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Thermodynamic analysis of K^+- and Mg^{2+}-induced polymerization of actin at the temperature of 298.15 K[1]

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Abstract

Polymerization of actin was investigated by a microcalorimetric method at 298.15 K. The calorimetric curves showed that polymerization of actin in the presence of Mg^{2+} at the concentration range 1.00–6.00 mM was an exothermic process. There was only one exothermic peak in the thermal curves under each condition. The average molar enthalpy change of actin polymerization was -0.703 kJ mol^{-1}. It was still exothermic in the presence of K^+ when the concentration was in the range 25.0–200.0 mM. The average molar enthalpy change of polymerization was -3.03 kJ mol^{-1}. However, the calorimetric curves contained only one exothermic peak when the K^+ concentration was lower, in the range 25.0–75.0 mM; there were two peaks, first exothermic and second endothermic, in the calorimetric curves as the K^+ concentration was higher, in the range 100.0–200.0 mM. The result not only indicated that calorimetric curves of actin polymerization changed with different concentration of K^+ but also showed that there were some differences between K^+- and Mg^{2+}-induced polymerization of actin in the presence of 0.1 mM $CaCl_2$ and 0.2 mM ATP. The ΔG^0 of actin polymerization in each condition was also assayed at 298.15 K and ΔS^0 in the corresponding condition were consequently evaluated from the energy–entropy relation at constant temperature and pressure. © 1998 Published by Elsevier Science B.V.

Keywords: Microcalorimetry; Muscle actin; Polymerization

1. Introduction

Actin, one of the major proteins involved in muscle contraction, is also widely present in nonmuscle cells in which it is a component of cytoskeleton and a motile apparatus for motile activities such as organelle movement, phagocytosis and chromosome movement. Actin monomer (G-actin) can be polymerized into actin filament (F-actin) which functions in living cells. The transformation from G-actin to F-actin has received much attention and has been investigated in detail. The addition of salts such as potassium and magnesium to a solution of G-actin can induce the polymerization in vitro. It is now clear that actin polymerization proceeds in at least four reversible steps: activation; nucleation; elongation; and annealing [1]. The methods that have been used to characterize actin polymerization, e.g. differential spectrum, flow birefringence, fluorescence spectrum, light scattering and electron microscopy, are all based on the change in matter [2]. But the energetics of actin polymerization are not very clear. Thermodynamic parameters for actin polymerization have been derived from van't Hoff plotting of data taken at different temperatures [3–6]. However, they cannot show different kinds of processes of polymerization. Besides,

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use of the van't Hoff relation in determinations of enthalpy change of actin polymerization is a question at issue [7,8]. Holtzer [8] concluded that the only valid way of assessing micellar enthalpies is direct calorimetry. Up to now, the heat produced in actin polymerization has not been measured by calorimetry. An improved Calvet MS-80 microcalorimeter (Setaram, France) was used to measure the heat effect of actin polymerization in the presence of K^+ and Mg^{2+} in various concentrations in our study. This paper provides some new information on polymerization of muscle actin with emphasis on analysis of enthalpy changes. The results also show some thermodynamic properties of protein self-assembly through weak bonding force.

2. Experimental

2.1. *Preparation of actin*

Rabbit skeletal muscle actin was purified by the method of Spudich and Watt [9]. It was further purified by chromatography through a 2×90 cm column of Sephacryl S-200 (Pharmacia). The concentration of actin was spectrophotometrically determined at 290 nm, using extinction coefficient of $E^{1\%}(290)=6.20\ \mathrm{cm}^{-1}$. Actin was dissolved in the buffer containing 2.0 mM Tris, 0.05% NaN_3, 0.5 mM DTT, 0.2 mM ATP, 0.1 mM $CaCl_2$ with pH value of 8.0.

2.2. *Measurement of calorimetric curves and enthalpy changes of polymerization*

The calorimetric curves and the enthalpy changes of polymerization of actin were recorded with a Calvet MS-80 standard microcalorimeter at 298.15 K. The improved reaction cell was used in experiments [10]. The minimum heat of the amplifier was 2.745×10^{-5} J at 10 μV. The heat as less as 0.001 J can be measured with a precision of 6%. A 5.0 ml of actin (0.5 mg ml^{-1}) was added into both the stainless reaction cell and the control cell. An amount of 0.5 ml K^+ or Mg^{2+} solution was placed in the small glass cup in the reaction cell and 0.5 ml of H_2O was placed in the small glass cup in the control cell. The reaction was started by turning the body of microcalorimeter up and down five times (5×180°) or more until the machine came to thermal equilibrium. Each experiment was repeated at least twice.

2.3. *Determination of concentration of monomer actin at equilibrium of polymerization*

An amount of 5.0 ml G-actin (0.5 mg ml^{-1}) was mixed with 0.5 ml of KCl or $MgCl_2$ solution. The mixed solution was kept in a water bath for 30 min at 298.15 K first, and then spun at 150,000 g for 1 h. The actin in the supernatant layer was regarded as monomer actin. The difference between the initial and final concentration of actin in the supernatant layer was taken as the amount of actin polymerized in each condition.

3. Results

3.1. *Activation of K^+ on actin below critical concentration of polymerization*

No polymers are formed when actin concentration is below the critical concentration, but actin is activated by some metallic ions, such as K^+, Mg^{2+} etc., and becomes monomer actin (a new form) showing a negative absorption peak at 212–215 nm at 298.15 K [1,4,11,12]. The critical concentration of actin is 30 μg ml^{-1} in the presence of 0.1 mM K^+ at 298.15 K. We measured the heat effect of formation of K^+ monomer actin and found that it was an exothermic process (Fig. 1).

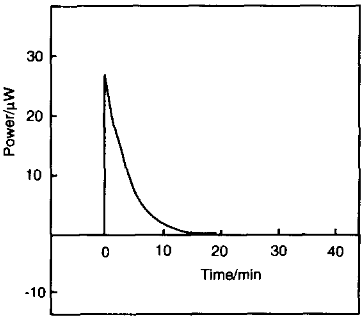

Fig. 1. Measured curve of activation of actin below critical concentration (30 μg ml^{-1}) in the presence of 100.0 mM K^+ at 298.15 K.

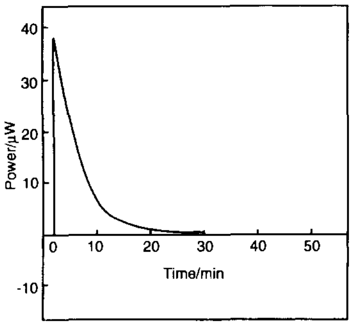

Fig. 2. Measured curve of actin polymerization in the presence of 50.0 mM K^+ at 298.15 K. The measured curves in the presence of 25.0 and 75.0 mM K+ are very similar to the above figure.

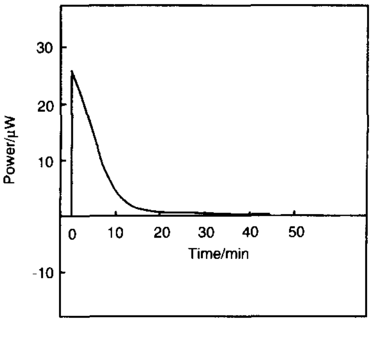

Fig. 4. Measured curve of actin polymerization in the presence of 3.00 mM Mg^{2+} at 298.15 K. The measured curves in the presence of 1.00, 2.00 and 6.00 mM Mg^{2+} are very similar to the above figure.

3.2. *Calorimetric curves of K^+- and Mg^{2+}-induced actin polymerization*

K^+-induced polymerization of actin has been well characterized by many researchers [2,11–14]. We measured the calorimetric curves of actin polymerization in the presence of K^+ from 25.0 to 200.0 mM. When the concentration of K^+ was from 25.0 to 75.0 mM, there was only one exothermic peak (Fig. 2). As the concentration of K^+ increases to 100.0, 150.0 and 200.0 mM, the calorimetric curves change. There were two peaks in the calorimetric curves, the first exothermic and the second endothermic (Fig. 3).

The mechanism of Mg^{2+}-induced actin polymerization was studied at length [15–17]. Mg^{2+} can cause conformational change in actin molecules before linking each monomer to form polymerized actin. Nucleation and elongation are involved in polymerization. However, there was only one exotherm in the thermal curves of Mg^{2+}-induced polymerization when the concentration of Mg^{2+} was in the range 1.00–6.00 mM (Fig. 4). Kinosian et al. [6] determined by the van't Hoff relation that Mg^{2+}-induced polymerization was endothermic. Our results showed that Mg^{2+}-induced polymerization was exothermic.

When K^+ and Mg^{2+} were added to actin at concentration of 100.0 and 2.00 mM, respectively, the calorimetric curve was similar to that in presence of 100.0, 150.0 and 200.0 mM K^+, in which there were two processes, the first exothermic and the second endothermic (Fig. 5).

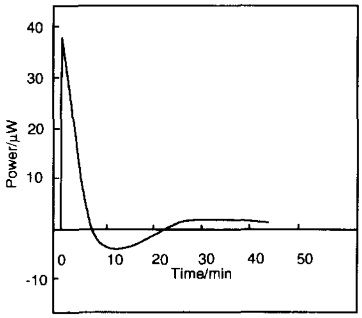

Fig. 3. Measured curve of actin polymerization in the presence of 150.0 mM K^+ at 298.15 K. The measured curves in the presence of 100.0 and 200.0 mM K^+ are very similar to the above figure.

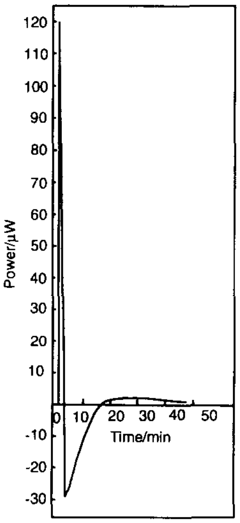

Fig. 5. Measured curve of actin polymerization in the presence of 100.0 mM K^+ and 2.00 mM Mg^{2+} at 298.15 K.

Table 1
Thermodynamic parameters of actin polymerizatiom in the presence of $MgCl_2$ at 298.15 K

Thermodynamic parameters	Concentration of $MgCl_2$ in mmol l^{-1}				Average value of each parameter
	1.00	2.00	3.00	6.00	
ΔG^0 (kJ mol^{-1})	−28.9	−29.5	−29.8	−30.1	−29.6
ΔH^0 (kJ mol^{-1})	−0.747	−0.659	−0.754	−0.652	−0.703
ΔS^0 (J mol^{-1} K^{-1})	94.3	96.9	97.3	99.0	96.9

3.3. *Thermodynamic parameters of actin polymerization*

The thermodynamic parameters of the polymerization of muscle actin at 298.15 K are listed in Tables 1 and 2.

The enthalpy change of actin polymerization ΔH^0 was measured by microcalorimetry directly. Table 1 shows that the ΔH^0 of Mg^{2+}-induced polymerization is −0.703 kJ mol^{-1} and is independent of the concentration of Mg^{2+} in this range of concentration. But the values of ΔH^0 determined by us with calorimetry are appreciably different from those determined by van't Hoff relation [5,6]. The latter is in positive values showing that polymerization is an endothermic process. In addition, the value derived by van't Hoff relation is much higher than our results. We can see in Table 2 that the average value of ΔH^0 of K^+-induced polymerization is −3.30 kJ mol^{-1} and is also independent of the concentration of K^+ in this range of concentration. The values of ΔH^0 listed in Table 2 are also appreciably different from those evaluated by van't Hoff relation [4–6]. Attention should be paid to the values of ΔH^0 that there are two peaks in the calorimetric curves in the presence of 100.0, 150.0 and 200.0 mM K^+ and that they are the sum of two kinds of heat effect, i.e.

$$\Delta H^0 = \Delta H^0\ (\text{exothermic}) + \Delta H^0\ (\text{endothermic}) \tag{1}$$

The polymerization of actin can be described by the following reaction

$$A_{(n)} + A_1 = A_{(n+1)} \tag{2}$$

The equilibrium constant is the reciprocal of the concentration of monomer ($[A]_{eq}$) which was determined by ultracentrifuge assaying, for $[A_{(n)}] \approx [A_{(n+1)}]$ when polymerization is at equilibrium. The value of ΔG^0 is derived from the equation of

$$\Delta G^0 = \mathrm{RT} \ln[\mathrm{A}]_{eq} \tag{3}$$

The value of ΔS^0 is calculated from the energy–entropy relation for changes at the temperature 298.15 K and normal atmosphere.

$$\Delta S^0 = (\Delta H^0 - \Delta G^0)/T \tag{4}$$

4. Discussion

Although there are many papers dealing with polymerization of actin, the reports on thermodynamics of the polymerization are quite limited. Our study is the first attempt to investigate the thermodynamics of actin polymerization with microcalorimetry. The results show that thermodynamics of actin polymerization are complicated. The calorimetric curves of actin polymerization presented in this paper included several interactions, which are interaction between actin and salt causing conformational change within

Table 2
Thermodynamic parameters of actin polymerizatiom in the presence of KCl at 298.15 K

Thermodynamic parameters	Concentration of KCl in mmol l^{-1}						Average value of each parameter
	25.0	50.0	75.0	100.0	150.0	200.0	
ΔG^0 (kJ mol^{-1})	−29.1	−29.5	−29.8	−30.1	29.8	−29.6	−29.7
ΔH^0 (kJ mol^{-1})	−3.11	−3.64	−3.11	−3.29	3.21	−3.43	−3.30
ΔS^0 (J mol^{-1} K^{-1})	87.2	86.9	89.5	89.9	89.2	88.4	88.5

actin molecules binding of ATP to actin, hydrolysis of ATP during polymerization and interaction among active actin monomers to form actin filaments through weak bonding force. Although the polymerization of actin has already been characterized by other methods, it is very hard to say which interaction is the strongest in contributing to the thermodynamics of actin polymerization. So the enthalpy changes reported in this paper are apparent values which are the sum of all interactions.

The heat effect of Mg^{2+}-induced actin polymerization is as small as that of K^+-induced polymerization in the presence of 0.2 mM $CaCl_2$ and 0.1 mM ATP. Our results indicated that the thermodynamics of K^+-induced actin polymerization was more complicated than that of Mg^{2+}-induced actin polymerization. In other words, the thermodynamics of K^+-induced actin polymerization is appreciably different from that of Mg^{2+}-induced actin polymerization.

van't Hoff plot was used by several researchers of actin to calculate the enthalpy change of actin polymerization. We agree with viewpoints of Weir and Frederiksen [7] that van't Hoff relation cannot be used for determining ΔH^0 of actin polymerization. Actin polymerization can be simply described by reaction (1). If critical concentration of polymerization (ccp) at two different temperatures is determined, and ΔG^0=RT ln ccp [4–6] is used to calculate free-energy change of two different reactions, in one case:

$$A_{(n)}(T_1) + A_1(T_1) = A_{(n+1)}(T_1) \qquad (5)$$

in the other case:

$$A_{(n)}(T_2) + A_1(T_2) = A_{(n+1)}(T_2) \qquad (6)$$

the two reactions are only the same if N (the number of monomers) is temperature independent. Certainly, $(\mathrm{d}N/\mathrm{d}T)_\mathrm{p}$ for actin polymerization cannot be zero. For example, the value of $(\mathrm{d}N/\mathrm{d}T)_\mathrm{p}$ is 0.4 for formation of micelle [8]. Thus, also, in Holtzer and Holtzer [8] equation

$$RT^2[\mathrm{d}\ln(\mathrm{ccp})/\mathrm{d}T]_\mathrm{p} = -\Delta H_N^\infty + T\{[\mathrm{d}G_{N+1}^\infty - \mathrm{d}G_N^\infty)/\mathrm{d}N]_{TP}\}(\mathrm{d}N/\mathrm{d}T)_\mathrm{p} \qquad (7)$$

the second term on the right cannot be zero. Therefore, it is clear that van't Hoff relation fails to determine the ΔH^0 of actin polymerization by measuring critical concentrations at different temperatures. In addition to this, ΔH^0 of actin polymerization may be temperature dependent. This can also be determined by means of microcalorimetry. The experimental results of ΔH^0 of actin polymerization measured by us in the range 293.15–310.15 K and pH values from 6.0 to 10.0 will be reported in another paper in the near future.

As to the calculation of free-energy change, Gordon et al. [4], Selden et al. [5] and Kinosian et al. [6] regard the reciprocal of critical concentration of polymerization (ccp) determined by viscometry as the reaction constant and thus calculated ΔG^0 by the equation ΔG^0=RT ln ccp [4–6]. Weir and Frederiksen [7] determined the concentration of monomer actin ($[A]_{eq}$) by ultracentrifugation after polymerization in the given condition overnight and calculated ΔG^0 according to the equation of $\Delta G^0=\ln[A]_{eq}$. In this experiment, we determined the concentration of monomer action in polymerized actin solution by ultracentrifugation according to the method of Weir and Frederiksen [7] after actin had been polymerized for 30 min. Certainly, some actin in supernatants could polymerize into filaments if kept in this solution for a longer time. Considering polymerization being near maximum extent and that its reaction equilibrium was reached after polymerization had undergone for 30 min, we determined the concentration of monomer actin at this point. So the time for both enthalpy and free-energy changes was not throughout, which was just like the case of common chemical reaction. The ΔG^0 values of Mg^{2+}-induced and K^+-induced polymerization of actin determined by us were quite close to those determined by Weir and Frederiksen [7].

Enthalpy change of actin polymerization determined by us with microcalorimetry were markedly different from those derived according to van't Hoff relation [3–6]. The above discussion, in our opinion, partially answers the discrepancy. And besides, the conditions of polymerization are different, which may also result in the difference. van't Hoff relation is commonly used to characterize the thermodynamic parameters of some chemical processes. Actin polymerization is a multistep reaction in which several interactions are involved. We believe that some modification should be made if it is used to determine the enthalpy change of actin polymerization. This paper is a primary study of the thermodynamics of actin polymerization. In order to get better understanding of the nature of this process, much work remains to be done. For instance, it is necessary to analyze how each factor which is related to actin polymerization contributes to thermodynamics.

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