Journal of Thermal Analysis and Calorimetry, Vol. 84 (2006) 3, 619–623

THERMAL CHARACTERISATION OF ACTIN FILAMENTS PREPARED FROM ADP-ACTIN MONOMERS

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The thermodynamic properties of the ADP- and ATP-actin filaments were compared by the method of differential scanning calorimetry. The lower melting point for the ADP-F-actin filament (58.4 *vs*. 64.5°C for ATP-F-actin) indicated that compared to the ATP-actin filaments its structure was less resistant to heat denaturation. The detailed thermodynamic characterisation of the proteins was carried out by the analysis of the calorimetric enthalpy, the entropy and the free enthalpy changes. All of the determined parameters gave lower values to the ADP-actin filaments than to the ATP-actin filaments. The calculated values of the activation energy also demonstrated that compared to the ADP-F-actin the ATP-F-actin was thermodynamically more resistant to the denaturing effect of heat.

Based on all of this information we have concluded that the actin filament prepared from ADP containing magnesium saturated actin monomers at pH 8.0 is thermodynamically less stable than the ones obtained from ATP-actin monomers.

Keywords: *actin, denaturation, DSC, nucleotides, thermodynamics*

Introduction

Actin as one of the most important parts of the cytoskeletal protein network was first discovered by Straub in 1943 [1]. Naturally in cells it can be found as a monomer or filament in complex with a divalent cation and a nucleotide in its central cleft between its large and small domain [2]. Its importance is significantly underlined by its involvement in different cellular processes (intracellular traffic, endo- and exocytosis and cell movements) [3–5].

While in the beginning its complex with myosin (acto-myosin complex) was the most frequent target of investigations, recently its complex with the actin binding proteins has come into the forefront of scientific investigations [6–9].

DSC is a sensitive and informative method to investigate the basic physico-chemical properties of the proteins. It is frequently used to characterize the actin and its complexes with other proteins on the molecular and supramolecular level as well [10–24]. As the actin is very sensitive to the physico-chemical characteristics of the surroundings [25–28] it is very important to explore the details of its properties by modelling different cellular conditions (pH, ionic strength). Beside the importance of the bound divalent cation the attached nucleotide is another crucial factor that greatly affects the molecular properties of the actin. Its influence on the dynamic and/or structural properties of the actin monomers and filaments was characterised by different laboratories [29–38].

The crystallographic structure of the ADP-actin monomer was determined in 2001 by Otterbein and colleagues in a 1.54 Å resolution [39]. The nucleotide exchange can induce a conformational change in the actin monomers, due to which compared to the ATP-actin monomers the affinity of the ADP-actin monomer for DNase I is lower [40]. Although this finding was explained by the internal movement of the subdomain 2, a more complex movement around more than one axis has also been implicated [41]. The affinity of the ADP-actin monomers for other actin binding proteins can also be different than that of ATP-actin monomers [2].

Under physiological conditions the steady-state critical concentration (C_c) for ATP containing actin monomers is around 0.07 μM while it is 1.1 μM for ADP-actin [42]. The association rate constants (k_+) of the polymerization at the barbed-end measured by Pollard in 1986 were 11.6 $\mu M^{-1}\ s^{-1}$ for the ATP-actin and 3.8 $\mu M^{-1}\ s^{-1}$ for the ADP-actin while the dissociation rate constants (k_-) were 1.4 and 7.2 s^{-1}, respectively. At the pointed end the determined association rate constants were 1.3 $\mu M^{-1}\ s^{-1}$ for the ATP-actin filament and 0.16 $\mu M^{-1}\ s^{-1}$ for ADP-actin. The dissociation rate constant was 0.8 s^{-1} for the ATP-actin and 0.27 s^{-1} for the ADP-actin [43]. These

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1388–6150/$20.00

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data suggest that the filaments containing ADP-actin monomers tend to depolymerise more easily, while in the case of ATP-actin containing monomers the elongation of the protein will dominate.

The ADP-actin monomers are natural components of the actin pool within the cells as they are produced normally during the thread-milling of the actin filaments along with the release of an inorganic phosphate (P_i) [44, 45]. As the actin filaments are getting older the ratio of the ADP-actin protomers to the total actin concentration is getting higher, which can alter the basic properties of the actin filaments [46].

In our work we characterised the effect of the nucleotide exchange on the thermal stability of the actin filaments. We described the difference in the thermodynamic state variables characteristic for the ADP- and ATP-actin filaments as well.

Experimental

Materials and methods

Chemicals

KCl, $MgCl_2$, $CaCl_2$, MOPS, hexokinase, glucose, and EGTA were obtained from Sigma (St. Louis, USA). ATP, ADP and β-mercaptoethanol were purchased from Merck (Darmstadt, Germany). NaN_3 was purchased from Fluka (Switzerland).

Actin preparation

Skeletal actin was prepared from acetone powder [47, 48] obtained from the rabbit skeletal muscle as described by Spudich and Watt [48]. The actin was stored in a 2 mM MOPS buffer containing 0.2 mM ATP, 0.1 mM $CaCl_2$, 0.1 mM β-mercaptoethanol, and 0.005% NaN_3 at pH 8.0 at the end of the preparation (buffer A).

The preparation of the magnesium saturated ADP-actin filaments

- Ion exchange on actin monomers: the calcium ion bound to the actin monomers was changed for magnesium by adding EGTA and $MgCl_2$ to the sample to get the final concentrations of 0.2 and 0.1 mM, respectively. After a 10 min incubation at room temperature the actin monomers were polymerized by adjusting the final concentration of the $MgCl_2$ and KCl to 2 and 100 mM, respectively [49]. The concentration of the actin monomers were calculated from the absorption at 290 nm by using the extinction coefficient of 0.63 mg^{-1} mL cm [50].
- Preparation of the ADP-F-actin: the actin monomer bound ATP was changed for ADP by the method of Drewes and Faulstich [51]. During the nucleotide exchange the final concentration of the hexokinase, the glucose and the ADP were adjusted in the sample to 1.65, 0.5 mg mL^{-1} and 1 mM, respectively. The samples were incubated for 1 h at 4°C. The polymerization of the ADP-actin monomers was initiated with the adjustment of the final concentration of $MgCl_2$ and KCl to 2 and 100 mM, respectively. In the case of the ADP-F-actin the length of the polymerization was 12 h at room temperature (22°C) as compared to the ATP-actin monomers the polymerization rate of the ADP-actin monomers is slower. In the case of the ATP-actin monomers the incubation time was 1 h at room temperature (22°C) [34].

DSC measurements

DSC measurements were carried out with a Setaram Micro DSC-II calorimeter. The applied temperature range was 0–100°C and the heating rate was 0.3 K min^{-1}. The polymerization buffer with no actin content was the reference solution during the DSC measurements. The actin concentration was 69 μM (3 mg mL^{-1}) during the experiments. All the DSC data were analyzed with the Microcal Origin software (version 6.0).

The calorimetric enthalpy change (ΔH_{cal}) was calculated by integrating the area under the heat denaturation curves.

The entropy change was calculated by using the following equation:

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

where ΔS is the entropy change in kJ K^{-1} mol^{-1}, ΔH_{cal} is the calorimetric enthalpy change in kJ mol^{-1} and the T_m is the peak transition point in K.

The free enthalpy change was calculated by using the following formula:

$$\Delta G=\Delta H_{cal}-T\Delta S \quad (2)$$

where ΔG is the free enthalpy change measured in kJ K^{-1} mol^{-1} at room temperature (20°C) point *T*.

The calculation of the activation energy was done by the method of Sanchez-Ruiz *et al*. [52]. Based on their work the following equation was applied during the analysis of the DSC data:

$$\ln\left[\ln\left(\frac{\Delta H_{cal}}{\Delta H_{cal}-\Delta H}\right)\right]=\frac{E_a}{R}\left(\frac{1}{T_m}-\frac{1}{T}\right) \quad (3)$$

where ΔH_{cal} is the total calorimetric enthalpy change, ΔH is the actual enthalpy change at a temperature point of *T*, E_a is the activation energy, *R* is the universal gas constant and T_m is the peak transition point. The slope and the intercept value of the fitted line on the plot of $\ln[\ln(\Delta H_{cal}/(\Delta H_{cal}-\Delta H))]$ *vs*. $1/T$

can inform us about the activation energy that is needed to initiate the thermal denaturation of the investigated protein. A higher value of the activation energy may indicate a thermodynamically more stable protein matrix [53].

Results and discussion

The detailed investigation of the actin filaments created from ADP and ATP bound magnesium saturated actin monomers were investigated with the method of differential scanning calorimetry at pH 8.0.

The analysis of the heat absorption curves revealed that the melting point for ADP-F-actin was 58.4°C compared to the value of 64.5°C for the actin filaments polymerised from ATP-actin monomers (Fig. 1; Table 1). This 6.1°C difference indicates that compared to the ATP saturated actin filaments the ADP-F-actin is less resistant to the heat denaturation. A detailed investigation of the thermodynamic state variables was done so that this conclusion could be further supported.

The calorimetric enthalpy change was determined by integrating the area under the heat denaturation curves belonging to the actin filaments polymerised from monomers with different nucleotide bound. The results of these calculations were 540 kJ mol^{-1} for the ADP-F-actin and 802 kJ mol^{-1} for the ATP saturated actin filament (Table 1).

The calculated entropy change reflected the same tendency in the difference between the two proteins as its value was 1630 kJ K^{-1} mol^{-1} for ADP-F-actin and 2375 kJ K^{-1} mol^{-1} for the ATP-F-actin (Table 1).

The free enthalpy change was calculated at 293 K by using the data of calorimetric enthalpy change and the value of the entropy change in Eq. (2). The values were 62 kJ mol^{-1} for the ADP-F-actin and 106 kJ mol^{-1} for the ATP-F-actin (Table 1).

Table 1 The thermodynamic properties of the ADP- and ATP-F-actin at pH 8.0 in the presence of magnesium ions. The free enthalpy change (ΔG) was calculated at 293 K

	ADP-F-action	ATP-F-actin
T_m/°C	58.4	64.5
ΔH/kJ mol^{-1}	540	802
ΔS/J K^{-1} mol^{-1}	1630	2375
ΔG/kJ mol^{-1}	62	106
E_a/kJ mol^{-1}	311	338

All these thermodynamic state variables showed the same qualitative difference as their value was consequently lower for the ADP-F-actin compared to the actin filaments formed from ATP-actin monomers.

The activation energy was also calculated with the Eq. (3) for the actin filaments by the method of Sanchez-Ruiz [52]. The calculated values were 311 kJ mol^{-1} for ADP-actin and 338 kJ mol^{-1} for ATP-actin (Fig. 2, Table 1). These values were in correlation with the data described above as their value was smaller for the ADP-F-actin than for ATP-F-actin. The difference in the activation energy suggested that compared to the ATP-F-actin the structure of the ADP-F-actin was less resistant to the denaturing effect of heat as the difference between the melting points has indicated previously.

All the measured and calculated thermodynamic state variables show that there is a significant difference between the ADP- and ATP-F-actin. Based on the values of the thermodynamic parameters determined

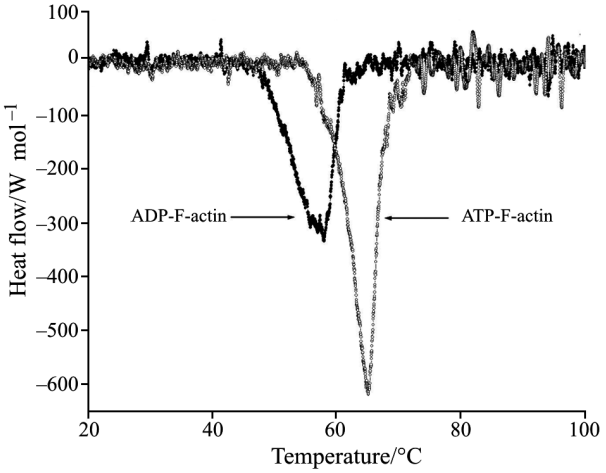

Fig. 1 The thermal denaturation curve of the filaments polymerised from magnesium bound ADP (dotted line with filled circles) or ATP (dotted line with empty circles) containing actin monomers at pH 8.0

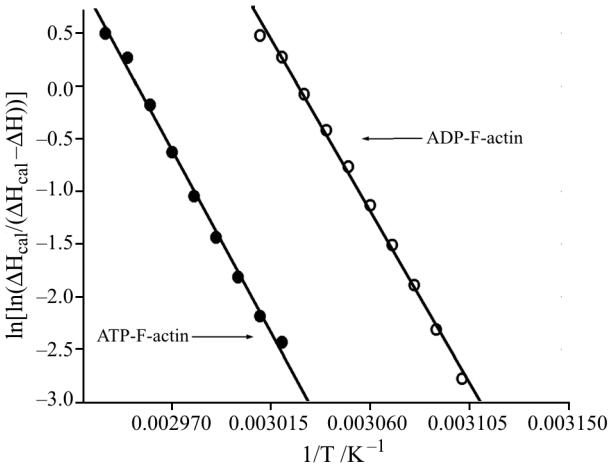

Fig. 2 The plots used for the calculation of the activation energy by the method of Sanchez-Ruiz [52] for the filaments polymerised from magnesium bound ATP (line with filled circles) or ADP (line with empty circles) bound actin monomers at pH 8.0

from the heat denaturation curves and the calculated values of the activation energies as well (Fig. 1; Table 1) this difference can be interpreted that compared to the ATP-F-actin the ADP-F-actin has a thermodynamically less stable structure.

Conclusions

The thermodynamic characterisation of the actin filaments prepared from ADP bound magnesium-actin monomers has revealed that these filaments are thermodynamically less stable than the ATP-actin filaments under the applied experimental conditions.

This finding is in good agreement with previous works where in the case of ADP-actin filaments an increased inter-monomer flexibility was described with a fluorescence spectroscopic method [34].

The actin filaments polymerised from either ADP- or ATP-actin monomers mainly consist of ADP-actin protomers. Our work – in agreement with previous studies – has provided further evidence that the actin filaments have conformational memory, i.e. the conformation of the filaments can reflect the previous structural states of the actin monomers they were generated from, and also the various environmental factors that affected the monomer state and the process of polymerisation.

The accumulated data could suggest that the stability of the actin filament may play a role in the function of the actin cytoskeleton. For example, the altered stability of the protein matrix in the case of the ADP-actin filaments, and thus their different conformation may explain their altered affinity to the actin binding proteins as well.

The faster depolymerisation of the filaments composed from ADP-actin monomers may be involved in the equilibrium between the actin monomers and polymers as well. Considering that the ATP-actin – ADP-actin monomer ratio can change when cells are aging the nucleotide dependence of the structure of actin filaments may serve as part of the general biological clock mechanism within cells.

Acknowledgements

This work was supported by the Hungarian Academy of Sciences. The Setaram Micro DSC-II used in the experiments was purchased with a grant (CO-272) provided by the National Research Foundation.

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Received: November 10, 2005
Accepted: December 19, 2005
OnlineFirst: March 20, 2006

DOI: 10.1007/s10973-005-7442-9