Food Hydrocolloids 58 (2016) 179–183

Contents lists available at ScienceDirect

Food Hydrocolloids

journal homepage: www.elsevier.com/locate/foodhyd

Effects of cations on the "salt in" of myofibrillar proteins

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ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form
12 February 2016
Accepted 25 February 2016
Available online 27 February 2016

Keywords:
Myofibrillar proteins
"Salt in"
Electrostatic interaction
Hydrated cations
Hydrated anions

ABSTRACT

The solubilities of myofibrillar proteins (MPs) increased monotonically with increasing NaCl concentration at pH = pI (isoelectric point), but decreased monotonically with increasing NaCl concentration at pH < pI. At pH > pI, the solubilities decreased initially, but increased with increasing NaCl concentrations from 0.2 to 0.8 M. For chloride salts, the "salt in" effect of cations decreased in the order: $Li^+ > Na^+ > K^+ > Rb^+$. For sodium salts, the "salt in" effect of anions decreased in the sequence: $Cl > Br > I > SCN$. The "salt in" effect of cations were stronger than that of anions. No evidence of increased electrostatic interaction was found during "salt in". Presence of CH_3COO decreased the solubilization effect of Na^+. These results indicate the "salt in" of MPs is more affected by the cations with higher degree of hydration, which may be interfered by the presence of highly hydrated anions.

1. Introduction

Myofibrillar proteins (MPs) are the major fraction of muscle proteins that process excellent functionalities of gelling, emulsifying and film-forming (Gomez-Estaca, Montero, & Carmen Gomez-Guillen, 2014; Zhou, Zhao, Su, Cui, & Sun, 2014; Zorba, 2006). Functionalities of MPs are affected by many factors, such as pH, ionic strength, heat or hydrostatic pressure treatment, protein oxidation, protein or nonprotein additives (Bertram, Kristensen, & Andersen, 2004; Chapleau & de Lamballerie-Anton, 2003; Li, Xiong, & Chen, 2013; Sun & Holley, 2011). Among those factors, ionic strength is a deciding factor because MPs are believed to be salt soluble proteins (Sun & Holley, 2011), and solubilization or swelling of MPs are tightly linked to their water holding and gelation properties. The physiological ionic strength of muscle tissue is estimated to be about 0.15–0.30 M (Puolanne & Halonen, 2010). Extra salts, especially sodium chloride (NaCl) are frequently added during meat processing to improve flavor, texture, color and shelf life of muscle tissue foods. Usually, 2–3% (0.47–0.68 M) NaCl are needed to solubilize the MPs and achieve the desired functional properties (Xiong & Brekke, 1991). Selective binding of Cl rather than Na^+ on protein surface leading to the increase of electrostatic repulsion and consequently "salt in" of MPs is suggested because NaCl causes swelling of muscle myofibrils but sodium acetate does not (Hamm, 1960; Puolanne & Halonen, 2010).

In colloidal science, influence of ions on protein solubility can be derived from the DLVO theory, which states that the colloidal stability is governed by the balance of attractive van der Waals forces and repulsive double-layer electrostatic interactions. Protein can be considered as a macroion. In salt solution, it is surrounded by more counterions than coions leading to the shielding of protein surface charge. As the salt concentration increases, shielding of protein charge leads to the compression of electric double layers and decrease of repulsive term of the DLVO theory. Thus decrease of protein solubility at high salt concentrations is predicted. However, DLVO theory fails to explain the presence of strong short-range repulsive forces between proteins at high salt concentrations and the ion specific effects (Salis & Ninham, 2014; Valle-Delgado, Molina-Bolivar, Galisteo-Gonzalez, & Galvez-Ruiz, 2011).

Hydration force due to the absorption of hydrated ions on protein surface has been proposed to explain the repulsive forces between proteins at high salt concentrations. This hydration force is originated from the formation of structured water layer around protein surface, which can be enhanced by the absorption of hydrated ions at high electrolyte concentrations. The overlapping of two interaction hydration layers causes an increase in the free energy of the system, resulting in a strong short-range hydration repulsion as observed experimentally (Valle-Delgado et al., 2011).

The law of matching water affinities (LMWA) has been proposed as a rule to explain the ion specific effects (Collins, 1997). According to this rule, ions can be ordered by their degree of hydration. Only

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http://dx.doi.org/10.1016/j.foodhyd.2016.02.028
0268-005X/

these ions with matched water affinities (degree of hydration) can form stable contact ion pairs (Collins, 1997). The negatively charged groups on protein surface (due to Asp and Glu residues), which are similar to CH_3COO, are highly hydrated, and they tend to bind cations with higher degree of hydration (Salis & Ninham, 2014). The order of cation binding to the negatively charged groups of proteins decreases with their degrees of hydration along the series $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. The positively charged groups (due to Lys, His and Arg residues) on protein surface, which are similar to NH_4^+, are classified as weakly hydrated, and them tend to bind anions with a lower degree of hydration, and the order of anion binding is: $SCN > I > NO_3 > Br > Cl$, which is in a reverse order of their degrees of hydration (Salis & Ninham, 2014).

In meat industry, the "salt in" of MPs is explained by the selective binding of Cl^- rather than Na^+ on protein surface and the increase of electrostatic repulsion (Hamm, 1960). However, this is intuitive contrary to DLVO theory. At normal meat processing condition, pH is above the pI of MPs in which protein surface is negatively charged, thus binding of more counterions Na^+, not coions Cl^- should be expected. In this study, with a goal of drawing a more clear picture of the role of ions in the "salt in" of MPs, we have determined the solubilities and zeta potentials (evidence of electrostatic interactions) of MPs at varied pH, ion strength and salt combinations to explore the interactions between salts and MPs. The knowledge may be helpful in re-examining the technological effects of NaCl in meat processing, and understanding the functionalities of MPs as a food hydrocolloid.

2. Experimental details

2.1. Materials and methods

Materials: Longissimus muscles were obtained from pig about 6 months of age within 12 h of slaughter, sliced into 20 mm-thick chops, divided into 150 g portions, vacuum packed in polyethylene bags and stored at −25 °C prior to use. Analytical reagent grade 2, 2' - bicinchoninate acid (BCA), $LiCl \cdot H_2O$, NaCl, KCl, RbCl, CsCl, NaBr, NaI, NaSCN, CH_3COONa, NaN_3 and all other chemicals were purchased from Aladdin (Shanghai, China). 18 megOhm de-ionized (D.I.) water was used unless stated otherwise.

Extraction of MPs: 150 g frozen muscles were thawed at 4 °C for 12 h and minced twice by a tissue mincer (Grindomix GM200, Retsch, Haan, Germany) at 2000 rpm for a duration of 7 s each with an interval of 1 min. The minced muscles were mixed with 2 volumes (w/v) of isolation buffer (0.1 M KCl, 20 mM Na_2HPO_4, 2 mM $MgCl_2$, 1 mM ethylene glycol tetraacetic acid, 1 mM NaN_3, pH 7.0) and blended by a blender (HGB 550, Waring products, New Hartford, CT) at low speed for 10 s and then high speed for 20 s. 2 volumes of isolation buffer was added and blended again at low speed for 10 s. The suspension was then centrifuged (Avanti J-26S, G-10 rotor, Beckman Coulter, Palo Alto, CA) at 4 °C and 2000 g for 15 min. The supernatant was discarded and the pellet was mixed with 4 volume of isolation buffer, homogenized (Ultra-Turrax T25 digital, IKA, Staufen, Germany) for 20 s. The suspension was then filtered through one layer of gauze and then centrifuged at 4 °C and 2000 g for 15 min. The supernatant was discarded and the pellet was mixed with 4 volume of isolation buffer, homogenized (T25 digital) for 20 s, filtered through two layers of gauze and then centrifuged at 4 °C and 2000 g for 15 min. The supernatant was discarded and the pellet was mixed with 4 volume of 0.1 M NaCl, adjusted the pH to 6.2, homogenized (T25 digital) for 10 s, centrifuged at 4 °C and 2000 g for 15 min. The last step was repeated once to collect the protein pellet. The protein pellet was mixed with 4 volumes of water and transferred into a dialysis bag with molecular weight cutoff of 8, 000–10, 000 Da, and dialyzed against 5 mM NaN_3 water at 4 °C for 48 h with 5 mM NaN_3 water being replaced every 8 h. After dialysis, the MPs were diluted to a concentration about 2–3 mg/ml with water.

Sample preparation for solubility testing and zeta potential measurement: Desired amounts of salts were weighed and added into a 50 ml wide-neck volumetric flask, followed by adding 10 ml water to solubilize or wet the salts. Then 25 ml MPs at a concentration around 2 mg/ml was measured with a graduated cylinder and transferred into the volumetric flask, followed by using 10 ml water to rinse the cylinder and the rinsing water was transferred into the volumetric flask. HCl (from 0.1 M to 12 M) and NaOH (from 0.01 to 2 M) at varied concentrations depending on the type of salts were used to adjust the pH to the targeted values at room temperature using a standard pH meter (HI 2211, Hanna Instruments, Woonsocket, RI) with a plastic electrode. The protein salt suspensions were incubated in a shaking bath with a speed of 100 rpm/min and temperature of 4 °C for 18 h. Blank salt solutions or suspensions without MPs were prepared by the same procedure above and analyzed for protein content. After incubation, all samples were submitted to centrifugation at 2000 g for 15 min and the supernatant was used for BCA and zeta potential analysis.

Protein determination by BCA method: BCA assay reagent A was consisted of 1 g sodium bicinchoninate (BCA), 2 g sodium carbonate, 0.16 g sodium tartrate, 0.4 g NaOH and 0.95 g sodium bicarbonate in 100 ml water with pH adjusted to 11.25 with 10 M NaOH. BCA assay reagent B was 0.4 g $CuSO_4 \cdot 5H_2O$ in 10 ml water. Fresh final BCA reagent was prepared daily by mixing the BCA assay reagent A and reagent B in a ratio 50:1 (v/v). 0.1 ml supernatant of MPs was drawn, followed by the addition of 2.0 ml final BCA reagent, mixed by a vortex, incubated at 60 °C for 15 min, and then cooled in an iced bath to room temperature. The absorbance at 562 nm was then recorded by a spectrophotometer (UV Bluestar A, LabTech, Beijing, China) to calculate the protein solubility after subtracting the absorbance in salt control. It should be noted that the solubility measured here was not a true solubility of protein, which should be obtained when protein was in contact or equilibrium with its crystal in solution and much higher centrifugation force was used to separate soluble and insoluble proteins. However, it reflected the "salt in" status of MPs fairly well because most extractions methods used the same centrifugation force to separate salt soluble proteins and salt insoluble MPs.

Zeta potential measurement: Each sample was loaded into one disposable folded capillary cell (DTS 1070) and the zeta potential was measured using a particle electrophoresis instrument (Zetasizer Nano ZS90, Malvern Instruments Ltd., Worcestershire, UK) at 25 °C. Average values of three measurement was reported for each sample.

Sodium Dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A precast 7.5% polyacrylamide gel from Jiancheng Bioengineering Institute (Nanjing, China) was used in SDS-PAGE. The protein solutions at a concentration of 3 mg/ml approximately were mixed with SDS-PAGE sample buffer at a volume ratio of 1:1. After heating in boiling water for 5 min, 10 μL of each sample (5 μL protein standards) was loaded to the gel well for electrophoresis at 80 V for 30 min and then 120 V for 90 min. The gel was stained using Coomassie Blue (0.1% dye in solvent mixture containing 45% methanol and 10% acetic acid in water) for 1 h and destained using ternary solvent mixture containing methanol, acetic acid and water at a volume ratio of 1:1.5:20 until the bands became visible.

Data analysis: All solubilities experiments were repeated six times and the data were presented as mean ± standard derivation. Statistics analysis was not performed because the purpose of this study was to discuss the mechanism, not to compare the effects of different salt treatments.