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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Solubilisation of muscle proteins from chicken breast muscle by ultrasonic radiations in physiological ionic medium

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Abstract: Solubilisation of myofibrillar proteins in physiological or low ionic strength solutions is essential for their utilisation as supplementary protein food. In order to achieve low ionic strength solubility, ultrasonication as a physical force has been introduced as an effective method to shift the solubility range of myofibrillar proteins from high to low ionic medium. In this study, change in the solubility behaviour of extracted actomyosin by ultrasonication in tris-maleate (with/without 0.1 M NaCl) and water is studied. Our results demonstrate that ultrasonication solubilises actomyosin in all the three investigated systems i.e. tris-maleate (with 0.1 M NaCl), tris-maleate only (without 0.1 M NaCl) and water. A decreasing trend in the investigated biochemical parameters such as ATPases (Ca^{2+}, Mg^{2+}) and turbidity was observed as a result of ultrasonic exposure. Analysis of SDS-PAGE profiles showed least solubility of myosin heavy chain in water compared to tris-maleate (with/without 0.1 M NaCl), while results of electron micrographs reveal change in the degree of dissociation or disruption of actomyosin aggregates according to the time of sonication and the suspension-media type. In conclusion, the results of our study suggest that ultrasonication plays a significant role in solubilisation of major myofibrillar proteins most probably by altering the conformation of actomyosin complex.

Subjects: Bioscience; Food Science & Technology; Health and Social Care

Keywords: chicken actomyosin; solubility; ultrasonication; turbidity; physiological ionic strength

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PUBLIC INTEREST STATEMENT

Major portion of meat contains myofibrillar proteins that are insoluble in water and low ionic medium. Because of limited solubility, these high quality proteins have not been utilised as supplementary protein food. Ultrasonication has been introduced as an effective method in extraction and subsequent solubilisation of myofibrillar proteins. However, the role of ultrasonication in solubilisation of muscle proteins is not clearly demarcated. We studied the effect of ultrasonication on solubility of actomyosin, a major myofibrillar protein, after extracting at high salt concentration. Our study design rules out the involvement of protease and other structural proteins in solubilisation. Results of our study clearly demarcate the role of ultrasonic radiations in solubilisation of muscle proteins.

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1. Introduction

Meat is a rich source of protein containing all essential amino acids. However, due to limited solubility of myofibrillar proteins, these high quality proteins of meat have not been utilised as a supplementary protein food. Myofibrillar proteins are generally insoluble in solutions of low ionic strength, and a relatively high concentration of salt is required to solubilise them (Wu & Smith, 1987). Attempts have already been made on solubilisation of these myofibrillar proteins in low ionic strength solutions by employing both chemical as well as physical methods. More than 90% of myofibrillar proteins from cod (Stefansson & Hultin, 1994), chicken breast (Krishnamurthy et al., 1996) and mackerel light muscles (Feng & Hultin, 1997) were solubilised in low ionic strength solutions after following an established order of treatment. Reports claim that ultrasonication (as a pure physical force) is effective in solubilising more than 80% of myofibrillar proteins in low ionic strength solutions from various vertebrate muscles (Cho et al., 2007; Ito, Tatsumi, Wakamatsu, Nishimura, & Hattori, 2003). However, prior washing with L-histidine and heating at high temperature is important to retain the optimum solubility (Ito et al., 2004). More insight into solubility behaviour can be gained by investigating the impact of low intensity sonic radiation on myofibrillar proteins in buffers of near physiological ionic strengths, or in alternative solubility states. Moreover, myofibrils are complex and well organized structure and an interesting alternative will be to use myofibrillar proteins extracts which are devoid of myofibrillar anchorage.

Actomyosin, the main contractile apparatus, constitutes major portion of the myofibrillar proteins extracted by high salt concentration. Extraction of natural actomyosin at high salt concentration is an important attribute for further purification and to study other biochemical properties (Gordon & Barbut, 1992). Response of actomyosin as a basic myofibrillar protein to low frequency ultrasonic radiations is important to understand the principle of solubilisation behaviour *in vitro*. We, therefore, intended to investigate the way ultrasonication, as a pure physical force, will affect solubility behaviour of actomyosin in different chemical environments.

2. Materials and methods

2.1. Chemicals

Acrylamide, bis-acrylamide, phenyl methane sulfonylfluoride (PMSF), adenosine 5′-triphosphate disodium salt (ATP), ammonium per sulphate and N,N,N′,N′-Tetramethylethylenediamine (TEMED) were procured from Sigma-Aldrich chemicals Pvt. Ltd. 1-Amino-2-naphthol-4-sulphonic acid, potassium chloride, bovine serum albumin and Tris buffer were purchased from SRL, India. All other chemicals and reagents used were of analytical grade.

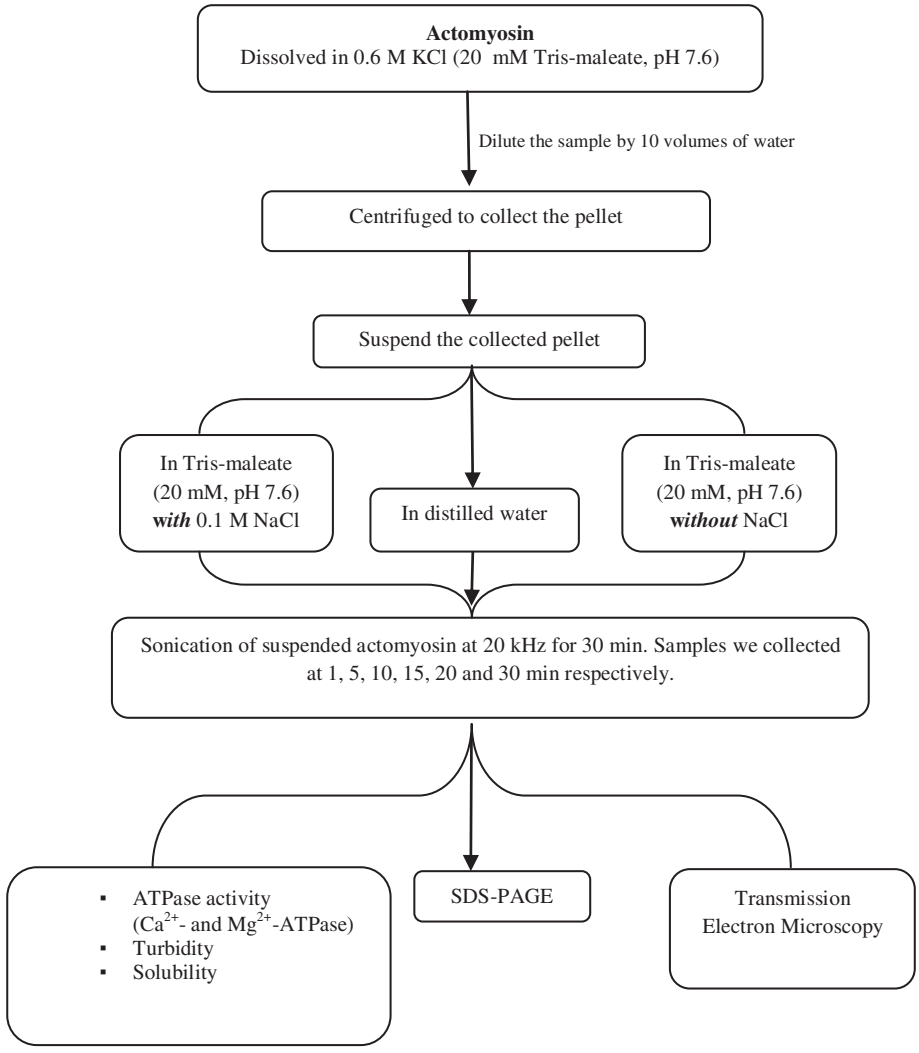
2.2. Preparation of actomyosin

Breast muscles of freshly sacrificed broiler chicken (5–6 months old) were obtained from local market and transported to lab in ice box. Actomyosin from breast muscles (*Pectoralis major*) was prepared according to the protocol outlined by Ahmad and Hasnain (2006) with minor modifications. Briefly, muscles were thoroughly chopped and minced with sharp knife. The minced muscles were washed three times with five volumes of sodium phosphate buffer (50 mM, pH 7.6) containing 2 mM PMSF. Afterwards, muscles were kept overnight in extraction buffer (0.6 M KCl in 20 mM tris-maleate, pH 7.6) at 4°C. The viscous extract thus obtained was filtered through fine gauze and actomyosin from filtrate was precipitated in 10 volumes of chilled distilled water (0–4°C). Actomyosin was collected after centrifugation at 5,000 rpm for 20 min at 4°C and dissolved in 0.6 M NaCl (20 mM tris-maleate, pH 7.6). Free myosin was removed by giving two washes in 0.2 M NaCl (tris-maleate, pH 7.6). Actomyosin thus obtained was washed two times with 10 volumes of chilled distilled water before finally dissolving in 0.6 M NaCl (20 mM tris-maleate, pH 7.6). After overnight dialysis with tris-maleate buffer (20 mM, pH 7.6) containing 0.6 M NaCl, the actomyosin was ready to use.

2.3. Protein estimation

Protein concentrations of all samples were determined by the method of Lowry, Rosebrough, Farr, and Randall (1951) taking bovine serum albumin as standard. Absorbance (optical density) was recorded at 660 nm on Genesys 10 UV scanning spectrophotometer. All the values presented here are mean of three replicates.

2.4. Study design



2.5. Solubilisation of actomyosin in tris-maleate (with/without 0.1 M NaCl) and water

Prior to suspension in tris-maleate (with/without 0.1 M NaCl) and water, actomyosin dissolved in 0.6 M NaCl (tris-maleate 20 mM, pH 7.6) was precipitated with 10 volumes of chilled distilled water and centrifuged at 5,000 rpm for 15 min to collect the pellet. Washing with chilled distilled water was repeated three times to remove the traces of salt and buffer. Finally the pellet, so collected, was suspended in tris-maleate (20 mM, pH 7.6) with/without 0.1 M NaCl and water, respectively.

Actomyosin (2.85 mg/ml) suspended in tris-maleate (with/without 0.1 M NaCl) and water was sonicated at low frequency (20 kHz) for 30 min. During ultrasonic treatment, the probe was immersed in the actomyosin solution and ultrasonic pulse mode of on-time 20 s and off-time 10 s was used. In order to ensure better homogeneity, the sample was gently stirred by glass rod for 15 s after every 1 min during application of ultrasound treatment. Aliquots collected at 1, 5, 10, 15, 20 and 30 min were centrifuged at

10,000 rpm for 15 min. The protein content in the supernatant was taken to calculate solubility of actomyosin. The solubility was calculated as the ratio of protein concentration in the supernatant after centrifugation and total protein concentration of actomyosin before centrifugation as described by Liu et al. (2011).

$$\text{Solubility}\,\% = C_s/C_o \times 100$$

where C_s is the protein concentration in the supernatant after centrifugation and C_o is the protein concentration before centrifugation.

2.6. Turbidity measurement

Turbidity of sonicated samples was determined by taking absorbance at 320 nm on Genesys 10 UV scanning spectrophotometer. All treated samples were diluted to constant protein concentration of 0.20 mg/ml before taking the measurement.

2.7. Assay of ATPase activity

Ca^{2+}- and Mg^{2+}-ATPase activities were determined by measuring inorganic phosphate released during ATP hydrolysis by actomyosin using the method of Hasnain, Samejima, Takahashi, and Yasui (1979) with slight modifications. Briefly, reaction mixture (2 ml) for Ca^{2+}- and Mg^{2+}-ATPase assay contain 0.2 ml sonicated actomyosin, 5 mM of $CaCl_2$ (for Ca^{2+}-ATPase assay) or 5 mM of $MgCl_2$ (for Mg^{2+}-ATPase assay) in 25 mM Tris-HCl buffer (pH 7.6). Mg^{2+}-ATPase and Ca^{2+}-ATPase assays were carried out in reaction mixture with final ionic strength of 0.02 M KCl and 0.03 M KCl, respectively. The reaction carried out at 20°C was initiated by adding 1 mM ATP (as final) to reaction mixture and incubated for 5 min at 20°C. Finally, the reaction was stopped by adding 15% trichloroacetic acid. The reaction mixture was centrifuged at 2,000 rpm for 10 min and liberated inorganic phosphate (P*i*) in supernatants was determined by the method of Fiske and Subbarow (1925).

2.8. Profiling of sonicated sample by SDS-PAGE

The treated actomyosin samples were separated into supernatants (soluble) and pellets (insoluble) by centrifugation. Supernatants (soluble fraction) were processed for electrophoretic profiling. Sodium dodecyl sulphate (SDS) polyacrylamide gels were prepared and run according to Laemmli (1970). Vertical slab gels contain 10% acrylamide concentration in separating and 4% in stacking gels, besides 0.1% SDS and 10% glycerol. The electrophoretic runs were made for three hours (15 mA/gel) at room temperature. When the run was over, the gels were washed with solution containing methanol (40%) and acetic acid (10%). Washed gels were stained in a solution containing 0.1% (w/v) Coomassie brilliant blue R-250 (CBBR250), 40% (v/v) methanol and 10% (v/v) glacial acetic acid. Gels were then destained overnight with 5% (v/v) glacial acetic acid.

2.9. Documentation and quantitative assessment of PAGE profiles

CBB-stained acrylamide gels were documented using digital camera (Zoom-4X, 14.1 Megapixels) and by direct scanning on an all-in-one (F370) computer assembly. Best gels were selected for densitometry by Scion Imaging software program (Scion Corporation: Beta release 4.0).

2.10. Transmission electron microscopy

Sonicated samples of actomyosin (2.85 mg/ml) were diluted to 0.4 mg/ml. Fixation of samples was carried on carbon-coated grid for 4 min. Samples were negatively stained with 5% lead acetate for 10 min, washed and cleaned by distilled water. The specimens were visualised using a transmission electron microscope (JEOL Ltd, Tokyo, Japan) at an accelerating voltage of 110 kV.

2.11. Statistics analyses

All experiments were carried out in quadruplet and analytical measurements of each were made in triplicates. Student's *t*-test was employed for statistical comparisons and values were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Change in solubility

Changes in solubility of actomyosin complex due to ultrasonic radiations are shown in Figure 1. Marked increase in total protein solubility (13 ± 1.21%) was observed when actomyosin, in tris-maleate in presence of 0.1 M NaCl, was exposed to ultrasonic radiations for 30 min. However, in absence of 0.1 M NaCl there is only 8 ± 1.01% increase in total protein solubility. Effect of ultrasonic radiations on solubility of actomyosin was noted as the least in water with only 6.8 ± 1.14% of actomyosin soluble after 30 min of sonication. Compared to control, in all suspending media (i.e. in tris-maleate with/without NaCl and water) significant amount of protein is soluble within 15 min of ultrasonic exposure. Although there was gradual increase beyond 15 min of sonication in tris-maleate with 0.1 M NaCl, but insignificant variation in protein solubility was observed in water and tris-maleate beyond 15 min of sonication ($p < 0.05$).

Solubility profile of our results follows the order: tris-maleate (with 0.1 M NaCl) > tris-maleate only > water. Since solubility of myofibrillar proteins depends on their source, freshness, pH of muscle, type of solvent and the ionic strengths (Wu, Atallah, & Hultin, 1991; Wu & Smith, 1987), the observed variation in solubility profile of sonicated actomyosin is quite obvious. Results of our study further reveal that sodium chloride significantly improves ultrasonication mediated solubilisation of various proteins from actomyosin complex which is in agreement with the study of Chang, Feng, and Hultin (2001), where it was observed that sodium chloride is fairly effective in solubilising substantial amount of major myofibrillar proteins after extraction. Filament formation, which is associated with low solubility, is mediated by electrostatic interactions (McLachlan & Karn, 1982) and hence we presume that ultrasonic radiations act as an additional force to augment the inhibitory action of sodium chloride on filament formation. Our results further reveal that compared to water, solubility in tris-maleate (without NaCl) is higher. Few reports suggest that in addition of salts, buffers also play an important role in solubilisation of muscle proteins (Guo, Peng, Zhang, Liu, & Cui, 2015; Hayakawa, Wakamatsu, Nishimura, & Hattori, 2009, 2010); while pH-related difference in solubility by ultrasonication has also been reported previously in myofibrillar proteins of spent hen (Cho et al., 2007). We, therefore, suggest that the variation in

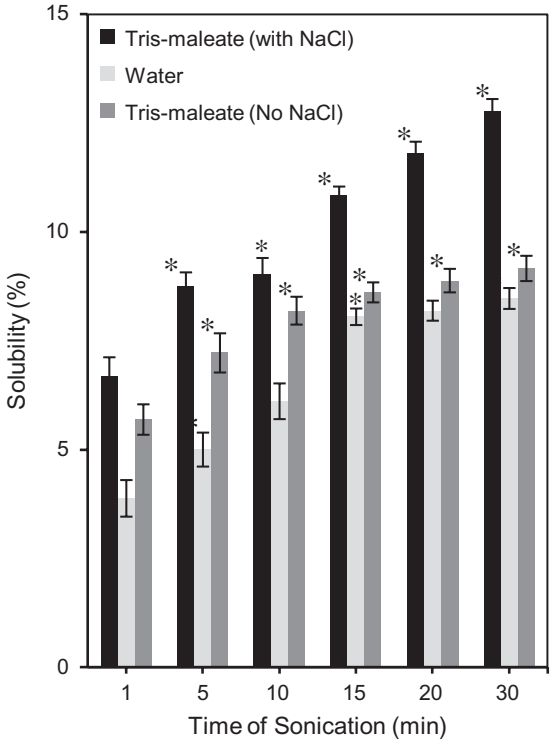


Figure 1. Change in solubility of chicken actomyosin treated with low frequency ultrasonic radiations (20 kHz) for different time intervals.

Notes: Solubility variation by ultrasonication was studied in tris-maleate (with 0.1 M NaCl), tris-maleate (without NaCl) and water. Solubility in respective controls was: tris-maleate with NaCl = 3.56 ± 0.31%, tris-maleate only = 2.01 ± 0.29% and water = 1.98 ± 0.32%. Solubility is expressed as the relative per cent value. *$p < 0.05$ vs. respective controls.

solubility of actomyosin in water (pH 6.9–7.0) and tris-maleate (pH 7.6) are pH dependent. We further demonstrate that only 6.8% proteins are solubilised in water compared to 80% as reported previously by Ito et al. (2003). According to these authors, the role of ultrasonication in muscle protein solubilisation is largely associated with destruction of myofibrillar structure to release the major myofibrillar proteins. Extraction of natural actomyosin at high salt concentration rule out the involvement of ultrasonication in destruction of myofibrillar structure, rather, more specifically, it implies the role of ultrasonic radiations in solubilisation. Direct exposure of actomyosin to ultrasonic radiations, carried out during the present study, reveals that ultrasonication significantly affects solubility, most probably by causing conformational changes in actomyosin complex.

3.2. Change in turbidity

Change in turbidity of actomyosin complex with ultrasonic radiations is shown in Figure 2. Turbidity of actomyosin complex shows gradual decrease with increasing time of exposure to ultrasonic radiations. Comparatively, actomyosin complex suspended in water shows maximum absorbance whereas actomyosin suspended in tris-maleate with 0.1 M NaCl shows minimum absorbance at 320 nm. In all cases, exposure of ultrasonic radiations for 30 min shows significant decline in turbidity ($p < 0.05$). Actomyosin is a turbid protein complex and decrease in turbidity may indicate dispersion of the actomyosin complex while increase in turbidity indicates aggregation (Juan, Pan, Shen, & Luo, 2012; Saleem, Hasnain, & Ahmad, 2014). At physiological ionic strength, actomyosin forms aggregates due to low solubility. Protein aggregates could cause light scattering (Gill, Chan, Phonchareon, & Paulson, 1992; Visessanguan, Ogawa, Nakai, & An, 2000) and may therefore increase the turbidity. Ultrasonic radiations have been extensively used for many years to disrupt cells and disperse aggregated materials (Dolatowski, Stadnik, & Stasiak, 2007). Our results indicate that under the influence of physical force applied by low frequency ultrasonic radiations, actomyosin aggregates get dispersed that might have resulted in more transparency.

3.3. Change in Ca^{2+}- and Mg^{2+}-ATPase activity

The effect of low frequency ultrasonic radiations on Ca^{2+}- and Mg^{2+}-ATPase activity is shown in Figure 3. It is observed that compared to untreated control actomyosin, ultrasonic radiations significantly inactivate ATPase activities in treated samples. Both Ca^{2+}- and Mg^{2+}-ATPase activity showed decreasing

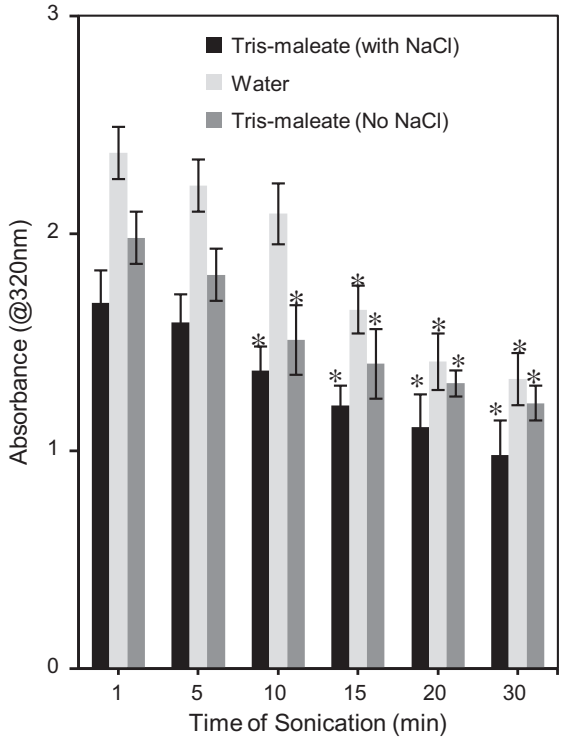


Figure 2. Changes in turbidity of chicken actomyosin treated with low frequency ultrasonic waves for different time intervals.

Notes: Turbidity in respective controls was: tris-maleate with NaCl = 2.29 ± 0.28%, tris-maleate only = 2.43 ± 0.34% and water = 2.51 ± 0.42%.
*$p < 0.05$ vs. respective controls.

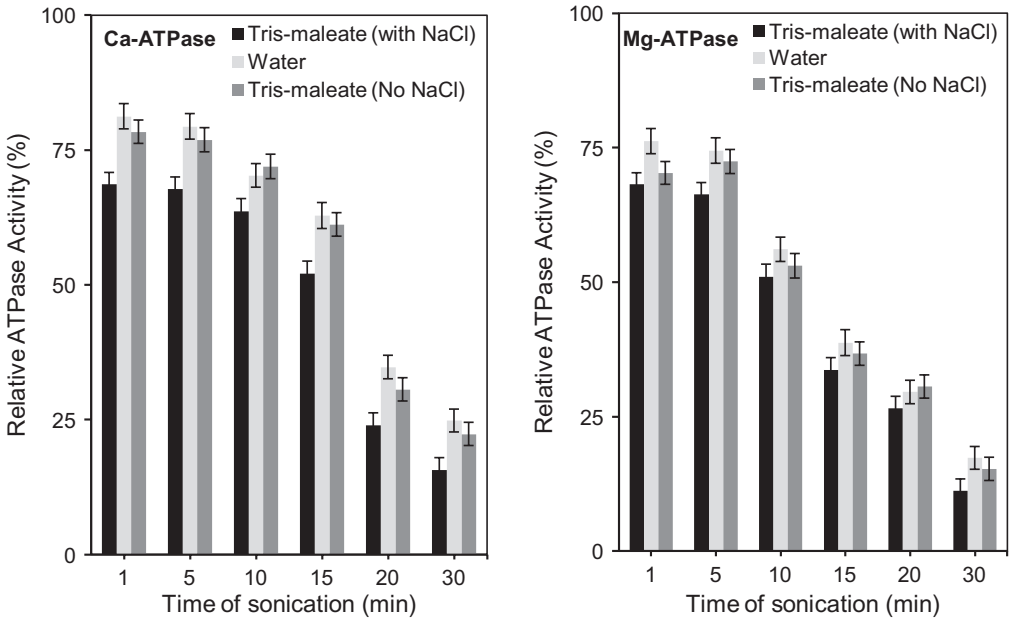


Figure 3. Changes in Ca^{2+}- and Mg^{2+}-ATPase activities of chicken actomyosin treated with ultrasonic radiations for different time intervals.

Notes: Actomyosin was sonicated in three different medias i.e. tris-maleate (with 0.1 M NaCl), tris-maleate (without NaCl) and water. ATPase activity in both cases is expressed as relative per cent.

trend parallel the time of sonication. Addition of sodium chloride (0.1 M) significantly increased the drop of Ca^{2+}- and Mg^{2+}-ATPase activity compared to tris-maleate only and water ($p < 0.05$). The differences in enzymatic activities were insignificant in case of water and tris-maleate only ($p > 0.05$). Comparatively, Mg^{2+}-ATPase activity indicated more sensitivity to ultrasonic radiations. Ca^{2+}-ATPase activity shows insignificant ($p > 0.05$) whereas Mg^{2+}-ATPase activity displays significant ($p < 0.05$) decline during first 10 min of sonication. Prolonged exposure of ultrasonic radiations, up to 30 min, significantly declines Ca^{2+}-ATPase with a noticeable decrease between 15 and 20 min ($p < 0.05$). Overall decline of 55 to 57 ± 1.9% was observed in Ca^{2+}- and Mg^{2+}-ATPase activity between 1 and 30 min of sonication.

Earlier reports on myosin, heavy meromyosin (Bárány, Bárány, & Oppenheimer, 1963) and actomyosin (Ahmad & Hasnain, 2013a) suggest the sensitivity of enzymatic activity to low frequency ultrasonic radiations. So far as functional relevance is concerned, Ca^{2+}-ATPase activity is an index of the structural state of myosin, whereas Mg^{2+}-ATPase depicts the nature of interaction between actin and myosin in actomyosin complex (Montecchia, Roura, Roldan, Perez-Borla, & Crupkin, 1997). Decrease in Ca^{2+}-ATPase activity during sonication indicates that ultrasonic radiations notably perturbed the myosin. Release of myosin light chains, as evident from SDS-PAGE profile (Figure 4), from actomyosin complex by ultrasonic radiations also supports the possible perturbations in myosin. In addition, Mg^{2+}-ATPase activity measurements indicate that interaction between actin and myosin markedly gets disturbed by ultrasonic radiations. Earlier reports about the susceptibility of actin to ultrasonic waves (Asakura, 1961) are in agreement with our results. As actin is reported to be the activator of myosin Mg^{2+}-ATPase (Torigai & Konno, 1996), it is reasonable to infer that decrease in Mg^{2+}-ATPase activity may be due to the effect of sonication on actin. Hence, the results indicate that ultrasonic radiations effectively alter the conformation of myosin and disturb the interaction between actin and myosin.

3.4. Electrophoretic profiling of actomyosin treated with ultrasonic radiations

SDS-PAGE results of actomyosin treated with low frequency ultrasonic radiations for different time intervals are shown in Figure 4. Various polypeptides corresponding to approximate molecular weights documented in various previous studies are: myosin heavy chain (MyHC) (~200 kD), actin (~42 kD), tropomyosin (~37 kD), troponin-T (~35 kD), troponin-I (~23 kD), troponin-C (~20 kD) and myosin light chains I, II and III (~25 kD, 18 kD and 16 kD) (Abbasi, Ahmad, & Hasnain, 2012; Ahmad & Hasnain, 2013b; Hasnain & Ahmad, 2006). All the polypeptides of known molecular weight present in the typical SDS-PAGE profiles of chicken actomyosin were intact and we do not find any extra fragment in treated

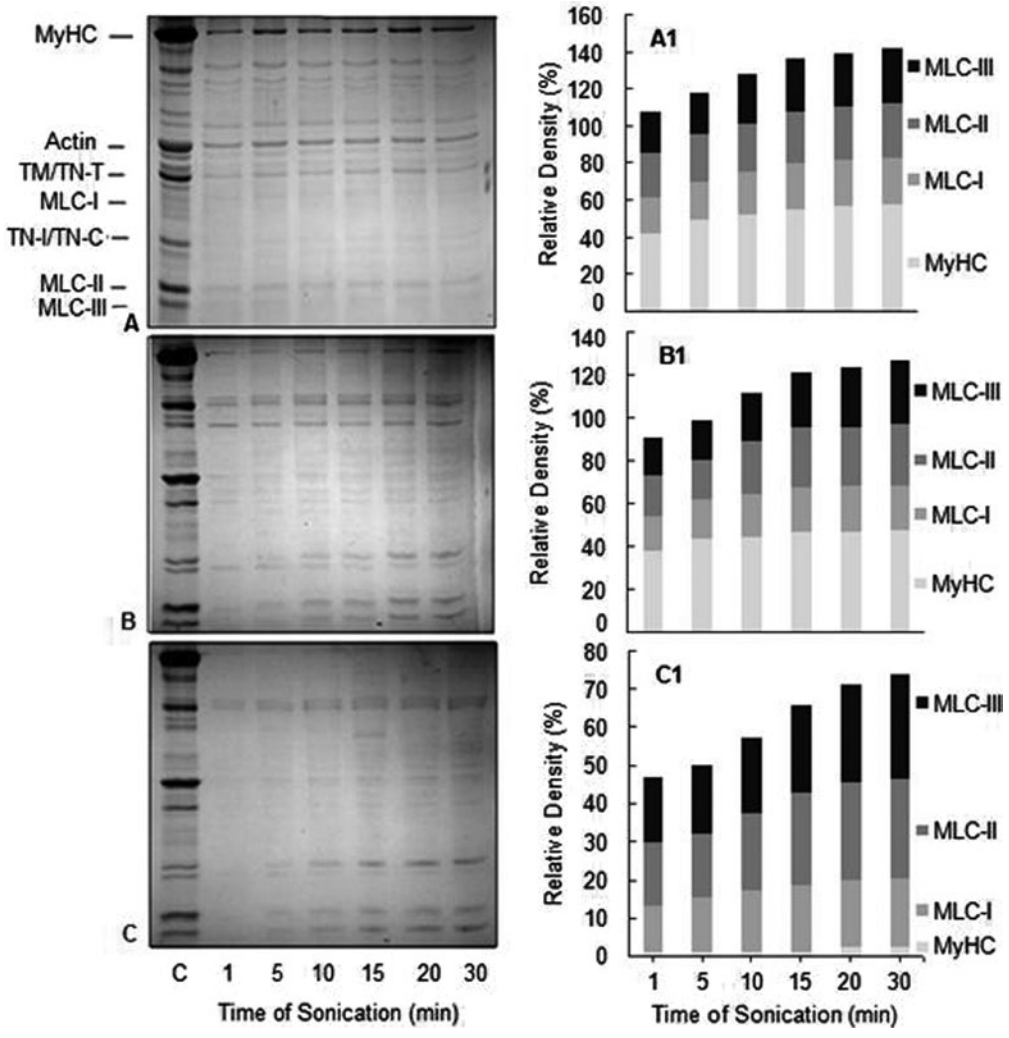


Figure 4. SDS-PAGE (10%) profiles of chicken actomyosin treated with low frequency (20 kHz) ultrasonic radiations for 30 min.

Notes: Treated actomyosin was fractionated by centrifugation at 10,000 rpm for 15 min into supernatant and pellet. Samples of supernatants were loaded on SDS gels in equal volume (10 µl). Supernatants of actomyosin sonicated in (A) tris-maleate (20 mM, pH 7.6) with 0.1 M NaCl (B) tris-maleate (20 mM, pH 7.6) only (C) water. C is the Control, Lane 1, 5, 10, 15, 20 and 30 is time of sonication in minutes, respectively. A1, B1 and C1 are densitometry profile of MyHC, myosin light chains (MLC)-I, II and III of A, B and C gels, respectively. MyHC is the myosin heavy chain; TM is the tropomyosin; TN is the troponin; MLCI is the myosin light chain I; TN-I is the troponin-I; TN-C is the troponin-C; MLC-II is the myosin light chain II and MLC-III is the myosin light chain III.

samples. Our results are in agreement with previous reports where no fragmentation of myofibrillar proteins was observed by ultrasonication (Ahmad & Hasnain, 2013a; Lyng, Allen, & McKenna, 1997, 1998). Figure 4(A) shows the proteins solubilised in tris-maleate (with 0.1 M NaCl) by ultrasonication. As apparent from SDS-PAGE and supported by densitometry graph (Figure 4(A1, B1)), there is a gradual increase in solubility of actin, MyHC along with light chains with the increasing time of sonication. MyHC was least soluble in water with very small amount following 20–30 min of sonication, however, intensity of myosin light chains appear increasing from 5 to 30 min of sonication (Figure 4(C1)). In addition to substantial amount of actomyosin proteins like actin, MyHC along with light chains were solubilised in tris-maleate (without 0.1 M NaCl) buffer (Figure 4(B1)). Ito et al. (2003) also showed increased solubility of myosin, actin and α-actinin from myofibrils by ultrasonication after washing with L-histidine. Our study demonstrates that myosin heavy chain from extracted actomyosin was fairly less solubilised in water by ultrasonication even after 30 min of sonication.

3.5. Transmission electron micrographs of actomyosin as affected by ultrasonication

Microstructure of actomyosin treated with low frequency ultrasonic radiations in tris-maleate (with/without 0.1 M NaCl) and water for different time intervals are illustrated in Figure 5. The actomyosin suspended in tris-maleate (with/without 0.1 M NaCl) and water was found as filament aggregates. Filament structure was apparent during initial sonication but with the increasing exposure of ultrasonic radiations, filaments were dispersed thereby decreasing degree of aggregation. Transmission electron microscopy has been used to study the structural changes induced by physical treatment in actomyosin filaments

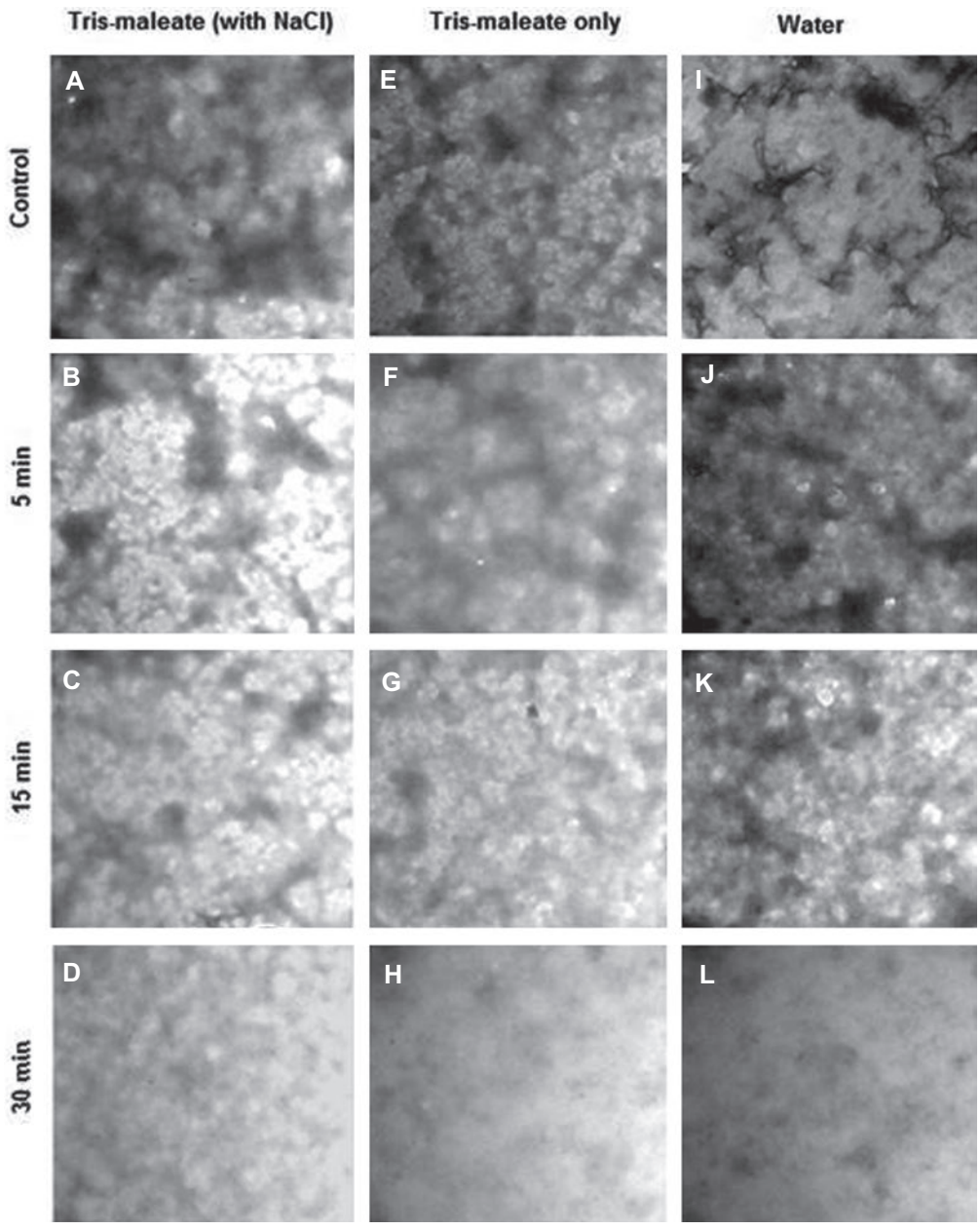


Figure 5. Transmission electron micrographs of actomyosin sonicated in tris-maleate (with NaCl), tris-maleate only and water.

Notes: 5, 15 and 30 min are the time of sonication. Magnification = 15,000 X.

(Kuo, Hwang, Yu, & Jao, 2007). Physical force like hydrostatic pressure is reported to cause shortening of actomyosin filaments by dissociation of myosin and depolymerisation of actin (Ko, Tanaka, Nagashima, Mizuno, & Taguchi, 1990). We are of the opinion that ultrasonication, as a physical force may cause disruption of aggregates in the similar manner. Results of our ATPase activity (Figure 3) together with SDS-PAGE (Figure 4) reveal that ultrasonication not only causes dissociation of actomyosin complex but also indicates the possible structural changes in myosin and actin. Further, in presence of sodium chloride actomyosin under the influence of ultrasonic radiations is comparatively dissociated or disrupted more easily due to increased ionic interactions. Actomyosin aggregates were least dispersed in water whereas in tris-maleate (With NaCl) aggregates were intensively disrupted. Higher disruption or dissociation of actomyosin complex in presence of sodium chloride is also supported by our solubility (Figure 1) and turbidity (Figure 2) profiles. Results of electron micrographs obtained here indicate that degree of dissociation or disruption of actomyosin aggregates depends upon the time of sonication and on the medium of suspension.

4. Conclusion

The study indicated that irrespective of the nature of suspending medium, low intensity ultrasonic radiations are effective in shifting the solubility of actomyosin from high salt to low salt concentrations. ATPase activity, turbidity and solubility profiles suggest that ultrasonic radiations have maximum effect in presence of sodium chloride. SDS-PAGE reveals that ultrasonic radiations solubilise least amount of MyHC in water whereas substantial amount of MyHC is solubilised in tris-maleate with and without sodium chloride. Disaggregation, as evident from electron micrographs, strongly support ultrasonication induced changes in turbidity and solubility profile of actomyosin complex. Overall, the results of our study suggest that in addition to structural disorganisation of myofibrillar complex, ultrasonication plays a significant role in solubilisation of major myofibrillar proteins most probably by altering the conformation of actomyosin complex.

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Competing interests
The authors declare no competing interests.

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