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The effect of *jasplakinolide* on the thermodynamic properties of ADP.BeF$_x$ bound actin filaments

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Abstract

The effect of BeF$_x$ and a natural toxin (*jasplakinolide*) was examined on the thermal stability of actin filaments by using differential scanning calorimetry. The phosphate analogue beryllium fluoride shifted the melting temperature of actin filaments (67.4 °C) to 83.7 °C indicating that the filaments were thermodynamically more stable in their complex with ADP.BeF$_x$. A similar tendency was observed when the *jasplakinolide* was used in the absence of BeF$_x$. When both the ADP.BeF$_x$ and the *jasplakinolide* bound to the actin filaments their collective effect was similar to that observed with ADP.BeF$_x$ or *jasplakinolide* alone. These results suggested that ADP.BeF$_x$ and *jasplakinolide* probably stabilize the actin filaments by similar molecular mechanisms.

Keywords: α-Skeletal actin; ADP.BeF$_x$; DSC; *Jasplakinolide*; Thermal stability; Thermodynamics

1. Introduction

The cytoskeleton is a three-dimensional network of proteins which is responsible for many functions in the organisation of the structure and dynamics of living cells [1–4]. The cytoskeleton is built up from three different filament systems, the microtubules, the intermediate filaments and the microfilaments. The microfilament system is assembled from the actin and actin-binding proteins. Actin is a 42 kDa globular protein that can be found in monomer (G-actin) or polymer (F-actin) form in cells. The actin monomer binds an ATP and a divalent magnesium ion in its central part under physiological conditions [5]. The ATP is hydrolyzed during the polymerization of the actin. The products of this hydrolization are ADP and inorganic phosphate (P$_i$). The phosphate dissociates from the actin filaments while the ADP remains bound to the protein. The intermediate states of the hydrolization process can be modelled with the help of different specific chemicals. Beryllium fluoride (BeF$_x$) is a widely used phosphate analogue and together with ADP is able to mimic the F-ADP.P$_i$ intermediate state of the ATPase cycle. The BeF$_x$ has a similar effect on the actin filaments as the P$_i$ and can bind to the actin filaments with relatively high affinity ($K_D = 2\ \mu M$) [6–8]. The association and dissociation rate constants for BeF$_x$ binding to and dissociating from ADP–actin are $4\ M^{-1}\ s^{-1}$ and $8 \times 10^{-6}\ s^{-1}$, respectively [9]. Based on these facts it was concluded that the beryllium fluoride could be a reliable probe to test the conformation of the ADP.P$_i$–actin filaments [9].

Jasplakinolide is a cyclic peptide isolated from a marine sponge (*Jaspis johnstoni*) that is able to bind and stabilize the filamentous actin *in vitro*. This actin-stabilizing toxin was effectively used previously to study the conformational and dynamic properties of actin filaments [10,11]. *Jasplakinolide* binds the actin filament with an affinity of approximately 15 nM and competes with phalloidin for the binding-sites on actin [12–14]. This toxin can decrease the amount of sequestered actin monomers by lowering the critical concentration of actin and enhances the polymerization of the filaments by accelerating the speed of the nucleation step [13]. Differential scanning calorimetry (DSC) studies revealed that *jasplakinolide* is able to stabilize actin filaments at sub-stoichiometric concentration as well [10,11].

Previous experiments showed that DSC is an effective method to study the thermal properties of actin [15–24]. The aim of our work was to investigate the effect of *jasplakinolide* on the thermodynamic properties of the ADP.BeF$_x$ bound actin filaments with differential scanning calorimetry (DSC). The calorimetric results obtained for the thermal stability of actin filaments

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in the presence of ADP.BeF_x and/or *jasplakinolide* provided the opportunity to analyse the mechanisms through which these compounds affect the structure and dynamics of actin filaments.

We found that the melting temperature (T_m) of actin filaments has changed from 67.4 °C to 83.7 °C after the binding of BeF_x to the actin filaments (ADP.BeF_x state). A similar shift in the melting temperature of F-actin was previously observed by Nikolaeva et al. [25]. In agreement with previous results the presence of *jasplakinolide* has shifted the T_m to 87.3 °C [10]. We also found that when the *jasplakinolide* bound to the ADP.BeF_x saturated actin filaments the melting temperature shifted to 85.6 °C, i.e. to a value similar to what was observed with either ADP.BeF_x or *jasplakinolide* separately. From these results we concluded that the ADP.BeF_x and *jasplakinolide* probably stabilizes the actin filaments by similar molecular mechanisms.

2. Materials and methods

2.1. Chemicals

KCl, $MgCl_2$, $CaCl_2$, Mops and NaF were purchased from Sigma (St. Louis, USA). ATP and β-mercaptoethanol were obtained from MERCK (Darmstadt, Germany). NaN_3 and $BeSO_4$ were purchased from FLUKA (Switzerland). The *jasplakinolide* was purchased from Molecular probes (Invitrogen).

2.2. Sample preparation

Skeletal actin was prepared from acetone powder of rabbit psoas muscle [26,27]. 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 mg^{-1} ml cm at 290 nm [28].

The actin bound calcium was changed for magnesium by incubating the samples for 5 min with 0.2 mM EGTA and 0.1 mM $MgCl_2$ at room temperature [5]. The actin monomers were polymerized by the addition of 2 mM $MgCl_2$ and 100 mM KCl.

ADP.BeF_x–actin filaments were prepared from the ADP containing actin filaments by the simultaneous addition of 10 mM NaF and 3 mM $BeSO_4$ to the actin solution. The samples were incubated for 2 h at room temperature followed by an overnight incubation at 4 °C. To create *jasplakinolide*-bound ADP.BeF_x–actin filaments *jasplakinolide* was added to the actin after the 2-h long incubation period at room temperature. To create *jasplakinolide*-bound actin filaments *jasplakinolide* was added to the actin at the same time as the $MgCl_2$ and KCl and the samples were incubated overnight at 4 °C [10,11]. These preparation strategies assured that the equilibrium between the actin filaments, the drug and the nucleotide analogues was established before the measurements.

2.3. DSC measurements and theoretical considerations

The calorimetric measurements were performed with a SETARAM Micro DSC-II Calorimeter. The actin concentration was 3 mg/ml (∼69 μM) during the measurements. When applied, the *jasplakinolide* was added in a 1:1 *jasplakinolide*:actin protomer concentration ratio. The DSC measurements were done in the temperature range of 0–100 °C and the heating rate was 0.3 K/min.

3. Results and discussion

The calorimetric experiments were completed by using 69 μM F-actin. The critical concentration of actin is low under the applied experimental conditions (0.04–0.1 μM) [29] compared to the applied actin concentration so the contribution of the actin in its monomeric form to the heat denaturation curve was hardly detectable. During the DSC measurements the sample and the appropriate reference solution was heated in the range of 0–100 °C under isobaric conditions. The difference between the energy uptake of the sample and the reference cell was recorded and plotted as a function of temperature.

The thermodynamic curves for actin filaments showed an endothermic phase transition in all cases (Figs. 1–3). The transition curves can be characterized by determining the melting temperature (T_m) defined as the temperature where the curves reach their minimum value. Comparing the different T_m values it is possible to characterize the thermodynamic stability of proteins under different conditions [24,30]. A higher T_m value is typically correlated with a thermodynamically more stable protein structure [30,31]. The significant advantage of exploring the differences between the melting temperatures is that the determined position of them is largely unrelated to the applied thermodynamic model describing the denaturation process [32].

In the absence of BeF_x and *jasplakinolide* the T_m was 67.4 °C for the actin filaments (Fig. 1). When the sample was prepared in the presence of BeF_x or *jasplakinolide* the peak of the phase transition shifted to higher temperatures (Figs. 1 and 2).

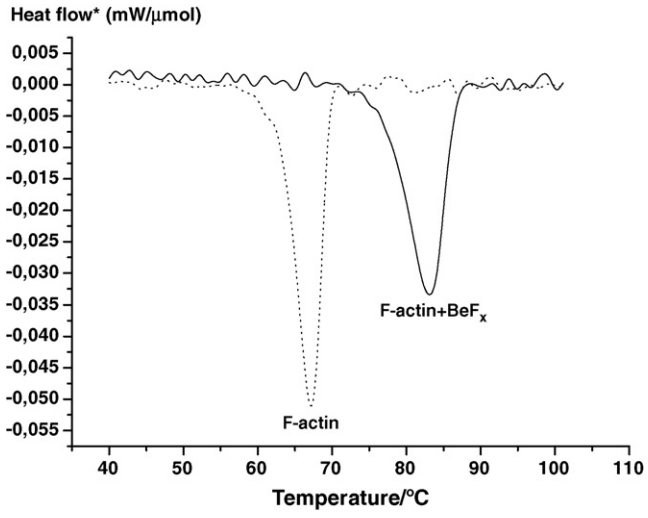

Fig. 1. The DSC curves were recorded in the presence (solid) and absence (dot) of BeF_x. The heat-flow* (mW/μmol) is the form of the heat-flow (mW) normalized with the amount of the applied protein (μmol).

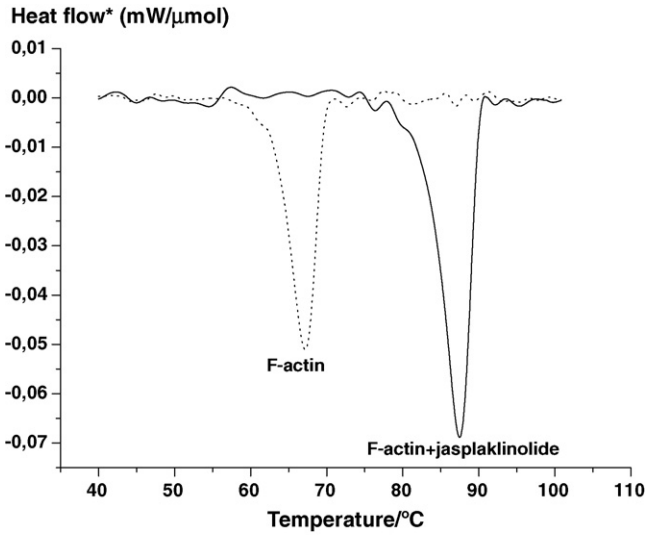

Fig. 2. The recorded DSC thermograms in the presence (solid) and absence (dot) of *jasplakinolide*. The heat-flow* (mW/μmol) is designated the normalized form of the heat-flow (mW) for the amount of the applied protein (μmol).

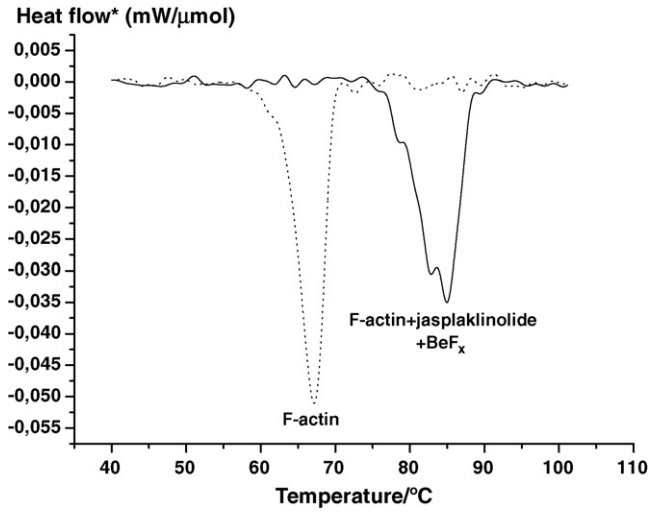

Fig. 3. The combined effect of *jasplakinolide* and BeF_x on the actin filament. The DSC curves were recorded in the presence (solid) and absence (dot) of *jasplakinolide* and BeF_x. The heat-flow* (mW/μmol) is representing the normalized form of the heat-flow (mW) for the amount of the applied protein (μmol).

It was shown previously that BeF_x could replace the P_i of the ADP.P_i complex within the nucleotide-binding region of actin (Fig. 4). When the BeF_x was applied as a P_i analogue the melting temperature shifted from 67.4 °C to 83.7 °C (Fig. 1), in agreement with earlier studies [25]. This result indicated that the beryllium fluoride was able to affect the conformation of the actin filaments by stabilizing the structure of the filaments.

As it was previously reported, *jasplakinolide* is able to bind to the actin filaments with high affinity (K_D = 15 nM) [12]. The time

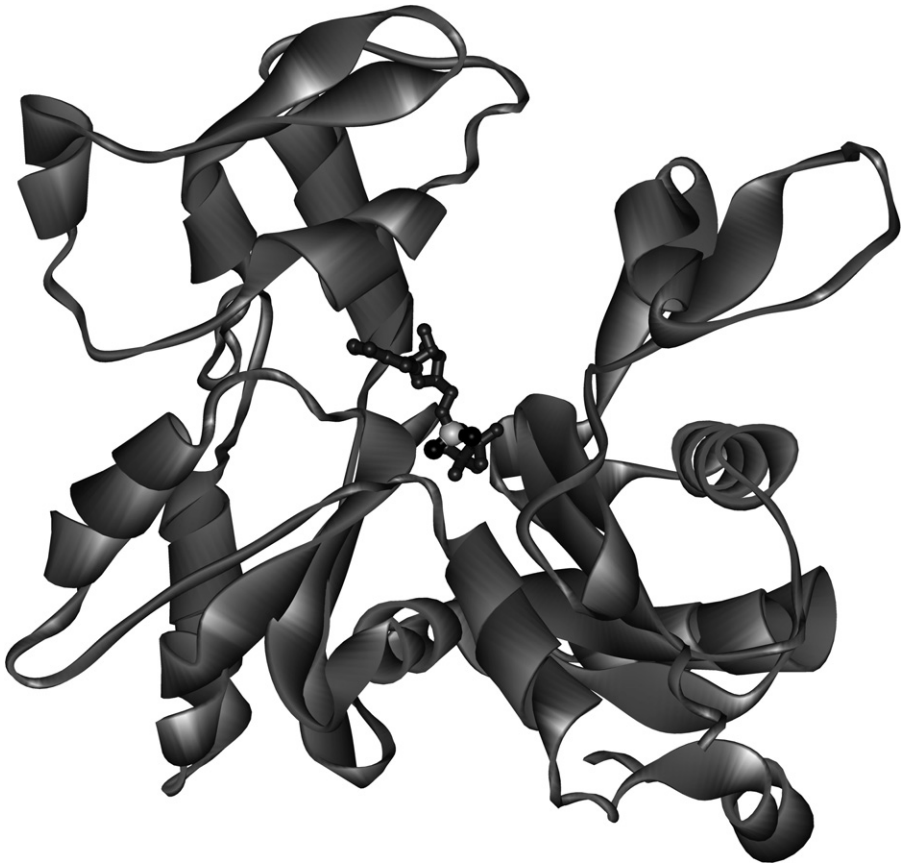

Fig. 4. Schematic picture of the actin monomer (Protein Data Bank: 1NWK) binding the P_i analogue ADP.BeF_x (Protein Data Bank: 4UKD) in the nucleotide-binding cleft.

required to achieve the *jasplakinolide* effect on the F-actin is not longer than the time required to mix the constituents of the sample [13]. Adding *jasplakinolide* to the actin filaments increased the T_m in our experiments to 87.3 °C. This observation was in agreement with our previous results [10,11] and indicated that the toxin could effectively stabilize the actin filaments (Fig. 2).

These results demonstrated that both compounds used during the DSC experiments possess the ability of stabilizing the actin filaments. The melting temperature of the F-actin increased approximately to the same extent when BeF_x and *jasplakinolide* was used separately (Figs. 1 and 2). These observations suggested that the beryllium fluoride and the *jasplakinolide* affected the heat stability of the actin filaments through similar molecular mechanisms.

If these two actin-binding compounds can stabilize the actin filaments by the same mechanism, then none of the two should be able to further stabilize the filaments once the other one has reached its maximal effect. To test this prediction the calorimetric experiments were repeated when both *jasplakinolide* and BeF_x were present in the sample (Fig. 3). The thermogram from these experiments showed that the melting temperature of the filaments in complex with ADP.BeF_x and *jasplakinolide* was 85.6 °C (Fig. 3), which is nearly identical with the values obtained in the presence of the beryllium fluoride or the toxin alone. This observation corroborated our previous conclusions that ADP.BeF_x and *jasplakinolide* probably altered the thermal stability of the F-actin through similar molecular mechanisms. One possible explanation behind the similar molecular background can be a competition between the BeF_x and the *jasplakinolide* for the same binding site as it was previously described in details for cofilin and BeF_x by Muhlrad et al. [33].

4. Conclusion

Differential scanning calorimetry is a widely used method to study the phase transitions and conformational changes within biological macromolecules [34]. In our experiments we used beryllium fluoride as a phosphate analogue to simulate the ADP.P_i intermediate step of the ATPase cycle of actin. We applied *jasplakinolide* as well to examine its impact on the thermal stability on the actin filaments. The heat-flow diagrams were recorded from 0 °C to 100 °C in the presence of BeF_x and/or *jasplakinolide*. The melting temperatures were nearly identical when the heat denaturation curves were recorded by using the different compounds separately. These findings suggested that the beryllium fluoride and the toxin affected the stability of the actin filaments through similar molecular mechanisms. To test this assumption we measured the thermodynamic stability of the filamentous actin when both the phosphate analogue and the drug were present in the sample solution. The melting temperature in this case was approximately the same as it was with either ADP.BeF_x or *jasplakinolide*. This observation corroborated our presumption that the *jasplakinolide* affects the stability of the actin filaments through the same molecular mechanism as the beryllium fluoride.

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