightly as

Table 2
Zeta (ζ) potential of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at different concentrations in the presence or absence of 2.5% NaCl.

Treatments	Sodium bicarbonate (M)	Zeta potential (mV)
$NaHCO_3$ without NaCl	0	$-12.12 \pm 0.07^{e\dagger}$
	0.2	-12.94 ± 0.43^{e}
	0.4	-16.41 ± 0.40^{d}
	1	-22.13 ± 0.22^{b}
$NaHCO_3$ with 2.5% NaCl	0	-17.19 ± 0.18^{d}
	0.2	-17.22 ± 1.20^{d}
	0.4	-20.86 ± 0.81^{c}
	1	-26.98 ± 1.02^{a}

Different subscripts in the same column indicate the significant differences ($P < 0.05$).
† Mean ± SD ($n = 3$).

the concentration of $NaHCO_3$ increased ($P < 0.05$), suggesting the partial denaturation of myosin heavy chain, especially at the head portion. At the same $NaHCO_3$ concentration, no differences were found between samples treated without and with 2.5% NaCl ($P > 0.05$). Prevalent bicarbonate ion (HCO_3^-) might induce denaturation of Ca^{2+}-ATPase by unfolding of protein, the exposure of hydrophobic residues, etc. Induced dissociation of the actomyosin complex by $NaHCO_3$ at high concentration might release free myosin, which underwent denaturation easily. Xiong, Lou, Wang, Moody, and Harmon (2000) reported that chicken myofibrillar proteins treated with 0.2 to 0.4 M NaCl had the higher contents of extractable actin and α-actinin. Tropomyosin and troponin-T were extracted at 0.3 M NaCl, whilst the noticeable extraction of myosin was detected at 0.4 M NaCl. Extraction of the both major (myosin and actin) proteins and other myofibrillar components gradually increased at higher salt levels (Wu & Smith, 1987).

The effects of $NaHCO_3$ at different concentrations in combination with or without 2.5% NaCl on Mg^{2+}-ATPase activity of NAM from Pacific white shrimp muscle are shown in Fig. 3B. In the absence of 2.5% NaCl, Mg^{2+}-ATPase activity of NAM decreased as $NaHCO_3$ concentrations increased up to 0.8 M ($P < 0.05$). Thereafter, no further decreases were noticeable when $NaHCO_3$ concentrations increased up to 1.0 M ($P > 0.05$). The result indicated that $NaHCO_3$ at higher concentrations was effective in dissociating the actomyosin complex, as evidenced by the lower Mg^{2+}-ATPase activity retained. The decrease in Mg^{2+}-ATPase can be used as an indicator for the selective denaturation of actin, which is reported to be the activator for myosin Mg^{2+}-ATPase (Torigai & Konno,

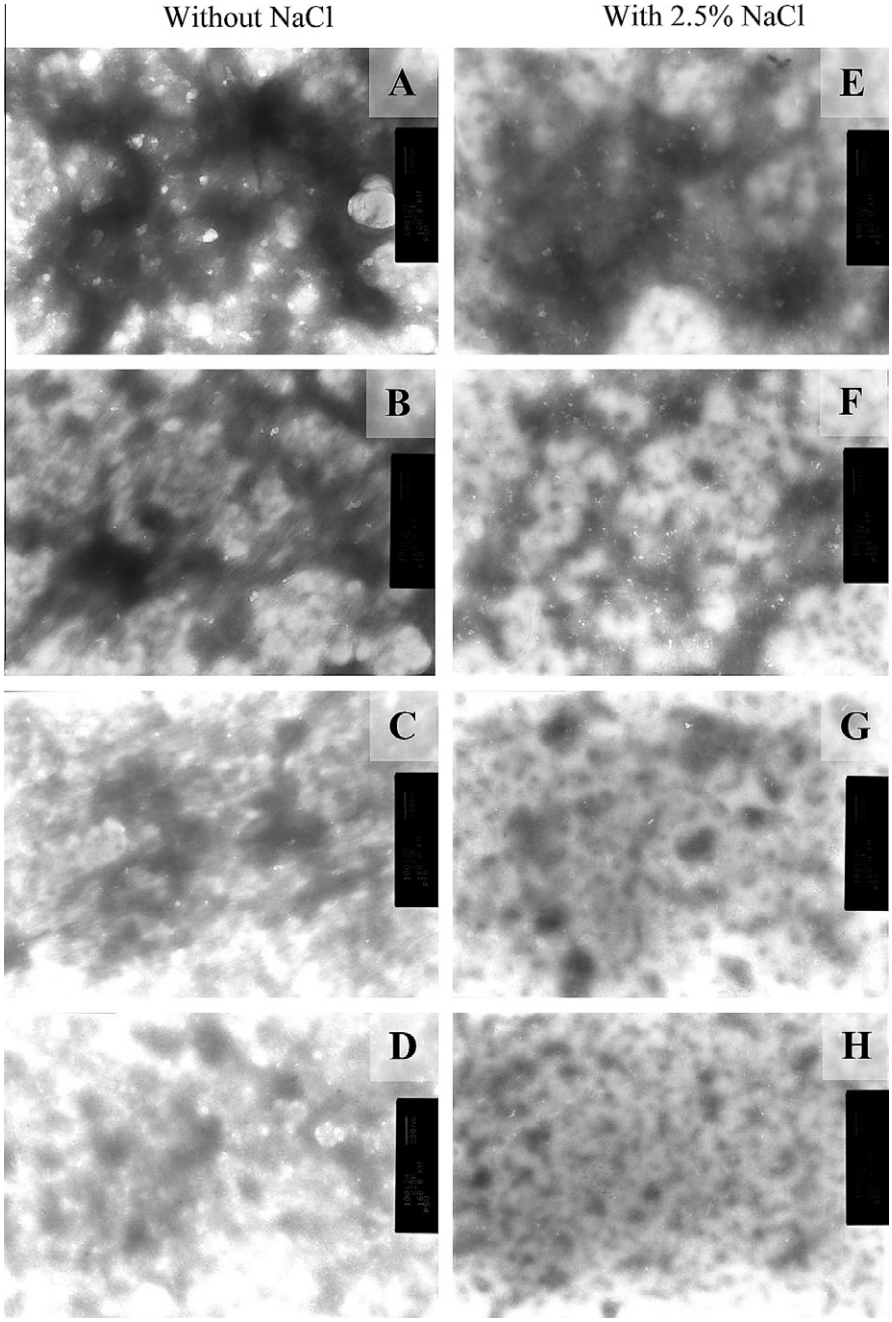

Fig. 4. Transmission electron micrograph of natural actomyosin from Pacific white shrimp treated with sodium bicarbonate at various concentrations in the presence or absence of 2.5% NaCl (A and E: 0 M $NaHCO_3$; B and F: 0.2 M $NaHCO_3$; C and G: 0.6 M $NaHCO_3$; D and H: 1 M $NaHCO_3$). Magnification: 25,000×.

1996). For NAM treated with $NaHCO_3$, Mg^{2+}-ATPase activity was lower in the sample with 2.5% NaCl, compared with those without NaCl. In the presence of 2.5% NaCl, Mg^{2+}-ATPase activity decreased as $NaHCO_3$ concentrations increased up to 0.6 M ($P < 0.05$) and remained constant when the concentration was in the range of 0.8–1 M. The result suggested that $NaHCO_3$ could promote the release of actin and make actin more susceptible to denaturation, especially in the presence of 2.5% NaCl. In general, myosin plays a role in the protection of actin from salt denaturation (Torigai & Konno, 1996). Therefore, the repulsive force mediated by $NaHCO_3$ at high concentration more likely contributed to the dissociation of actomyosin complex. This was confirmed by the increased solubility, decreased turbidity as well as the changes in S_oANS and SH group content.

3.4. Zeta potential of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

The zeta potential (ζ) representing the surface charge of NAM of Pacific white shrimp suspended in $NaHCO_3$ at various concentrations in combination with or without 2.5% NaCl is shown in Table 2. NAM solutions turned to become more negatively charged ranging from −12.12 to −26.98 as $NaHCO_3$ concentrations increased. In the presence of 2.5% NaCl, higher negative charge was obtained in all samples, in comparison with the absence of NaCl, when the same $NaHCO_3$ concentration was used. NaCl could facilitate the solubilisation or unfolding of protein molecules, where the $COOH^-$ group of side chains could be deprotronated with ease. Also, at the higher $NaHCO_3$ concentration, the ability of proteins to obtain the negative charge could be more pronounced. As a result, the higher negative charge was obtained in NAM treated with $NaHCO_3$ at high concentrations, particularly in the presence of NaCl. Benjakul et al. (2010) reported that a protein in an aqueous system has a zero net charge at its isoelectric point (pI), when the positive charges are balanced out by the negative charges and noted that the differences in net surface charge at different pHs were most likely governed by the different unfolding or exposure of charged amino acids, in which protonation or deprotonation could take place at different degrees. Thus, distinct negative net charge on the surface of the protein might enhance the dissociation of actomyosin complex, leading to the increased solubility of proteins. This might be associated with the increased water holding capacity or yield of Pacific white shrimp treated with $NaHCO_3$ (Chantarasuwan et al., accepted for publication).

3.5. Transmission electron micrograph of NAM from Pacific white shrimp as affected by sodium bicarbonate at different concentrations

Microstructures of NAM from Pacific white shrimp treated with $NaHCO_3$ at various concentrations in the presence or absence of 2.5% NaCl are illustrated in Fig. 4. The NAM suspended in water (without 2.5% NaCl) was found as the filamental aggregates. When 2.5% NaCl was incorporated, filaments were more dispersed and the aggregation was lowered. NaCl at a level of 3.0% has been shown to solubilise surimi and NAM from ling cod (*Ophiodon elongatus*) (Sultanbawa & Li-Chan, 2001). Transmission electron microscopy is used to obtain information on the changes in the shape of the actomyosin filaments caused by chemical treatment (Hsu, Hwang, Yu, & Jao, 2007). This filamental structure of actomyosin was still observed after being treated with $NaHCO_3$ at low concentrations (0.2 M), but was more disrupted with increasing $NaHCO_3$ concentrations. In the presence of 1.0 M $NaHCO_3$ and 2.5% NaCl, filamental structure was intensively disrupted (Fig. 4H). The less disruption was found in the absence of 2.5% NaCl when 1 M $NaHCO_3$ was used. Actomyosin filaments were shortened by salt treatment, probably due to the dissociation of myosin subunits and depolymerisation of actin (Ko, Tanaka, Nagashima, Mizuno, & Taguchi, 1990). The NAM of Pacific white shrimp treated with $NaHCO_3$ could undergo more dissociation or disruption, mainly caused by the increased negative charge (Table 2). Furthermore, under the high ionic strength condition, hydrophobic interactions stabilising the protein structure might be destroyed to some extent, leading to the dissociation of filament structure. Destructibility of protein was associated with the lost of intermolecular covalent and noncovalent interactions, including disulphide bonds and hydrophobic interactions (Lee & Lanier, 1995). The higher dissociation was in accordance with the increases in solubility (Fig. 1B) and lower turbidity (A660 nm) (Fig. 1A). The degree of unfolding or destruction of actomyosin varied depending on $NaHCO_3$ concentration used. Many proteins are partially unfolded into a compact state called 'molten globule', which retains most of the secondary structure whilst losing their tertiary structure (Mohan, Ramachandran, Sankar, & Anandan, 2007). In the presence of 2.5% NaCl, the attachment of the cross-bridges is further weakened as the Cl^- causes the increased electrostatic repulsive forces. If the lattice swells appreciably, the cross-bridges cannot remain attached (Ko et al., 1990). However, the excessive disruption of filamental structure contributed to mushy texture of shrimp or transparency in appearance as reported by Rattanasatheirn et al. (2008) and Chantarasuwan et al. (accepted for publication).

4. Conclusions

Sodium bicarbonate can be used as non-phosphate compounds to increase the yield of Pacific white shrimp. It caused the dissociation of filamental structure of actomyosin complex associated with the increased solubility. The actions could be enhanced with the aid of 2.5% NaCl or increasing the concentration of sodium bicarbonate used. However, the high concentration could lead to the loss in yield due to the excessive solubilisation or disruption of protein filaments.

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