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MICROCALORIMETRIC INVESTIGATIONS OF K^+- AND Mg^{2+}-INDUCED POLYMERIZATION OF ACTIN AT TEMPERATURES FROM 293.15 K TO 310.15 K

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Abstract

Microcalorimetric measurements of the polymerization of actin in the presence of 100 mM KCl and 2 mM $MgCl_2$ were carried out with a Calvet MS-80 microcalorimeter at temperatures from 293.15 to 310.15 K. It was observed that the polymerization of actin was endothermic and the enthalpy change for actin polymerization was temperature-dependent. The enthalpy change ΔH^0 was fitted to ΔH^0 (kJ mol^{-1})=434.0–1.16 (T/K) and the change in heat capacity ΔC_p° calculated from ΔH° was −1.16 kJ (mol K)$^{-1}$ in the above range of temperatures. The direct calorimetry results showed that the enthalpy and entropy change for actin polymerization could not be obtained from measurements of the critical concentration and the only way to assess the enthalpy change for the polymerization of actin and similar reactions lies in the use of calorimetry.

Keywords: microcalorimetry, muscle actin, polymerization, van't Hoff relation

Introduction

The addition of salts of cations such as potassium and magnesium to a solution of G-actin can induce the self-assembly of the monomer into filaments, F-actin, *in vitro* [1]. The investigation of actin polymerization *in vitro* is of great significance for observing and studying the effects of different kinds of factors, including some additional compounds, and for maintaining the normal activities of cells by adopting certain adjustable and controllable steps.

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The polymerization of actin is considered to proceed in at least four reversible steps: activation, nucleation, elongation and annealing [2]. Above the critical concentration, actin polymer in solution with monomer in the presence of ATP hydrolyzes ATP continually; this is therefore not a true thermodynamic equilibrium, but is considered to be a steady state. However, the hydrolysis of the actin-bound ATP is not tightly coupled to polymer formation and at the steady state it is generally slow. Thus, analysis of the apparent equilibrium between ATP-actin monomer and polymer is considered to be a useful method of estimating the thermodynamic parameters of the polymerization reaction for ATP-actin [3]. Much interest has been shown in the thermodynamics of actin polymerization in past years [4–8]. Asakura *et al.* [4] and Gordon *et al.* [6] obtained the free-energy change ΔG of actin polymerization from measurements of the critical concentration (C_c) via the following expression:

$$\Delta G = -RT\ln K = RT\ln Cc \tag{1}$$

ΔH was derived from the slope of a van't Hoff plot:

$$\Delta H = \delta(\Delta G/T)/\delta(1/T) = R\delta(\ln Cc)/\delta(1/T) \tag{2}$$

and ΔS was calculated from the energy-entropy relation:

$$\Delta S = (\Delta H - \Delta G)/T \tag{3}$$

Kinosian *et al.* [8] established the following equation for the free-energy change with temperature of actin polymerization from measurements of the critical concentration:

$$\Delta S = A + BT + CT^2 \tag{4}$$

The values of ΔH and ΔS were determined from the derivatives of the free-energy change, and the heat capacity ΔC_p was calculated from the derivative of ΔH:

$$\Delta H = \delta(\Delta G/T)/\delta(1/T) - A - CT^2 \tag{5}$$

$$\Delta S = \delta(\Delta G)/\delta T = -B - 2T \tag{6}$$

$$\Delta C_P = \delta(\Delta H)/\delta T = -2CT \tag{7}$$

However, Holtzer and Holtzer [9] and Weir *et al.* [10] objected to such a use of the van't Hoff relation for determination of the enthalpy change for actin polymerization, as this involved some mistakes. Holtzer and Holtzer [9] concluded that the only valid way to assess micellar enthalpies is direct calorimetry.

In previous work by this laboratory, the enthalpy change for actin polymerization and the effects of various Pt(II) complexes on it in the presence of K^+ and Mg^{2+} at 310.15 K were measured by direct calorimetry [11–13], and experimen-

tal thermodynamic analysis of actin polymerization in the presence of K^+ and Mg^{2+} in various concentrations at 298.15 K was also carried out [14]. In the present study, an MS-80 Calvet microcalorimeter (Setaram, France) was employed for direct measurement of the enthalpy change for actin polymerization in the presence of 100 mM KCl and 2 mM $MgCl_2$ at temperatures from 293.15 to 310.15 K. The aims of this study were to determine whether the enthalpy and entropy change for actin polymerization could be obtained from its free-energy change determined as a function of temperature, and to acquire direct information about the energetics of actin polymerization by means of microcalorimetry.

Materials and methods

Materials

Disodium adenosinetriphosphate (Na_2ATP), a Sigma reagent, and other reagents of A.R. grade from the Chinese market were used. Actin was isolated and purified from rabbit skeletal muscle according to the Pardee method [15]. Its purity was identified by SDS-PAGE with a single 42 kD band. The concentration of actin was determined spectrophotometrically by using a value of $A^{1mg/ml}=0.63$ at 290 nm [16]. Actin was dissolved in a buffer solution containing 2 mmol l^{-1} Tris·HCl (pH=8.0), 0.2 mmol l^{-1} Na_2ATP, 0.5 mmol l^{-1} 2-mercaptoethanol, 0.2 mmol l^{-1} $CaCl_2$ and 0.005% NaN_3.

Calorimetry

The thermal curves and the enthalpy changes for actin polymerization were recorded with a Calvet MS-80 standard microcalorimeter at temperatures of 293.15, 298.15, 313.15 and 310.15 K. The improved reaction cell is shown in Fig. 1. The minimum heat measured was 6.863×10^{-5} J in the range of 25 μV of the amplifier. The baseline deviation of the instrument was within 1 μW/48 h.

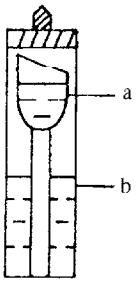

Fig. 1 A sketch of the improved reaction cell: (a) small glass cup (1 ml); (b) stainless reaction cell (15 ml)

In the experiments, 4 ml of actin solution was placed in the reaction cell and 4 ml of buffer in the reference cell; 0.5 ml of solution containing 4.2 mol l^{-1}

Na_2ATP, 42 mol l^{-1} $MgCl_2$ and 2.1 mol l^{-1} KCl was added to the small glass cups in the reaction cell and in the reference cell. It took about 90 min for the thermal equilibrium of the instrument to be attained. The reaction was then started by turning the microcalorimetric body down and up five times ($5\times180^\circ$) or more in order to ensure full mixing of the liquid in the bottom of the stainless steel cell with that in the small glass cup. The experiments at each temperature were repeated three to five times.

Spectrophotometry

A DU-7 UV-visible spectrophotometer (Beckman Company, U.S.A.) was used to determine the concentration of G-actin and the critical actin concentration at 290 nm at 298.15 K. After each calorimetric experiment, the solution in the reaction cell was 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 the final concentrations of actin in the supernatant layer was taken as the amount of actin polymerized in each run.

Viscometry

The change in viscosity during actin polymerization was measured with an Ubbelohde viscometer with an outflow time of 53.20 s for water at 298.15 K. The measurements were made under the same conditions as for the reaction of actin polymerization in the microcalorimeter, i.e. before measurements the sample was kept at the experimental temperature for 90 min. This was done to make sure that polymerization was achieved and to observe the tendency to change of the viscosity of the polymers with time.

Results and discussion

The calorimetric curve for actin polymerization at 298.15 K is shown in Fig. 2. The calorimetric curves at 293.15, 303.15 and 310.15 K were very similar. Figure 2 indicates that the self-assembly of purified G-actin into F-actin is a complex process involving both endothermic and exothermic reactions at the same time in the presence of K^+ and Mg^{2+} in the above range of temperatures. The experimental results also further confirmed some previous reports [1, 2], i.e. the polymerization of actin is not a single reaction, but a process with multiple steps. As there are two peaks, the first a large endothermic one, and the second a smaller exothermic one, in the calorimetric curves, the total enthalpy change for actin polymerization should be the sum of two kinds of heat effects and endothermic:

$$\Delta H^\circ = \Delta H^\circ(\text{endothermic}) + \Delta H^\circ(\text{exothermic}) \quad (8)$$

The enthalpy changes for actin polymerization at temperatures from 293.15 to 310.15 K are listed in Table 1 and the temperature dependence of the enthalpy

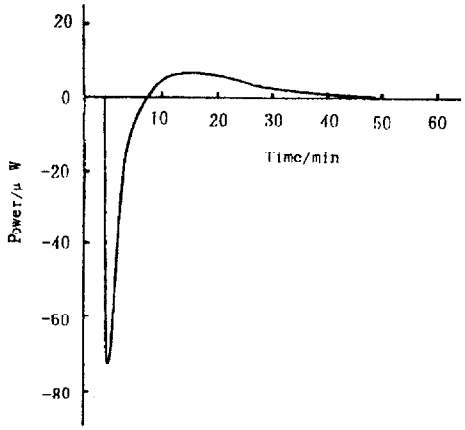

Fig. 2 The calorimetric curve of actin polymerization in the presence of 100 mM KCl and 2 mM $MgCl_2$ at the temperature 298.15 K. The concentration of monomer actin was 1.02 mg ml^{-1}

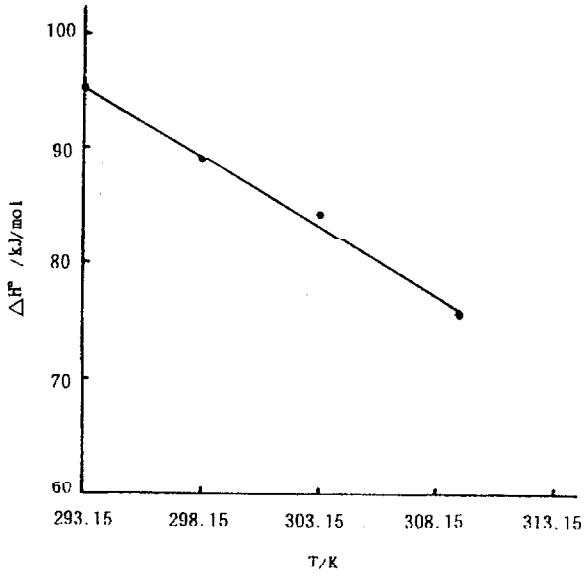

Fig. 3 The temperature dependence of the enthalpy change of actin polymerization in the presence of 100 mM KCl and 2 mM $MgCl_2$ in the range of temperatures from 293.15 K to 310.15 K

changes are shown in Fig. 3. The experimental enthalpy changes are fitted to the relation

$$\Delta H^{0}(\text{kJ mol}^{-1}) = 434.0 - 1.16(T/\text{K})(293.15\ \text{K} \leq T \leq 310.15\text{K}) \quad (9)$$

and the heat capacity ΔC_p° can be further calculated from the derivation of ΔH°:

$$\Delta C_P^\circ(\text{kJ(mol K)}^{-1}) = \delta\Delta H^\circ/\delta T = -1.16(293.15\ \text{K} \le T \le 310.15\text{K}) \quad (10)$$

The enthalpy change for actin polymerization obtained by Asakura *et al.* [4] at temperatures from 273.15 to 293.15 K was 50 kJ mol^{-1} and that proposed by Gordon *et al.* [6] at temperatures from 273.15 to 308.15 K was 63 kJ mol^{-1}. As compared with the previous work, there are some clear differences between the enthalpy change for actin polymerization from the van't Hoff plot and those from the direct calorimetry here. The latter is markedly temperature-dependent and the results derived from the van't Hoff plot are smaller than those presented here.

Table 1 The enthalpy changes of actin polymerization at temperatures from 293.15 K to 310.15 K

T/ K	$C_{G\text{-actin}}$/ mg ml^{-1}	ΔH°/ kJ mol^{-1}
293.15	1.60	95.3
298.15	1.02	89.0
303.15	1.17	84.1
310.15	1.32	75.4

For all experiments, the polymerization of actin was induced by 100.0 mmol KCl and 2.0 mmol $MgCl_2$

According to the measurements of critical concentration in the range of temperatures from 278.15 to 308.14 K, Kinosian *et al.* [8] calculated the enthalpy change of actin polymerization as ΔH=4.18 kJ mol^{-1} from Eq. (5), and the heat capacity as ΔC_p=–0.209 kJ (mol K)$^{-1}$ from Eq. (7) for Mg-ATP-actin at 298.15 K. As compared with the results from direct calorimetry, the enthalpy change obtained by Kinosian *et al.* seems to small. Though ΔC_p from ΔH for Mg-ATP-actin suggested by Kinosian *et al.* shows that it was temperature-dependent, there is still a quantitative difference between the results of Kinosian *et al.* and those in this study.

At the steady state, the polymerization of actin can be described by the following reaction:

$$A_1 + A_{N'} = A_{(N'+1)} \quad (11)$$

where A_1 is a monomer of actin and $A_{N'}$ and $A_{(N'+1)}$ are the polymers of actin with N' and N'+1 monomers, and where N' is the most probable degree of polymerization of F-actin species. The standard free energy change for the above reaction can be estimated via

$$\Delta G_{N'}^\infty = RT\ln C_c \quad (12)$$

According to the expression of standard partial molal Gibbs free energy, Holtzer and Holtzer [9] derived the enthalpy change for the above reaction:

$$\Delta H^{\infty}_{N'} = -RT^2\left(\frac{\partial \ln(C_c)}{\partial T}\right)_P + T\left(\frac{\partial(G^{\infty}_{N'+1} - G^{\infty}_{N'})}{\partial N'}\right)_{TP}\left(\frac{\partial N'}{\partial T}\right)_P \quad (13)$$

To compare Eq. (13) with Eq. (2), it can be seen that the right hand side of Eq. (13) has one more term than Eq. (2). Holtzer and Holtzer considered that it was this additional term that made the large difference between the micellar enthalpies from the experimental quantity and that given by the van't Hoff relation. According to Eq. (2), $(\partial N'/\partial T)_P$ should be zero, i.e. the most probably micelle number is independent of temperature. Holtzer and Holtzer further proved that the magnitude of the electrical part of the last term of Eq. (9) would be about 105 kJ at about 300 K, and therefore it must be taken into account. Kodama [17] also pointed out that, even if the van't Hoff plot can be used to calculate the value of the enthalpy change, the error accompanying such estimates usually exceeds ±10%.

Our experimental results from direct calorimetry support the view of Holtzer and Holtzer, i.e. the measurements of critical concentration (C_c) can be used only to estimate the standard free energy change for actin polymerization, but, determined as a function of temperatures, it cannot be used to calculate the standard enthalpy and entropy for actin polymerization. If no temperature dependence of the most probable micelle number is taken into account, the van't Hoff relation fails to estimate the standard enthalpy and entropy in the system of actin polymerization as attempted by Asakura *et al.* [4] and Gordon *et al.* [6].

The thermodynamic parameter of actin polymerization, ΔG°, derived from measurements of the critical concentration at 298.15 K, is listed in Table 2. The experimental results show that the polymerization of actin is an endothermic entropy-driven reaction under those conditions. This is in agreement with references [4–6].

Table 2 The thermodynamic parameters of actin polymerization at the temperature 298.15 K. The concentration of G-actin (C_c) was 1.02 mg/ml and the polymerization of actin was induced by 100.0 mmol KCl and 2.0 mmol $MgCl_2$

C_c/ 10^{-6}mol l^{-1}	ΔG°/ kJ mol^{-1}	ΔH°/ kJ mol^{-1}	ΔS°/ J $(K\ mol)^{-1}$
1.06	−34.1	89.0	413

The time dependence of the reduced viscosity of the G-F-actin transformation at 298.15 K in Fig. 4 shows similar results to those in reference [18]. From Fig. 4, it is observed that the reduced viscosity of actin displays a maximum about 6 min after the polymerization of actin starts; it then falls a little and con-

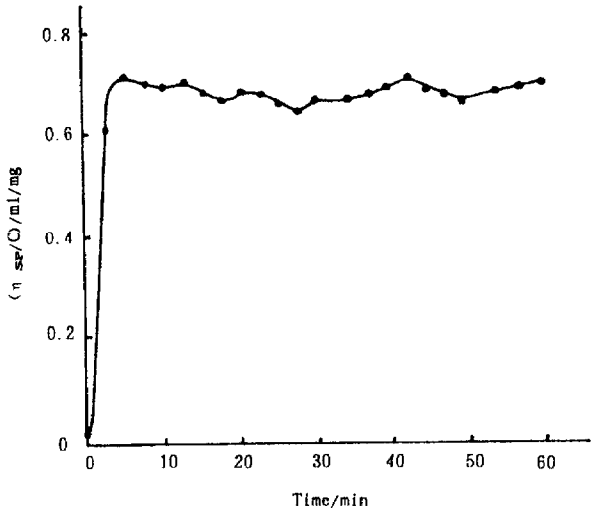

Fig. 4 Time dependence of reduced viscosity of actin polymerization in the presence of 100 mM KCl and 2 mM $MgCl_2$ at the temperature 298.15 K. The concentration of monomer actin was 1.02 mg ml^{-1}. Before the measurement was started, the sample had been retained at the experimental temperature for 90 min

tinues an irregular change up and down for a long time (60 min). We suspect that such a change in the reduced viscosity of actin polymer could be related to the complex mechanism of actin polymerization. Though actin polymerization in the presence of ATP is not an equilibrium reaction but a steady state, the results from direct calorimetry at different temperatures are still very significant to analyze the mechanism of the self-assembly of actin.

The enthalpy changes presented in this study are larger than those from the critical concentration [4–7]. This means that actin polymerization is not a weak bonding combination from G-actin monomer into F-actin filaments. As actin polymerization in the presence of ATP is a multistep reaction involving hydrolysis of the actin-bound ATP, the binding of nucleotides and divalent cations to monomeric actin, the binding of divalent cations and nucleotides to F-actin and so on [19, 20], the enthalpy change for actin polymerization from direct calorimetry should be the sum of the heat effects from all these steps. For a complete understanding of the complex reaction mechanism of actin polymerization, it is necessary to establish the contribution of each reaction step to the polymerization. Our further studies will include microcalorimetric measurements of the active enthalpy change for monomer actin below the critical concentration and for Mg-ADP-actin, an equilibrium reaction in the absence of ATP, under the same conditions as for Mg-ATP-actin.

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