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Nucleotide dependent differences between the α -skeletal and α -cardiac actin isoforms

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Abstract

The thermodynamic properties of the actin filaments prepared from cardiomyocytes were investigated with differential scanning calorimetry. This method could distinguish between the α -cardiac and α -skeletal components of the actin filaments polymerised from ADP-actin monomers by their different melting temperatures (T_m). Similar separation was not possible with filaments polymerised from ATP-actin monomers. Further analyses revealed that the activation energy (E_{act}) was greater for filaments of α -skeletal actin than for α -cardiac actin monomers when the filaments were polymerised from ADP-actin monomers. These results showed that the α -cardiac actin filaments were thermodynamically less stable than the filaments of α -skeletal actin and their difference was nucleotide dependent. Based on these results and considering previous observations it was concluded that the existence of two actin isoforms and their nucleotide dependent conformational differences are part of the tuning regulatory mechanism by which the cardiac muscle cells can maintain their biological function under pathological conditions.

Keywords

Calorimetry; Thermodynamics; Stability; Nucleotides; Actin filament; Protein conformation

Actin is an important intracellular protein with versatile cellular functions [1-4]. In the heart muscle cells both α -cardiac and α -skeletal isoforms of actin can be found [5,6]. The contribution of the skeletal actin isoform in the cardiac muscle is 16–24% of the total actin [5,6]. The actin binds an ATP in complex with a divalent magnesium ion under physiological circumstances [7]. The ATP is hydrolysed during the polymerisation of the actin monomers and the filaments contain mainly ADP in their matured form [8]. The role of the nucleotide in the mechano-chemical properties of the actin filament was emphasized by Janmey and colleagues when they suggested that the energy utilized during the breakdown of the ATP could be stored in the protein [9]. The characterisation of the ADP-actin revealed a change in the subdomain structure and the dynamics of the protein as well [10,11]. Increased inter-monomer flexibility and decreased stiffness were observed when the actin filaments were polymerised from ADP-actin monomers [9,12].

The work of some actin-binding proteins (e.g. thymosin β 4, ARP 2/3 and cofilin) in the cardiac muscle tissue is strongly influenced by the quality of the actin-bound nucleotide [13-15]. Based on the essential roles of the actin-binding proteins in the eukaryotic cells a detailed investigation of the different forms of their targeted actin isoforms is essential to understand the complex work of the cytoskeletal network. In the non-muscle cells the ATP

concentration is influenced by several intracellular factors [16]. The cellular ATP depletion can be the consequence of hypoxia or mitochondrial dysfunction that can cause an increase in the concentration of the ADP-F-actin while the cells become more susceptible to apoptosis [16-18]. The ATP depletion inside the cardiac cells can be the consequence of ischemic cardiac diseases and other heart diseases as well [19].

Differential scanning calorimetry (DSC) is able to determine directly the global thermodynamic properties of a protein and reveal differences on the sub-molecular level as well [20]. In this work the thermodynamic features of actin filaments polymerised from ADP containing actin monomers of heart muscle cells were characterised with the method of differential scanning calorimetry. The measured T_m values and the determined activation energies indicated that the filaments of ADP- α -cardiac actin monomers were thermodynamically less stable than the actin filaments prepared from ADP- α -skeletal actin monomers. The difference disappeared when the filaments were polymerised from ATP-actin monomers. For both the α -cardiac and the α -skeletal isoforms the filaments were more stable when polymerised from ATP-actin monomers than those from ADP-actin. The observed nucleotide and isoform dependent differences in the conformation of the actin filaments may play important roles in the function of muscle cells.

Materials and methods

Sample preparation

The cardiac actin was prepared from acetone powder of bovine heart muscle [21,22]. The calcium saturated actin monomers were stored in a 2 mM Mops buffer (pH 8.0) containing 0.2 mM ATP, 0.1 mM CaCl_2 , 0.1 mM β -mercaptoethanol and 0.005% NaN_3 . The concentration of the actin monomers was calculated by using the extinction coefficient of $0.63 \text{ mg}^{-1} \text{ ml cm}^{-1}$ at 290 nm [23]. The actin bound calcium was changed for magnesium by incubating the samples for 5 min in the presence of 0.2 mM EGTA and 0.1 mM MgCl_2 at room temperature [24]. The exchange of the actin monomer bound ATP for ADP was done by incubating the actin for 1 h at 4 °C in the presence of 1.65 mg/ml hexokinase, 0.5 mg/ml glucose and 1 mM ADP [25]. The ADP-actin was polymerised for 12 h in the presence of 2 mM MgCl_2 and 100 mM KCl. The polymerisation of actin was tested by using pyren-labelled actin in the ratio of 5% as described previously [26]. The excitation wavelength was 365 nm and the emission was monitored at 407 nm.

DSC measurements and theoretical considerations

The calorimetric measurements were performed with a SETARAM Micro DSC-II Calorimeter in the temperature range of 0–100 °C with a constant heating rate of 0.3 K/min. The actin concentration was 69 μM . To analyse the results we applied the method established by Sanchez-Ruiz and his colleagues [27]. Their analysis was based on a model that was created by Lumry and Eyring [28]. The Lumry–Eyring model was successfully applied previously to reveal the thermodynamic properties of actin in solution [29] and in muscle fibres as well [30]. They proposed that the irreversible protein denaturation can be interpreted with a two step kinetic model [28]. In this model a reversible denaturation step was followed by an irreversible one that could be described by a reaction scheme as:

where N is the natural, D is the reversibly denatured and I is the irreversibly denatured form of the protein. The forward and backward rate constants of the reversible step are denoted as k_{+1} and k_{-1} while k_2 appertain to the irreversible formation of the end product from the intermediate D form of the protein. Valuable thermodynamic information about the

reversible unfolding can be obtained when the rate constant for the irreversible step (k_2) is much smaller compared to the reversible step, or when the irreversible process takes place above the T_m value where the main thermodynamic conversion of the protein has occurred. The activation energy of the denaturation process can be related to the calorimetric enthalpy change:

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

where R is the universal gas constant ($8.314 \text{ J mol}^{-1} \text{ K}^{-1}$), E_A is the activation energy and ΔH is the enthalpy change related to the T temperature [27,31]. The ΔH values were determined by calculating the under curve area until the temperature point of T . The activation energy can give information about the energy required to drive the protein through the thermal denaturation process.

Results

Sample characterisation

Actin filaments prepared from the actin monomers of cardiac muscle cells were investigated with the method of DSC. Before the polymerisation process the ATP was replaced with ADP in the binding-pocket of the actin monomers (Fig. 1). The critical concentration of the ADP-actin is $\sim 1 \mu\text{M}$ [32,33], therefore, the contribution of the ADP-actin monomers to the amount of the total actin was so small ($<1.5\%$) that their effect on the calorimetric results was negligible under the applied conditions. Polymerisation tests confirmed that the α -cardiac ADP-actin monomers were able to polymerise, and the polymerisation was completed within the applied time frame ($\sim 12 \text{ h}$) (Fig. 2). The SDS–polyacrylamide gel electrophoresis (SDS–PAGE) could not reveal any contaminating proteins in the samples (inset in Fig. 2).

DSC results on ADP-actin filaments

DSC experiments were carried out on actin filaments polymerised from ADP-actin monomers. The actin was prepared from cardiac muscle cells. The denaturation curve indicated an endothermic transition, and was composed of two distinct peaks (Fig. 3). The T_m values corresponding to the two peaks were $56 \pm 2 \text{ }^\circ\text{C}$ and $60 \pm 2 \text{ }^\circ\text{C}$. To analyse the results the measured heat transition curve was decomposed into two Gaussians (Fig. 3). Similar approach had been successfully applied previously for the analysis of complex heat absorption curves [34]. The Gaussian fit resolved the T_m values of $54.3 \pm 0.5 \text{ }^\circ\text{C}$ and $60.0 \pm 2.1 \text{ }^\circ\text{C}$, which were close to those obtained without the decomposition. Based on the areas under the fitted Gaussians the peaks with the lower and the higher T_m values contributed by $88 \pm 8\%$ and $12 \pm 8\%$ to the total area of the heat absorption curve, respectively.

We attempted to further analyse the DSC results by determining the activation energy (Eq. (2)) attributed to the denaturation process using a previously described method [27,28]. The peak with the higher T_m could not be analysed reliably because of its small size and the narrow temperature range it covered. The calculated activation energy for the first peak of the DSC curve was $254 \pm 11 \text{ kJ mol}^{-1}$ (Fig. 4). This value was smaller than the activation energy determined for the α -cardiac actin filaments from ATP-actin monomers (332 kJ mol^{-1}) [35], or for α -skeletal actin filaments of ADP-actin (311 kJ mol^{-1}), the data are also presented in Fig. 4) [29].

Discussion

The comparison of the α -cardiac and α -skeletal actin isoforms

One of the characteristic features of the DSC results was the appearance of the two peaks in the heat absorption curve (Fig. 3). A possible explanation for this observation could be that the nucleotide exchange was not complete during the preparation of ADP-actin monomers, and the samples contained ATP-actin before the polymerisation. It is unlikely that the method fails to replace the bound ATP with ADP in the case of the α -cardiac actin isoform because the method of nucleotide exchange is well established, and was previously successfully used in many cases for skeletal actin [36].

An alternative explanation for the appearance of the two peaks is based on earlier observations that the actin from cardiac muscle contains α -skeletal actin (16–24%) as well [5,6]. The T_m value of 60 ± 2 °C we observed for the higher temperature peak is close to the value of 58.4 °C, which was obtained previously for the filaments polymerised from α -skeletal ADP-actin monomers [29]. The proximity of these values suggests that the higher temperature peak in Fig. 3 belongs to the α -skeletal part of the actin pool within the cardiac cells. To test this possibility we determined the contribution of the areas belonging to the two peaks of the recorded DSC curve. The contribution of the peak associated with the higher T_m value was similar to the α -skeletal actin content of the heart muscle cells ($12 \pm 8\%$ vs. 16–24%) [5,6], which corroborates the conclusion that the higher temperature peak is attributed to α -skeletal actin. The appearance of the two peaks can also be caused by the differences in the thermodynamic properties of the different α -actin isoforms. Considering the T_m values it is possible to compare the thermodynamic stability of the different forms of proteins as the higher T_m value can be attributed to a thermodynamically more stable protein conformation [37,38]. The advantage of exploring the differences between the melting temperatures is that their position is largely independent of the applied thermodynamic model describing the process of the denaturation [39]. When the filaments were polymerised from ADP-actin monomers the lower T_m observed for α -cardiac actin filaments indicated that the protein conformation was thermodynamically less stable compared to the α -skeletal actin filaments. Analyses that were carried out according to the approach of Sanchez-Ruiz and colleagues [27] showed that the activation energy (E_{act}) was ~18 % smaller for the α -cardiac actin filaments (254 kJ mol^{-1} ; Fig. 4) than for the α -skeletal actin filaments (311 kJ mol^{-1}) [29], when both of them were prepared from ADP-actin monomers. The larger energy requirement could be related to the more heat resistant protein matrix, which supports the conclusion that the α -cardiac actin filaments are thermodynamically less stable than α -skeletal actin filaments, when both are prepared from ADP-actin monomers.

The effect of nucleotide exchange on actin monomers

When the actin filaments were prepared from cardiac ATP-actin monomers only one peak was previously found with a T_m value of 62.17 °C [40], so the two isoforms within the cardiac actin pool were indistinguishable. This result can indicate that the conformational differences between the filaments of α -cardiac actin and α -skeletal actin are nucleotide dependent, so they can only be observed when the filaments are polymerised from ADP-actin monomers.

It is well known that α -skeletal actin filaments can generate a more stable and rigid conformation when they are prepared from ATP-actin monomers compared to the filaments prepared from ADP-actin monomers [9–12]. There was previously no report about such nucleotide dependence in the case of the α -cardiac actin isoform. The observation that the T_m value was lower for the actin filaments polymerised from α -cardiac ADP-actin monomers (54.3 ± 0.5 °C) than from cardiac ATP-actin monomers (62.17 °C) (Fig. 3)

showed a thermodynamically less stable protein matrix when the actin filaments were prepared from ADP containing cardiac actin monomers. The calculated activation energy for the α -cardiac actin filaments was $254 \pm 11 \text{ kJ mol}^{-1}$ (Fig. 4). This value is smaller than the activation energy determined previously for the α -cardiac ATP-actin filaments (332 kJ mol^{-1}) [35], and thus the difference between the calculated activation energy values gives an additional evidence (similarly to the case of α -skeletal actin) that the actin filaments prepared from α -cardiac ATP-actin monomers are thermodynamically more stable than the filaments prepared from α -cardiac ADP-actin monomers.

Conclusion

In this study the comparison of the α -actin isoforms revealed that the filaments of the α -skeletal actin were more resistant to heat denaturation than the α -cardiac filaments, when they were polymerised from ADP-actin monomers. This difference was not found in filaments polymerised from ATP-actin monomers, which indicates that the conformational differences between the isoforms were nucleotide dependent. The structure and the dynamics of proteins can be related to their biological functions [41-43]. The proper function of the cytoskeletal and sarcomeric proteins are essential for the normal work of the cardiomyocytes [44]. Previous observations revealed that the isoform content of cardiac muscle cells could vary under various circumstances [5]. In diseased heart increased α -skeletal/ α -cardiac actin ratio was observed, which indicates that the altered stoichiometry of the actin isoforms can be a response to the altered demands against the cardiac muscle [6,45].

Our present results can assist to understand the molecular details behind the organisation of the actin filament pool in the cardiac muscle cells. It is reasonable to assume that the properties of the actin pool are optimal under physiological conditions. In this case the filaments are polymerised from ATP-actin monomers and their conformation seems to be isoform independent. The intracellular ATP depletion is a serious condition when the concentration of ADP-actin monomers is elevated. The filaments polymerised under these conditions from ADP-actin monomers are more flexible than those polymerised from ATP-actin monomers, so their conformation differs from their optimal physiological state. Although this fact is true for both α -skeletal and α -cardiac actin isoforms the change in the α -skeletal actin filaments is smaller due to the nucleotide dependent nature of the differences between the actin isoforms. The α -skeletal actin filaments remain more rigid, i.e. their conformation is more similar to the physiological state of them.

The elevated level of the α -skeletal actin isoform [6,45] can contribute to the tuning of the conformational properties of the total actin population. We can conclude that the existence of two actin isoforms and their nucleotide dependent conformational differences are part of the tuning regulatory mechanism by which the cardiac muscle cells can maintain their biological function under pathological conditions.

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Fig. 1.
The ribbon model of the α -skeletal actin monomer from rabbit skeletal muscle. ATP (represented with balls in the middle) in the nucleotide-binding cleft is shown (Protein Data Bank: 1NWK).

Fig. 2.

The polymerisation curve of the ADP-actin from bovine cardiac tissue. The polymerisation was started by adjusting the MgCl_2 concentration to 2 mM and the KCl concentration to 100 mM in the solution containing $69 \mu\text{M}$ actin (5% pyrene labelled). The inset shows the SDS-PAGE analysis of the actin sample on right side of the reference.

Fig. 3. DSC results on actin filaments. The DSC recording of α -actin filaments prepared from ADP containing actin monomers of the bovine cardiac tissue (○). The heat-flow* is representing the normalized version of the original heat-flow values (heat-flow* = heat-flow/ μ mol). The black line is the result of smoothing of the original curve while the dotted lines are indicating the fitted Gaussians. The T_m values with the error bars are shown on the figure as well.

Fig. 4.

Analysis of the DSC results. The calculation of the activation energy for actin filaments prepared from ATP ($\square$) or ADP ($\circ$) containing actin monomers of the bovine cardiac tissue based on the method of Sanchez-Ruiz.