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Calorimetric studies on actin polymerization and a comparison of the effects of cisplatin and transplatin [1]

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Abstract

The effects of cisplatin and its trans-isomer transplatin on the thermokinetics of actin polymerization have been studied by measurement with an MS-80 Calvet calorimeter (Setaram, France). The power–time curves show that transplatin inhibits the normal association of actin, promotes the abnormal association of actin even at lower concentrations; and induces the depolymerization of actin polymer in the G/F system at higher concentrations. However, cisplatin, at lower concentrations, mainly inhibits the polymerization of actin; at higher concentrations, it induces the crosslinking and depolymerization of actin polymer in the G/F system.

Keywords: Actin; Cisplatin; Microcalorimetry; Transplatin

1. Introduction

The polymerization and depolymerization of actin are fundamental processes for cellular functions. The linear self-association of the actin monomer (G-actin) to F-actin is the chemical basis of the formation of an intracellular microfilamentous system. The regulatory dynamic state of this system is crucial for a living cell [1, 2]. Diverse metal ions perturb the dynamic state either by disturbing the self-association or by reactions

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other than self-association. These effects are evidently related to the toxicity of these metals. It has been reported that cisplatin, the anti-tumor complex, and its analogues cause the microfilamentous network to be disorganized or even broken up [3]. The mechanism of these effects of heavy metals is not known precisely.

At present, although several methods have been developed to measure the actin polymerization and to study the effect of different factors on the process of actin polymerization [4], little is known about its effect on the thermokinetic process of actin polymerization. Here, the actin polymerization in the presence of cisplatin and transplatin was studied by a microcalorimetric method in order to understand the effects of cisplatin and its trans-isomer transplatin. Based on the power–time curves, we discuss the thermal assignments of the relevant steps in actin polymerization, the effects of cisplatin and transplatin on the microfilaments and the mechanisms of the processes.

2. Experimental

2.1. Materials

Disodium adenosinetriphosphate (Na_2ATP), Merck reagent, cisplatin, from Qilu Pharmaceutical Factory, and other reagents of A.R. grade were used. Actin was isolated and purified from rabbit skeletal muscle according to the Pardee method [5]. Its purity was identified by SDS-PAGE with a single 42KD band. The concentration of actin was determined by a UV spectrophotometric method [6] with BSA as standard. 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.

2.2. Apparatus and method

A Calvet MS-80 microcalorimeter (Setaram, France) with an improved design [7] was employed to record the thermal effects as a function of time for the whole process. The minimum value of the amount of heat measured in the calorimeter is 2.745 μJ with a precision of 3–5%. The base line drift of the recorder is ± 1 mW (48 h^{-1}) when the amplifier is at 50 μV.

Before measurement, the sample was placed in the stainless steel cell (15 ml) and the reactant in the small glass cup (1.5 ml). They were both placed in a block thermostated at 37 °C (± 0.001 °C). To start the reactions, the block was rotated three times ($3 \times 180°$) in order to ensure full mixing of the liquid in the stainless steel cell with that in the small glass cup. Because the reaction cell and reference cell were operated simultaneously under the same conditions, all the heat effects in both cells resulting from mechanical action cancel each other. So precision and accuracy can be ensured in the measurements. This instrument was calibrated by measuring the heat of neutralization and the heat of KCl dissolution; the values are in agreement with the reported values.

For the present experiments, 5 ml of actin solution was put in the sample cell and reference cell; 0.6 ml of polymerization solution, containing 18 mmol l^{-1} Na_2ATP, 10

mmol l^{-1} $MgCl_2$, 450 mmol l^{-1} KCl, with various concentrations of cisplatin or transplatin was added to the small glass cup in the sample cell; meanwhile, 0.6 ml of buffer with the same concentration of cisplatin or transplatin as that in the sample cell was added to the small glass cup in the reference cell. It took about 1 h to reach thermal equilibrium. The thermokinetic processes were recorded at 310.15 K. Amplification was set at 50 μV. The speed of recording was 1 mm min^{-1}. The experiment was repeated 2–3 times. The deviation of the measurement is within ± 2%.

3. Results

3.1. Comparison of the effects of cisplatin and transplatin at lower concentrations on the power–time curves of G-actin polymerization

The power–time curves of actin polymerization at lower concentrations of cisplatin and transplatin are given in Fig. 1 and related data are listed in Table 1. The results show significant differences. In the presence of cisplatin, the power–time curves of actin polymerization display endothermic and exothermic processes. First, exothermic peaks diminish rapidly with increasing cisplatin concentration, with the endothermic step probably being inhibited; then the heat effects in the endothermic process decrease rapidly with increasing cisplatin concentration, as reflected in the decrease in ΔH_2 (the change in enthalpy of the second peak in the power–time curve) values measured and the time taken for completion of the endothermic reaction (Table 1). However, in the

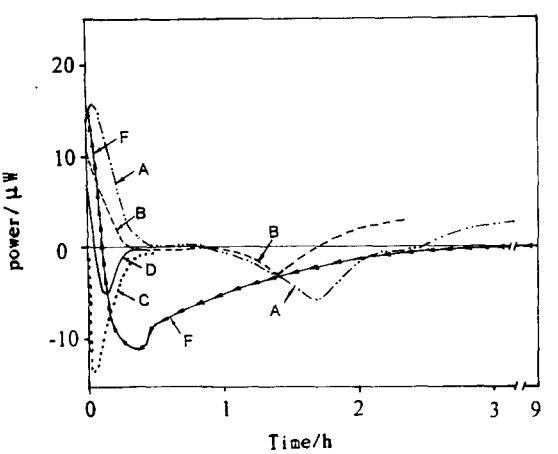


Fig. 1. Power–time curve of the polymerization of actin in the presence of cisplatin or transplatin in the range of lower concentrations.
Transplatin concentration group: —, 0.0178 mmol per mg P (D); ···, 0.003 mmol per mg P (C). Cisplatin concentration group: – – –, 0.110 mmol per mg P (B); –·–, 0.038 mmol per mg P (A). Control group: –▲–▲–; platinum, 0 mmol per mg P (F); actin concentration, 1 mg ml^{-1}; Mg^{2+}, 10 mmol l^{-1}; K^+, 450 mmol l^{-1}.

Table 1
The duration and ΔH values of the thermokinetic processes of actin and the effects of cisplatin and transplatin

System	C_{Pt} [a]	t_1/s [b]	t_2/s [b]	t_{total}/s [b]	ΔH_1/kJ mol^{-1} [c]	ΔH_2/kJ mol^{-1} [c]	ΔH/kJ mol^{-1} [c]
Actin	0	660	31740	32400	−3.93	52.01	49.26
Actin–cisplatin	0.038	1320	8280	9600	−2.53	7.60	5.07
	0.110	1320	7799	9119	−0.39	6.95	6.56
	0.158	–	5820	5820	–	6.67	6.68
	0.363	–	3600	3600	–	4.78	4.78
	0.517	5400	–	5400	−2.52	–	−2.52
	0.660	9200	–	9200	−8.11	–	−8.11
	1.275	10800	–	10800	−25.33	–	−25.33
	2.080	18000	–	18000	−57.12	–	−57.12
Actin–transplatin	0.001	840	11760	12620	−0.311	8.44	8.13
	0.003	–	2340	2340	–	3.01	3.01
	0.007	–	1500	1500	–	1.00	1.00
	0.018	–	1500	1500	–	1.09	1.09
	0.089	–	1500	1500	–	0.82	0.82
	0.134	5400	–	5400	−42.00	–	−42.00
	0.178	10800	–	10800	−43.26	–	−43.26
	0.223	10800	–	18000	−533.03	–	−533.03

[a] Platinum concentration (mmol l^{-1}) in the solution containing 1 mg protein.
[b] Duration (s) of the first peak, the second peak and the total process.
[c] The change in enthalpy respectively corresponding to the first peak, the second peak and the summation of the total reaction.

presence of transplatin at very low concentrations (about 1/12 the cisplatin concentration), the power–time curve of actin polymerization showed an endothermic curve in which the heat input in 3.0 kJ mol^{-1}, which is about 5 times that given by cisplatin of the same concentration. It seems that transplatin strongly affects the actin polymerization, which is different from cisplatin in the lower concentration range.

3.2. Comparison of the effects of cisplatin and transplatin at higher concentrations on the power–time curves of G-actin polymerization

The power–time curves of actin polymerization at higher concentrations of cisplatin and transplatin are given in Fig. 2 and related data are listed in Table 1. The results show different exothermic characters, in which the endothermic steps are all inhibited by cisplatin and transplatin. From Fig. 2 and Table 1, it is clear that the heat output powers increase rapidly with increasing concentration of transplatin. The heat output induced by transplatin is 16 times that of cisplatin (compared in Table 1). This indicates that transplatin induces the depolymerization of actin more strongly than cisplatin at higher concentrations. ΔH_m (molar enthalpy change) values as a function of cisplatin and transplatin concentration are shown in Fig. 3.

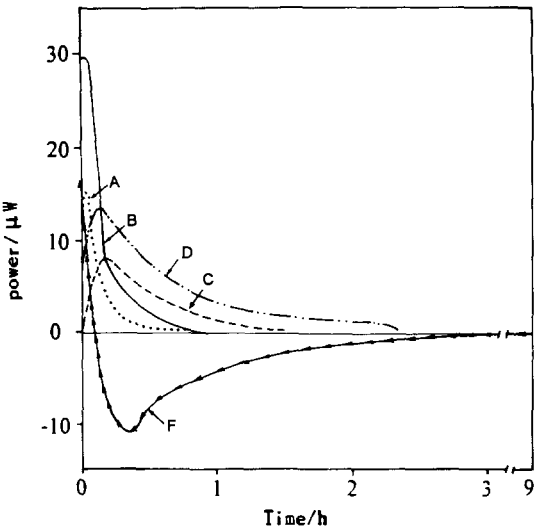


Fig. 2. Power–time curve of the polymerization of actin in the presence of cisplatin or transplatin in the range of higher concentrations.
Transplatin concentration group: –···–, 0.223 mmol per mg P (D); – – –, 0.178 mmol per mg P (C). Cisplatin concentration group: —, 0.660 mmol per mg P (B); ···, 0.517 mmol per mg P (A). Control group: – ▲ – ▲ –; platinum 0 mmol per mg P (F); actin concentration, 1 mg ml^{-1}; Mg^{2+}, 10 mmol l^{-1}; K^{+}, 450 mmol l^{-1}.

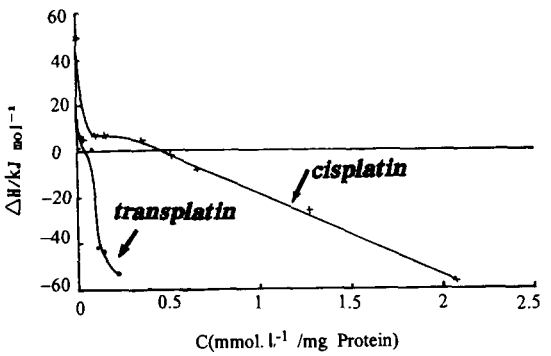


Fig. 3. Value of ΔH_m of polymerization of actin in the presence of cisplatin or transplatin as a function of concentration.

3.3. *The power–time curves of* F-*actin depolymerization in the presence of cisplatin or transplatin*

The power–time curves of F-actin depolymerization and the effects of cisplatin and transplatin are given in Fig. 4. They show that an exothermic process related to the depolymerization of F-actin at 37 °C appears after 32 h, but after 3 h in the presence of cisplatin, and from the beginning in the presence of transplatin.

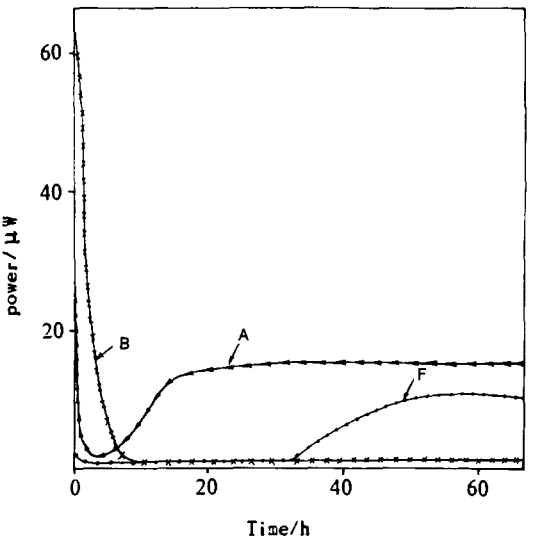


Fig. 4. Power–time curves of depolymerization of F-actin in the presence of cisplatin or transplatin: ●, F-actin, actin concentration, 8 mg ml^{-1} (F); ▲, F-actin–cisplatin, actin concentration, 8 mg ml^{-1}, cisplatin, 0.18 mmol mg^{-1} (A); ×, F-actin–transplatin, actin concentration, 8 mg ml^{-1}, transplatin, 0.18 mmol mg^{-1} (B).

4. Discussion

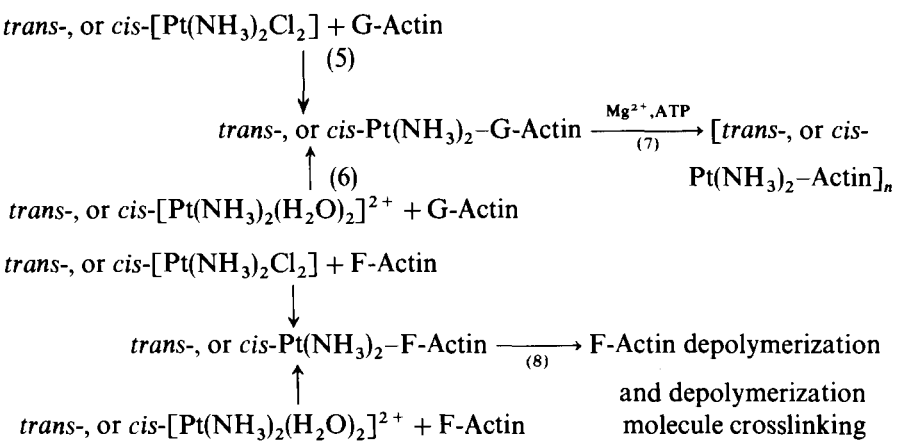
Actin polymerization can be divided into several steps [4] all of which are related to events with different heat effects: step (1) is mainly exothermic, and steps (2) and (3) mainly endothermic.

$$\text{Actin} \xrightarrow[(1)]{Mg^{2+}, \text{ATP, conformation change}} \text{Mg–ADP–Actin} \xrightarrow[(2)]{\text{self-association (elongation)}}$$

$$(\text{Mg–ADP–Actin})_n \xrightarrow[(3)]{\text{modulation of degree of association}} (\text{Mg–ADP–Actin})_{ni}$$

The power–time curve shows that the thermokinetic process of actin polymerization has two different thermal effects [8]; the first exothermic step is related to the net result of the reactions (metal ion (Mg^{2+}, K^+) binding to actin, the change in conformation of actin and the hydrolysis of ATP in actin); and the second endothermic step is mainly assigned to the results of association and length adjustment. In the presence of cisplatin or transplatin, the following reactions, in addition to the above steps, are involved, in which the polymerization (step (7)) should correspond to an endothermic process, and depolymerization (step (8)) to an exothermic process.

$$\textit{trans}\text{-, or } \textit{cis}\text{-}[Pt(NH_3)_2Cl_2] \Leftrightarrow \textit{trans}\text{-, or } \textit{cis}\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+} \quad (4)$$



The trans- or cisplatin reacts with actin in two ways: either directly or via hydrolysis products. It is known [9] that the processes of trans- or cisplatin hydrolysis (step (4)) and their binding to actin (steps (5), (6)) are both relatively rapid reactions and the corresponding heat processes are included in the first peak (exothermic) in the power–time curves of trans- or cisplatin–actin.

It has been reported that platinum binding to actin by Pt–S and Pt–N bonds [10] inhibits the actin polymerization and induces crosslinking and depolymerization [11], and their extents are related to the reactivity of the platinum compound. These two aspects can also be reflected in the related power–time curves (Fig. 1 and Fig. 2). When the concentration of cisplatin increases, in the G/F system, reaction (7) will be inhibited resulting in a difficulty for association of G-actin. Therefore, the decreasing of the endothermic effects indicates the concentration dependence of cisplatin, as shown in Fig. 1. With increasing concentration of cisplatin, the endothermic character changes to exothermic, indicating that the effect of platinum will induce the depolymerization of F-actin (as reaction (8)), and then the association of G-actin no longer occurs. The heat output correlates with the depolymerization of F-actin. There is probably crosslinking of depolymerized actin monomers in addition to depolymerization of F-actin in this system. Transplatin is similar to cisplatin in the following aspects: first, inhibiting the association of actin via a crosslinking step, resulting from high reactivity at lower concentrations, and then inducing the depolymerization of actin polymer in the system with G/F equilibrium at higher concentrations. But its effects are more pronounced than those of cisplatin, both in the lower and higher concentration ranges.

The different effects of cisplatin and transplatin on the actin polymerization are due to the different types of reaction with actin. Raman spectra [12] revealed that, compared with cisplatin, there are various Pt–S and Pt–N binding sites in the transplatin–actin. Transplatin, therefore, strongly induces the conformational change of actin, resulting in the depolymerization of F-actin. This is also reflected in the power–time curves of transplatin–actin. The sign of ΔH_m for actin polymerization will be changed rapidly, even at lower concentrations of transplatin (as shown in Fig. 3), showing that transplatin induces the depolymerization of F-actin significantly. This is probably related to their pharmacological or toxicological action.

In summary, the actin polymerization process is featured in power–time curves by one exothermic and one endothermic step. The former is due to the net results of magnesium/potassium ion-binding, the hydrolysis of ATP and conformation changes, while the second step corresponds to the slower reactions of association and modulation of the degree of polymerization. Cisplatin inhibits polymerization at lower concentrations, but induces the depolymerization and possibly crosslinking at higher concentrations. Transplatin inhibits the normal association of actin and possibly promotes the abnormal polymerization, i.e. a polymerization process resulted from crosslinking at the lower concentrations and strongly induced the depolymerization of actin polymer (F-actin) at higher concentrations.

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