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Biophysics

Some Physical-Chemical Properties of Smooth Muscle Smitin

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ABSTRACT. Some physical-chemical properties of smitin were studied and the action of temperature on structural properties of smitin was investigated using the intrinsic fluorescence method. It is shown that amino acid residues of tryptophan and tyrosine play a key role in spectra intensity of the intrinsic fluorescence of smitin. It is shown that a rise in temperature from 40^0C to 60^0C results in a sharp change of fluorescence intensity. The calorimetry method was used to study thermal denaturation of smitin. Transition temperature is $T_{max}=55.2^0$C. Melting temperature interval is $T=30^0$C. Calorimetric enthalpy is equal aH = 6.4 cal/g. This value is rather low in comparison with the muscle proteins and probably reflects relatively low order of structural organization of smitin molecule. Circular dichroism spectrum of smitin shows strong negative bond at 226 nm, while molecular ellipsis of pure preparation is equal to - 2700. The secondary structures calculated from the circular dichroism spectrum of smitin shows: ~-helix 2.59%; -sheet 22.24%; random structure 75.17%. The experimental data allow us to conclude that the molecule of smitin is mainly represented as random structure. © *2017 Bull. Georg. Natl. Acad. Sci.*

Key words: smitin, titin, fluorescence intensity, circular dichroism (CD)

Recently it became known that apart from main contractile proteins the muscle also contains giant proteins with high molecular weights. Worthy of notice among these is an elastic protein-titin, nebuline (in striated muscle) and smitin (in smooth muscle). Titin was isolated by Maruyama [1] from the striated muscle. He called that protein "connectin". He determined the molecular mass of this protein, but other characteristics remained to be explored. Later Wang [2] showed that it was mix of two different proteins with molecular masses 3000 kD and 800 kD. He named the big protein "Titin" and the small one which is difficult to reveal – "Nebuline". These two proteins together compose 10-15% of myofibril proteins. In 2002 it was demonstrated that the smooth muscles contain a novel giant protein of 2000 KD molecular

mass which was called smitin (further c-titin) [3]. Titin was investigated in three countries (Japan, USA, England) by Maruyama [4], Wang [2], Trinick [5] and in our department. In our laboratory it was established that the molecule of connectin (titin) contains a collagen type structure (left helix) that determines firmness of myofibril [6]. The thermodynamic and hydrodynamic parameters of titin were also studied [7,8]. Titin molecule has the form of thread approximately with the length of 1.2 μm, and in skeletal and heart muscles it spans half a sarcomere (from M line up to Z disc). It connects the myosin end with Z disc and provides the tension transfers. Titin consists of 27 000 amino acids. It contains repetitive immunoglobulin (Ig G) sites consisting of 100 amino acids and unique fragment (PEVK) rich with proline, glutamine, valine and lysine. The structure and function of striated muscle titin was well studied. Today as opposed to titin there is scant information about smitin physical-chemical properties. It is only known that it interacts with myosin and α-actinin [9]. During interaction with myosin smitin unexpectedly revealed diversity having formed different structural units in natural conditions. Smitin in vitro binds with myosin filaments, while in vivo it forms irregular groups and contains many "side polar" myosin threads. At low ionic strength it forms a special structure containing myosin bipolar threads. By immunoreaction and sedimentation it was demonstrated that smitin and the smooth muscle myosin are associated with "side polar" and bipolar structures. It was shown that smitin plays a central role in the organization of myosin threads [3]. In our department smitin was extracted from chicken smooth muscle (stomach) and its physical parameters were investigated [10,11]. It is interesting to determine smitin role in smooth muscle tonic contraction, where as opposed to striated muscle, neither sarcomere is distinctly formed nor Z disc presents and consequently contraction degree is different. Hence, it is important to study smitin interaction with other proteins of smooth muscle and its function in it. The issue is extremely topical considering that smitin, likewise titin, as been noted above, has similar molecular morphology and location within the contraction apparatus, but its role in the smooth muscle tonic contraction is unexplained (there, in contrast to the striated muscle, the sarcomere is not distinctly formed and the contraction character is different).

This work was carried out on smooth muscle pure smitin and the temperature action on structural properties was studied using intrinsic fluorescence, calorimetry and circular dichroism methods.

Materials and Methods

Smitin was isolated from chicken smooth muscle, particularly from stomach [10]. Myofibrils were obtained according to Wang [2]. Extract of myofibrils was loaded on the toyopearl HW65 (fine) column, obtained fractions containing smitin, myosin and other proteins. Protein concentration was determined by the burette method. Purity of the preparations was examined by the electrophoresis method in the polyacrilamide gel gradient (3-15%) in the presence of Na-dodecyl sulfate. Circular dichroism (CD) spectra of smitin were obtained by Jasco spectropolarimeter J-500A. CD spectra of smitin's secondary structure was calculated by the program developed in Johannes Gutenberg University Mainz (JGU) .The fluorescence spectra was measured with the RF-500 "Shimadsu" spectrofluorimeter. Spectra position and maximum intensity was determined automatically. During the experiment the rate of cuvette heating was of the order of 1-2^{0}K/min. Calorimetric measurements were taken using a DASM-4 type microcalorimeter, with heating rate of 1^{0}K/min. The use of similar calorimeters rules out such effects of heat capacity that are related to changes in viscosity of smitin giant molecule occurring in the process of denaturation.

Results and Discussion

The action of temperature on smitin structural properties was studied by the intrinsic fluorescence method.

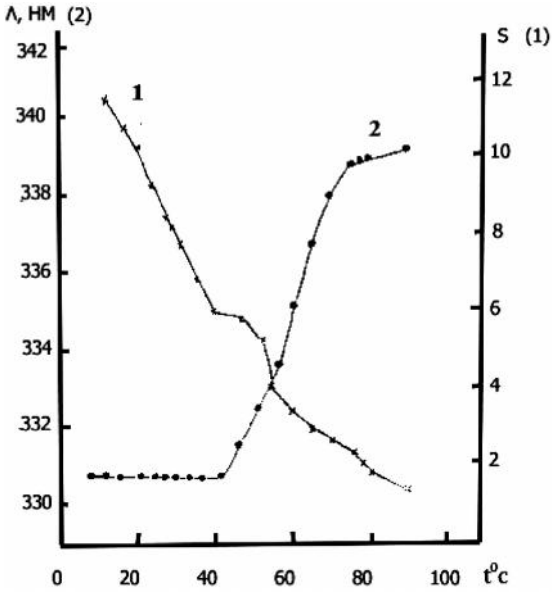

Fig. 1. Curves showing temperature dependence of smitin fluorescence spectrum position (1) and relative quantum yield (2). Excitation wave length 280.4 nm (smitin-0.05 mg/ml, 0.6MKCl, 30mM K^+ phosphate buffer, pH 7.2).

The presence of fluorescent amino acids in the smitin molecule (tryptophane, tyrosine) allowed to study the action of temperature on the quantum yield and spectrum position at 0.1-0.05 mg/ml concentrations. Comparison between spectra at λ=280nm and λ=296 nm shows that tryptophane and tyrosine residues both contribute to the intensity of smitin intrinsic fluorescence spectra (Fig.1). A rise in temperature is attended with a sharp change of quantum yield at 40-60^{0}C and a 6 nm shift of the spectrum to the long wave band, which is due to relocation of fluorescent amino acids from internal position to the surface, i.e. a partial unfolding of the smitin molecule takes place. This finding is in good agreement with elasticity of the smitin molecule, which in its turn, is conditioned by the β-sheet structure. According to our data thermal denaturation (Fig.2.) of smitin solution represents a complicated process with following characteristic features. The partial heat capacity of protein solution increases linearly from room temperatures up to 40^{0}C. Such a rise of heat capacity before denaturation is specific for many proteins, but in our case it is much more expressed. The possible reason of such behavior may be a partial unfolding of smitin molecule, including the changes in exposition of non-polar groups and even the changes in orientation of distinct domains, without disruption of intramolecular bonds. One may interpret such a process as smooth configurational transition, having nonenthalphic nature in the range of 40^0-70^{0}C a real conformational transition from native to denatured state takes place. The melting curve characterizing this process represents a set of peaks. These heat absorption peaks must reflect the denaturation of some cooperative subunits with definite composition and conformation and these subunits probably are

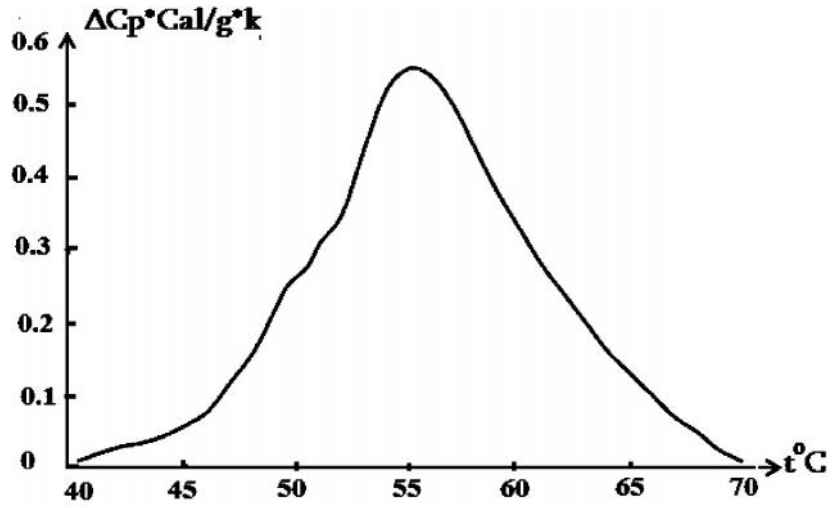

Fig.2. Thermogram of smitin heat absorption during thermal denaturation, protein concentration 1.6 mg/ml, 0.6M KCl, 30 mM K^+ phosphate buffer, pH 7.2.

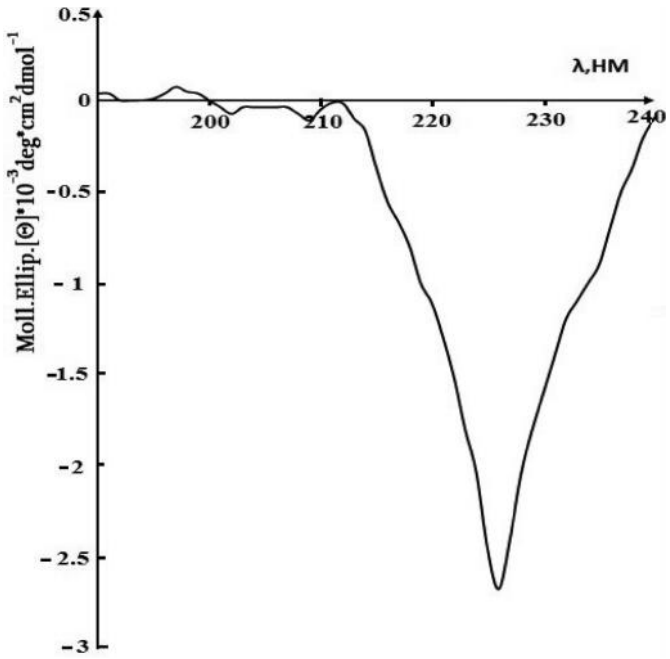

Fig.3. CD – spectrum of pure smitin (mol.ellip.- 2700), smitin- 0.3mg/ml, 0.1M KCl, 0,15M KH_2PO_4, pH 7.

the immunoglobuline domains and PEVK region. Calorimetric studies show that thermal denaturation of smitin starts at 40^0 C and ends at 70^0 C (Fig.2) .Transition temperature of smitin is T_{max}=55.2^{0}C. Melting temperature interval is T=30^0 C.Calorimetric enthalpy of smitin according to our data is equal to ΔH=6.4±0.5 cal/g. This value is rather low in comparison with the muscle proteins (for myosin ΔH=25 cal/g.) and probably reflects relatively low order of structural organization of smitin molecule. Although the smitin molecule in solution represents globular-like formation its calorimetric enthalpy is much more than Vant Hoff's one. This fact indicate, that denaturation of smitin can not be considered as a transition between two states in contrast with small globular proteins. In 2016, by Bobylev et al. [12] CD spectrum of smitin before and after the formation of aggregates was shown. The secondary structure after chromatography had 6.1% α-helix and 2.5 % β-structure, while helix and β-structure content in aggregated smitin was 5.5% and 40.2%, accordingly. No changes were detected in secondary structure on formation of aggregates. In our data CD spectrum of smitin shows strong negative bond at 226 nm, while molecular ellipsis of pure preparation is equal to - 2700. (Fig.3.) The secondary structures calculated from the CD spectrum of smitin are as follows: α-helix 2.59%; β-sheet 22.24%; random structure 75.17%. Difference between our and Bobylev et al. data about smitin secondary structure can be caused by the fact that their experiments were carried out on the isoform of smooth muscle smitin. Obtained results confirm that in smooth muscle smitin has the same function as titin has in skeletal muscle.

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ბიოფიზიკა

გლუვი კუნთის სმიტინის ზოგიერთი ფიზიკურ-ქიმიური თვისებები

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შესწავლილია სმიტინის ზოგიერთი ფიზიკურ-ქიმიური თვისება. საკუთარი ფლუორესცენციის მეთოდით ნაჩვენებია ტემპერატურის გავლენა სმიტინის სტრუქტურულ თვისებებზე. დადგენილია, რომ ამინომჟავები, ტრიფტოფანი და ტიროზინი მნიშვნელოვან როლს ასრულებენ სმიტინის საკუთარი ფლუორესცენციის სპექტრის ინტენსივობაში. ტემპერატურის გაზრდისას ადგილი აქვს კვანტური გამოსავლის მკვეთრ ცვლილებას 40°C-დან 60°C-მდე და სპექტრის წანაცვლებას გრძელ ტალღოვან უბანში.

კალორიმეტრული მეთოდით შესწავლილ იქნა სმიტინის თერმული დენატურაცია. გადასვლის თერმული ტემპერატურა არის T=55,2°C. ლღობის ტემპერატურის ინტერვალი არის 30°C. კალორიმეტრული ენთალპია aH = 6,4 კალ/გ. ეს მონაცემები ბევრად დაბალია კუნთის სხვა ცილების დენატურაციის ენთალპიასთან შედარებით. შესაძლებელია, ეს შედეგია ტიტინის მოლეკულაში რეგულარული სტრუქტურის შედარებით დაბალი შემცველობისა. სმიტინის წრიული დიქროიზმის სპექტრი უჩვენებს ძლიერ უარყოფით ზოლს 226 nm-ზე და მოლეკულური ელიფსურობა ტოლია -2700. წრიული დიქროიზმის სპექტრით გამოთვლილ იქნა სმიტინის მეორეული სტრუქტურა: ~-სტრუქტურა 2,59%, -ნაკეცები 22,24%, მოუწესრიგებელი გორგალი 75,17%. მიღებული ექსპერიმენტული მონაცემები საშუალებას გვაძლევს დავასკვნათ, რომ სმიტინის მოლეკულა ძირითადად წარმოდგენილია მოუწესრიგებელი გორგლის სახით.

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