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Editors
A. Antić-Jovanović and S. Anić

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SONOLYTIC DEGRADATION OF OVOMUCIN BASED PROTEIN MATRIX OF HEN EGG WHITE

D. Filipović and M. B. Radojčić

Laboratory of Molecular Biology and Endocrinology
VINČA Institute of Nuclear Sciences,
P.O.Box 522, 11001 Belgrade, Serbia and Montenegro

Abstract

Sonication of ovomucin based protein matrix of the thick fraction of egg white with the therapeutic ultrasound of 23 kHz frequency and 5μm amplitude causes irreversible decrease of its viscosity down to the limit value of 2.1mPa·s. The ultrasound treatment does not affect structure of proteins with lower molecular mass (Mm < 270,000 g/mol), such as ovalbumin, conalbumin and ovoglobulins, which reside within the protein matrix, but it leads to the fragmentation of ovomucin fibers (Mm > 3 x 10^6 g/mol) which form the thick egg white matrix. The results suggest that in analogy with avian egg white matrix, sonication-induced changes in mammalian mucin matrix of joints and tendons may constitute the therapeutical action of ultrasound.

Introduction

Ultrasound (ν=0.75-3MHz) is used for physical therapy of damaged ligaments, joint capsules, or tendons and for emulsification of cataracts and repair of soft tissue injuries [1]. Its beneficial effect is taught to be based on the local increase in tissue temperature. Many of the treated tissues are composed of ductular and tubular structures lined with epithelial cells embedded in extra cellular jelly-like protein matrix composed of mucins [2]. The molar mass (Mm) of mucins is *cca* 10-15x10^6 g/mol, and they compose surfactants, molecular sieves, or supportive and protective matrix structures. Although not precisely defined, the changes in mucin matrix are taught to play important part in ultrasound therapy [3]. Mammalian mucins are not easily available in high quantities, but their avian complement ovomucin (Mm ≅ 3x10^6 g/mol) is easily obtained from the thick fraction of hen's egg white. Egg white ovomucin forms fibrillar matrix (Mm ≅ 40x10^6 g/mol) encompasing other egg white globular proteins [4]. Sonochemistry postulates the existence of a critial molar mass (Mmc), termed lower molar mass limit (LMmL), according to which the molecules whose Mm is below LMmL remain unaffected by ultrasound of certain frequency. Using synthetic polymeres the Mmc of 274,000 g/mol was determined for ν=23kHz, A=5μm laboratory ultrasound [5]. As Mm of ovomucin monomer, as well as Mm of ovomucin matrix are above Mmc, it is of interest to characterized its changes induced by the treatment with ν=23kHz, A=5μm laboratory ultrasound.

Experimental

Fresh Brown Leghorn (*Gallus gallus)* farm hen's eggs were used as a source ovomucine based matrix. The thick fraction of egg white was separated by filtration on a Büchner funnel, placed in a treatment tube and sonified for 20-300 s (in 20s or 40-60s pulses followed by 30 or 60 min breaks) using Soniprep 150 apparatus (ν=23 kHz, A=5μm). The viscosity was measured by an Ostwald viscometer at 20.0 ^{0}C. After 300 s treatment samples (1ml) were dissolved in in 0.1M Tris-HCl pH 6.8 containing 4% SDS, 5% β-mercaptoethanol, 6M urea, 20% glycerol and 0.1% brom-phenol-blue (1:1= vol: vol), boiled 2 min at 100^{0}C and analysed by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The gel was calibrated using chicken muscle myosin heavy chain (Mm=223,000g/mol) and ovalbumin (Mm=43,500g/mol) as standards. A linear relationship between log Mm and protein mobility was established, and used to estimate the Mm of egg white protein peaks. Resolved proteins were stained by 0.125 % Commasie Brilliant Blue and scanned by UltraScan-XL densitometer. The quantification was performed by the comparison of the respective integral area of control and treated sample. The experimental error of the measurements was ≤ 8%.

Results and Discussion

The viscosity measurements of the thick egg white ovomucin matrix treated with ν=23kHz, A=5μm ultrasound for various time periods, showed an ubrupt decrease in viscosity to 2.61±0.09 mPas (mean ± SEM, n=5) during the first 60s of ultrasonification. Prolonged ultasound treatment (>60s) lead to further decrease in viscosity which was much slower and which finally reached a minimum value of 2.08±0.03 mPas after120s ultrasonication (*Figure 1*). Densitometric scan of SDS-PAGE separated proteins *(Figure 2)* indicated *cca* 26% decrease in ovomucim matrix proteins, corresponded to Mm>40x10^6 at the entrance of the stacking gel, indicating partial disruption of the matrix upon 300 s ultrasound treatment. The decrease in ovomucin monomer peak (Mm = 3x10^6 g/mol) with mobility between 0.01 and 0.03 was *cca* 14%. The other thick egg white matrix proteins with Mm < Mmc (274,000 g/mol): conalbumin (Mm = 78,000 g/mol), avidin (Mm = 64,000 g/mol), ovalbumin (Mm = 43,500 g/mol), and ovomucoid (Mm = 28,000 g/mol) were not fragmented (*Figure 2*) after 300 s ultrasound treatment. The ovalbumin peak was even somewhat increased (*cca* 9%), which was interpreted as a release of this most abundant protein (68% of total egg white proteins) upon disruption of ovomucin matrix. The ultrasound generated ovomucin fragments <10,000g/mol were not detectable under these experimental conditions.

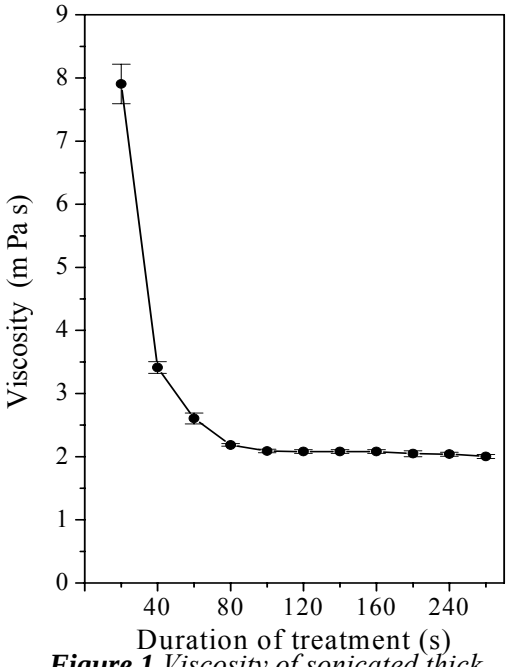

Figure 1 *Viscosity of sonicated thick egg white ovomucin matrix*

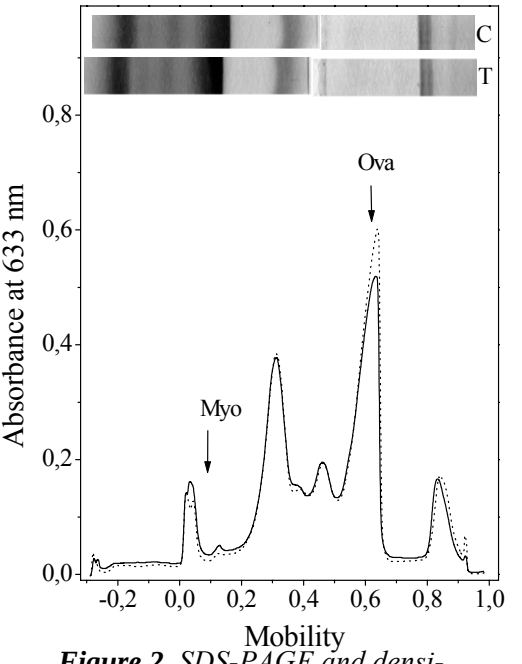

Figure 2 *SDS-PAGE and densitogram of thick egg white proteins: C-control; T-sonicated*

Conclusions

The results indicated that ovomucin matrix of the thick fraction of egg white is partly disrupted after treatment with ν=23kHz, A=5μm laboratory ultrasound. They confirmed that CMm hypothesis is valid for the natural mucin based structures, such as hen thick egg white matrix. In analogy with the avian matrix, sonolytic treatment of mammalian mucous tissues may also cause partial disruption of their matrix. The resulting changes in protein structure and in the related viscosity of extracellular tissue milieux may consitute the beneficial therapeutic action of ultrasound.

References

[1] M.Dyson, In: *Biological effects of Ultrasound* (Ed. Nyborg and Ziskin) Churchil-Livingston, New York, 1986, 121-33.
[2] W.L.terHaar, In: *Physics in Medicine and Biology*, Encyclopedia. (Ed. McAinsh) Pergamon Press, Oxford, 1986, 843-6.
[3] M.C.Rose, W.A.Voter, C.F.Brown, B.Kaufman, Biochem.J. 1984, 222, 371-7.
[4] M.Vučković, M.Radojčić, B.H.Milosavljević, J .Serbian Chem.Soc. 2000, **65**, 157-66.
[5] D.Filipović, M.Radojčić, B.H.Milosavljević, J.Serb.Chem.Soc. 2000, **65**,123-30.