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THE EFFECT OF pH ON THE THERMAL STABILITY OF α-ACTIN ISOFORMS

G. Papp[2], *Beáta Bugyi*[2], *Z. Ujfalusi*[1], *Sz. Halasi*[2] *and J. Orbán*[2*]

[1]Research Group for Fluorescence Spectroscopy, Office for Academy Research Groups Attached to Universities
[2]University of Pécs, Faculty of Medicine, Department of Biophysics, Pécs, Szigeti str. 12, 7624, Hungary

The effect of pH was characterised on the thermal stability of magnesium saturated skeletal and cardiac α-actin isoforms with differential scanning calorimetry (DSC) at pH 7.0 and 8.0. The calorimetric curves were further analysed to calculate the enthalpy and transition entropy changes. The activation energy was also determined to describe the energy consumption of the initiation of the thermal denaturation process.

Although the difference in T_m values is too small to interpret the difference between the α-actin isoforms, the values of the activation energy indicated that the α-skeletal actin is probably more stable compared to the α-cardiac actin. The difference in the activation energies indicated that lowering the pH can produce a more stable protein matrix in both cases of the isoforms. The larger range of the difference in the values of the activation energies suggested that the α-cardiac actin is probably more sensitive to the change of the pH compared to the α-skeletal actin.

Keywords: *actin isoform, activation energy, calorimetry, divalent cation, pH*

Introduction

The 42.3 kDa actin was described first by Straub and his colleagues [1]. The atomic structure of this protein is available since 1990 in a 2.8 Å resolution [2]. The monomer (globular or G-actin) and the polymer form (filamentous or F-actin) of this protein can be identified within the cells of the eukaryotes. The globular form can be divided into two large domains with a cleft between them containing a bound nucleotide and a divalent cation. The large domains can be further divided into two subdomains [3].

The environment of the actin within the cells can affect the structural and dynamic properties of the actin filaments. One important feature is the flexibility of the protein that can be altered under different conditions [4–16]. The binding of different ligands to the actin filament can alter its properties as well [17, 18].

The cation dependence of the flexibility of actin filaments has been studied previously [4, 19–22]. It was found that the dynamics of the actin filaments can be affected by applying calcium or magnesium ions during the polymerization process [23, 24]. It was shown that the replacement of the bound calcium for magnesium decreased the flexibility of the carboxyl-terminal region (located in subdomain 1) of the actin monomer [22]. It has been demonstrated that both the intra-monomer and the inter-monomer flexibility of the actin filaments are larger in the calcium bound F-actin than in the magnesium bound form of the protein. The inter-monomer flexibility was proved to be larger than the intra-monomer in the case of the calcium-F-actin and the magnesium-F-actin as well [4].

According to previous studies another important factor that can affect the flexibility of actin filaments is the change of the concentration of the H^+-ion around the actin filaments. The intracellular pH can change under physiological and pathological conditions as well. In muscle cells under physiological conditions the pH can decrease from 7.1 to 6.5 after intense exertion [25]. This kind of fall in the pH is considered to be caused by the continuous and increased ATP hydrolysis and the shift of the cellular energy production from the aerobic to the anaerobic way [26, 27]. According to the work of Hild and colleagues the inter-monomer flexibility of the skeletal actin filaments was larger at pH 7.4 than at pH 6.5 in the case of the magnesium F-actin while this difference was not detected in the case of the calcium bound actin filaments [19].

Actin isoforms exhibit different biophysical and biochemical properties in vitro under certain experimental conditions. The different actin isoforms are classified as α-, β-, γ-type based on the differences in their isoelectric points [28, 29]. In our experiments we examined two α-actin isoforms, the α-skeletal and the α-cardiac muscle actins. Although they are able to form copolymers with each other, there are subtle dif-

* Author for correspondence: jozsef.orban@aok.pte.hu

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Akadémiai Kiadó, Budapest, Hungary
Springer, Dordrecht, The Netherlands

ferences in their own filamental structure and larger differences in their ability to bind actin binding proteins as well [30]. In a normal heart there is a specific skeletal/cardiac actin stoichiometry. Hewett and colleagues could demonstrate functional correlation between the skeletal α-actin isoform content and the contractile function of the heart. It was suggested that the α-skeletal actin may promote the increased contractile function in the heart compared to the α-cardiac actin isoform [31].

To better understand the relationship between the flexibility of different actin isoforms and the pH of the surroundings, we examined the thermal stability of the magnesium saturated cardiac and skeletal α-actin isoforms at pH 7.0 and 8.0 as well. The differential scanning calorimetric experiments could demonstrate that the thermal stability of actin filaments was greater at lower pH value in both cases of the actin isoforms. We could also present that the α-skeletal actin is more resistant to heat denaturation than the α-cardiac isoform independently from the applied pH value.

Materials and methods

Actin preparation

The skeletal and cardiac α-actins were prepared from acetone powder of rabbit skeletal and bovine heart muscle according to the method of Spudich and Watt [32].

Magnesium bound actin was prepared from calcium bound actin monomers by the method of Strzelecka *et al.* [11]. The calcium containing actin monomers were incubated with 100 μM $MgCl_2$ and 200 μM EGTA for 5 min at room temperature. The actin was polymerised in the presence of 2 mM $MgCl_2$ and 100 mM KCl for at least two hours at room temperature before the experiments.

The concentration of the actin monomers was determined spectrophotometrically by using a Shimadzu UV-2100 spectrophotometer. The absorption coefficient of 0.63 mL mg^{-1} cm^{-1} [33] and the molecular mass of 42.3 kDa [34] was used during the calculations.

Differential scanning calorimetry

The thermal denaturation of the two kinds of α-actin isoforms was investigated with a SETARAM Micro DSC-II calorimeter between 0 and 100°C. The heating and cooling rates were 0.3 K min^{-1}. Conventional Hastelloy batch vessels were used with 950 μL sample volume. The concentration of the different actin isoforms was 69 μM. The experimental buffer without the protein was used as a reference during the measurements. The sample and reference vessels were balanced with a precision of ±0.1 mg. The data were processed with the Microcal Origin 6.0 software.

The calorimetric enthalpy changes (ΔH_{cal}) were determined by calculating the area under the heat absorption curves. The transition entropy changes were calculated with the following formula:

$$\Delta S=\Delta H_{cal}/T_m \quad (1)$$

where ΔS is the entropy change, ΔH_{cal} is the calorimetric enthalpy change and the T_m is the peak transition temperature.

The activation energy of the thermal denaturation process can be calculated based on the theory of Sanchez-Ruiz *et al.* [35]. The theory assumed that the thermal transition of a protein could be interpreted as a kinetic process. The kinetic constant of this transition can be analysed by using the Arrhenius equation. Based on their work the following equation can be used to determine the activation energy of the calorimetric transition of a protein:

$$\ln\left(\ln\left(\frac{\Delta H_{cal}}{\Delta H_{cal}-\Delta H}\right)\right)=\frac{E_A}{R}\left(\frac{1}{T_m}-\frac{1}{T}\right) \quad (2)$$

where ΔH is the enthalpy change corresponding to the T temperature [35, 36] and ΔH_{cal} is the same as in the Eq. (1). The activation energy can inform us about the energy needed to initiate the thermal denaturation of the protein. The larger energy need for the denaturation may suggest a protein matrix which is more resistant to heat denaturation.

Results and discussion

To characterize the effect of pH on the thermal stability of different type of magnesium saturated α-actin isoforms differential scanning calorimetry experiments were performed at two different pH values. The samples at pH 7.0 and pH 8.0 were heated from 0 to 100°C to define the melting temperature of the proteins. The heat absorption curves were further processed to calculate the calorimetric enthalpy and entropy changes. The activation energy was also calculated to characterize the energy requirement to initiate the thermal denaturation of the proteins.

The heat absorption curves of the α-cardiac and α-skeletal actins showed that the T_m value was higher for the α-skeletal actin than for the α-cardiac isoform at pH 7.0 and 8.0 as well (Figs 1a and b). The difference in the T_m values was 1.96°C at pH 7.0 and 1.88°C at pH 8.0. The enthalpy and entropy changes also reflected the differences between the different actin isoforms (Table 1). The calculated values of the activation energy were higher for the α-skeletal actin isoform at both pH values. The difference was

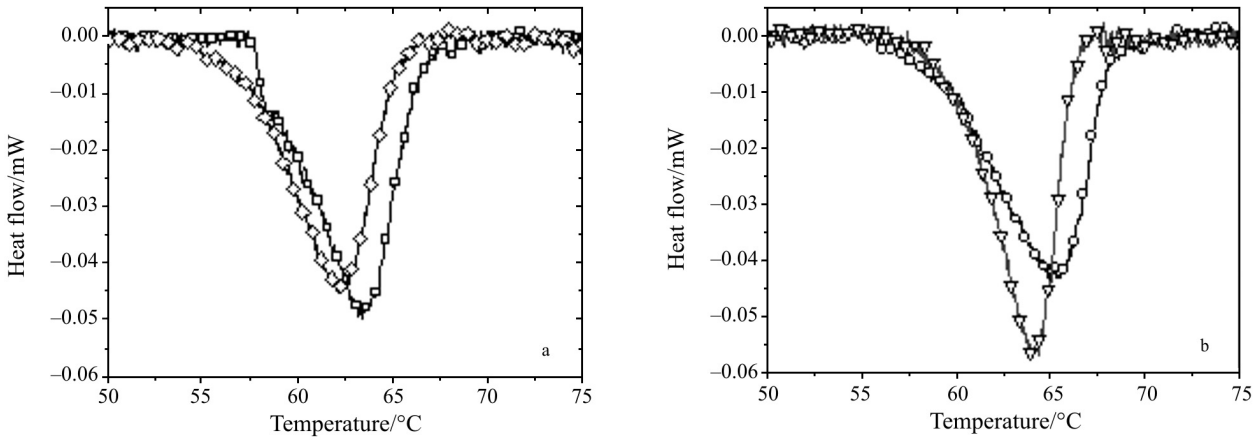

Fig. 1 The thermal denaturation curve of the different magnesium bound α-actin isoforms at pH 7.0 pH 8.0. a – The α-cardiac actin at pH 7.0 is represented with (□), at pH 8.0 has a symbol (◇), b – The α-skeletal actin at pH 7.0 is indicated with (○) and at pH 8.0 the α-skeletal actin has a symbol (▽)

Table 1 The calculated values of the thermodynamic variables for the α-cardiac and α-skeletal actin filaments saturated with magnesium ions at pH 7.0 and 8.0

	α-cardiac actin		α-skeletal actin	
	pH 7.0	pH 8.0	pH 7.0	pH 8.0
T_m/°C	63.38	62.17	65.34	64.05
ΔH_{cal}/kJ mole^{-1}	806.4	768.4	932.5	872.0
ΔS/J K^{-1}	2397	2296	2756	2587
E_A/kJ mole^{-1}	483.7	331.9	547.0	446.7

63 kJ mole^{-1} at pH 7.0 and 115 kJ mole^{-1} at pH 8.0. Although the difference in the T_m values was small the determined values of the activation energy could indicate that the α-skeletal actin was more resistant to heat induced denaturation than the α-cardiac isoform.

The calorimetric curves recorded at different pH values showed that at pH 7.0 the T_m value was higher than at pH 8.0 in the case of both isoforms (Figs 2a and b). The pH induced difference in the value of T_m was 1.21°C for the α-cardiac and 1.29°C for the α-skeletal actin. The enthalpy and entropy changes also indicated the difference induced by the altered pH values (Table 1). The calculated values of the activation energy were higher at pH 7.0 for both of the

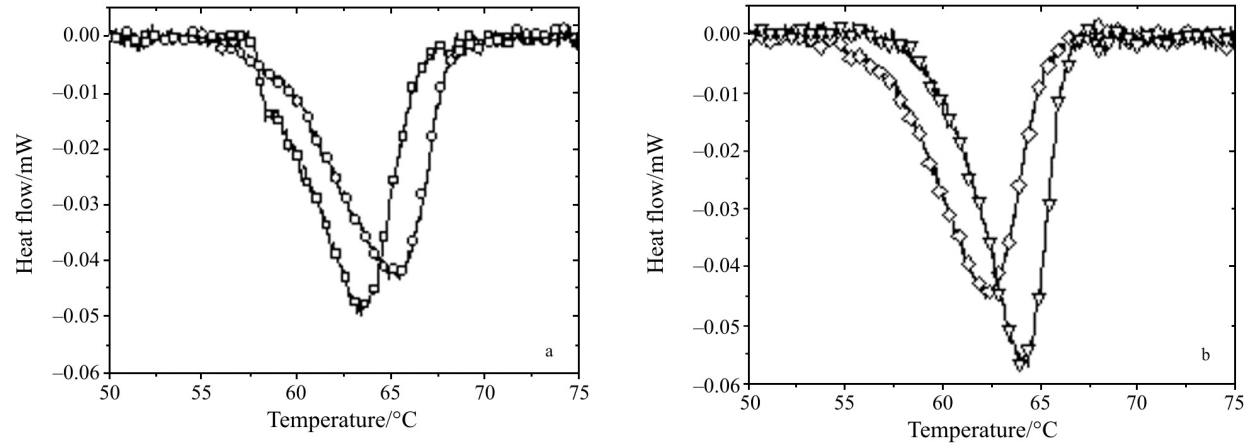

Fig. 2 The thermal denaturation curves recorded at pH 7.0 and 8.0 for the magnesium bound α-cardiac and α-skeletal actin isoforms; a – At pH 7.0 the magnesium bound α-cardiac actin is represented with (□) and the α-skeletal actin has a symbol (○), b – At pH 8.0 the magnesium saturated α-cardiac actin is indicated with (◇) and the magnesium bound α-skeletal actin has a symbol (▽)

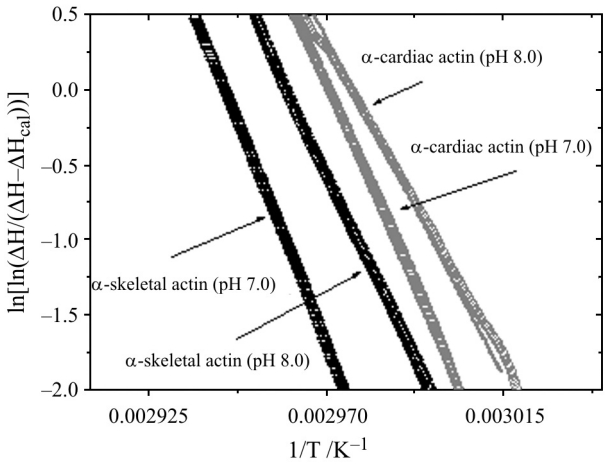

Fig. 3 The plot for the calculation of the activation energy based on the method of Sanchez-Ruiz [35] (α-cardiac actin at pH 7.0, α-cardiac actin at pH 8.0, α-skeletal actin at pH 7.0, α-skeletal actin at pH 8.0)

α-actin isoforms. The difference was 152 kJ mole^{-1} for the α-cardiac and 100 kJ mole^{-1} for the α-skeletal actin. These energy values could indicate that the structure of both of the actin filaments is more resistant against heat denaturation at pH 7.0 than at pH 8.0. This observation is in good agreement with a previous study which showed that the inter-monomer flexibility of the actin filaments decreased as a result of the decreased pH value around the protein matrix [19]. The comparison of the pH dependence of the activation energies for the two isoforms suggests that the α-cardiac actin was more sensitive to the pH changes compared to the α-skeletal isoform. A similar difference was reported previously for skeletal and cardiac myosin as well [37].

Conclusions

The differential scanning calorimetry measurements with the α-cardiac and α-skeletal actin filaments were performed at pH 7.0 and 8.0. The effect of the pH on the melting temperature of the denaturation curves was relatively small. Parallel to the T_m value changes the calculated enthalpy and entropy changes could show a clear difference between the cardiac and skeletal isoforms at different pH values. We also calculated the activation energies to further characterise the recovered differences. The calculated activation energies required for the heat denaturation of the proteins proved to be higher for the α-skeletal actin isoform than for the α-cardiac one. At lower pH values more energy was required to initiate the denaturation process in both cases of the isoforms. These observations suggest that the α-skeletal actin is more resistant to thermal denaturation than the α-cardiac actin at both pH values. The stability of the two isoforms was greater at pH 7.0 than at pH 8.0.

Our results showed a difference in the thermal stability of the cardiac and skeletal actin isoforms. We have also shown that the heat denaturation process was pH dependent for both actin isoforms. These results can demonstrate that the effect of the small difference between the amino acid sequences of α-actin isoforms can be detected by using the method of differential scanning calorimetry. The results also showed that the pH dependences of the thermal stability of the α-cardiac and α-skeletal actin isoforms were similar, which can be interpreted considering their common muscle origin, i.e. their similar biological function.

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