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Thermodynamic studies on the effects of cisplatin or its analog complexes on actin polymerization

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Abstract

The effects of cisplatin or its analog complexes on actin polymerization have been studied by the microcalorimetric and viscosity methods at the temperature of 310.15 K. The thermodynamic parameters and the power–time curves of actin polymerization in the presence of cisplatin or its analog complexes (*cis*-DBDP, *cis*-DIDP, *cis*-DADP) are measured. The results show that there are different interaction mechanisms between actin and cisplatin or its analog complexes. The relative viscosity of actin polymer decreases in the presence of cisplatin or its analogs, and also shows Pt concentration dependence. It indicates that these compounds affect actin polymerization. It has been discussed from the experimental results that the presence of cisplatin or its analog complexes cause different polymerization mechanism of actin. © 2001 Published by Elsevier Science B.V.

Keywords: Cisplatin or its analog complexes; Actin polymerization; Microcalorimetry; Viscosity

1. Introduction

Actin polymerization plays an important role in the generation of cell shape and underlying a cell motile function [1]. The effects of cisplatin, an anti-tumor drug, and its *cis–trans*-isomer, transplatin, on actin polymerization have been studied by a microcalorimetric method [2,3]. The experimental results showed different thermokinetic characters of actin polymerization in the presence of cisplatin and transplatin, which were closely related to interaction mechanism between the compounds and actin. In the present study, the thermodynamic parameters of actin polymerization in the presence of cisplatin or its analog complexes were obtained from the direct heat measurements and the viscosity measurements. The aims of the research were to make a systematic understanding about the extent of the influences that the different substituting groups X in the molecules of *cis*-$Pt(NH_3)_2X_2$ exert on actin polymerization, and then to give a reasonable explanation for the mechanism on the effects of cisplatin or its analog complexes on actin polymerization from the interaction between the compounds and actin. It will shed some light on the active action of cisplatin as anticancer drug and its further modification of cisplatin.

2. Experimental

2.1. Materials

Dissodium adenosinetriphosphate(Na_2ATP), Merck reagent, *cis*-DCDP[cisplatin or *cis*-diamminedichloro-

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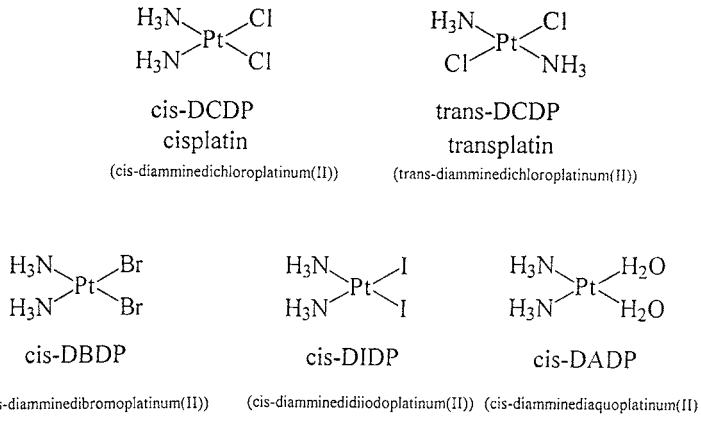


Fig. 1. The structure of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP and *trans*-DCDP.

platinum(II)], *cis*-DBDP[*cis*-diamminedibromoplatinium(II)], *cis*-DIDP [*cis*-diammineadiiodoplatinium(II)], *cis*-DADP[*cis*-diammineadiaquoplatinium(II)], *trans*-DCDP[trasplatin or *trans*-diamminedichloroplatinum] are available from Professor Dou Peiyan, Beijing Medical University. Their chemical structures are shown in Fig. 1. Other reagents are A.R. reagents.

Actin was isolated and purified from rabbit skeletal muscle according to Pardee method [4]. Its purity was checked by SDS-PAGE, yielding a single 42 KD band. 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. The concentration of actin was determined by UV spectrophotometric method [5] with BSA as standard. It was 1.6×10^{-5} mol l^{-1} (0.7 mg ml^{-1}) for the experiment.

2.2. Apparatus and methods

A Calvet MS-80 microcalorimeter (Setaram, France) was used for recording the power–time curves and the enthalpy changes of actin polymerization at 310.15 K [2,3]. The minimum value of heat measured in the calorimeter is 2.745×10^{-5} J. The base line deviation of the instrument was within 1 μW/48 h in the range of 50 μV of the amplifier. The heat as less as 0.001 J can be measured with a precision of 2%. In the experiments, 5 ml of actin solution were put in the bottom of the sample cell and the reference cell. The 0.6 ml of polymerization solution, containing 18 mmol l^{-1} Na_2ATP, 10 mmol l^{-1} $MgCl_2$ and 450 mmol l^{-1} KCl, with various concentrations of cisplatin or its analog complexes was added to the small glass cup in the sample cell. The 0.6 ml of buffer with the same concentration of cisplatin or its analog complexes 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 reaction was then started by turning the microcalorimetric body down and up five times ($5 \times 180°$) 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 speed of recording was 1 mm min^{-1}. Each experiment was repeated at least twice.

An Ubbelohde-type viscometer with an outflow time of 51.40 s for water at 310.15 K was employed for measuring the change in viscosity during actin polymerization in the presence and in the absence of cisplatin or its analog complexes, respectively. Before measurement, the viscometer was washed with dilute K_2CrO_4–H_2SO_4 three times and exhausted with a vacuum pump in the presence of C_2H_5OH. The measurements were made under the same conditions as the reaction of actin polymerization in the microcalorimeter, i.e., before measurements, the actin solution and the polymeriation solution with various concentration of platinum complex were kept at the experimental temperature for 60 min, respectively, then mixed them and recorded the relative viscosity of mixture with time until the relative viscosity reached a limiting value, which was considered as a measure of polymerization degree.

3. Results

3.1. Power–time curves of actin polymerization

The power–time curves of actin polymerization in the presence of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP and *trans*-DCDP at 310.15 K are shown in Fig. 2. The results show significant differences between the power–time curves of actin polymerization and those of actin polymerization in the presence of cisplatin or its analog complexes. Compared with the power–time curve of actin polymerization, *cis*-DCDP and *cis*-DBDP give a small effect on actin polymerization, i.e., induce the endothermic peak delayed and decrease the endothermic heat as reflected in the change of ΔH^0, but *cis*-DADP, *cis*-DIDP and *trans*-DCDP are quite different in the effects on the power–time curves of actin polymerization, which show exothermic heat effect.

3.2. Pt concentration dependence of relative viscosity of actin polymerization

Pt concentration dependence of relative viscosity of actin polymerization in the presence of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP and *trans*-DCDP at 310.15 K is shown in Fig. 3. From Fig. 3, it was observed that the polymerization degree of actin was changed in the presence of cisplatin or its analog complexes. The effects of four *cis*-compounds of platinum on polymerization degree increased with the order of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP. Transplatin not only made the polymerization degree of actin reduced, but also located the highest polymerization degree of actin at lower concentration of platinum complex.

3.3. Thermodynamic parameters of actin polymerization

The thermodynamic parameters of actin polymerization in the presence of cisplatin or its analog complexes at 310.15 K are listed in Table 1. The enthalpy changes of actin polymerization ΔH^0 are the sum of two kinds of heat effect, i.e.

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

The polymerization of actin can be described by the following reaction:

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

The equilibrium constant of actin polymerization K_p can be calculated by Scatchard method [6] using the Pt

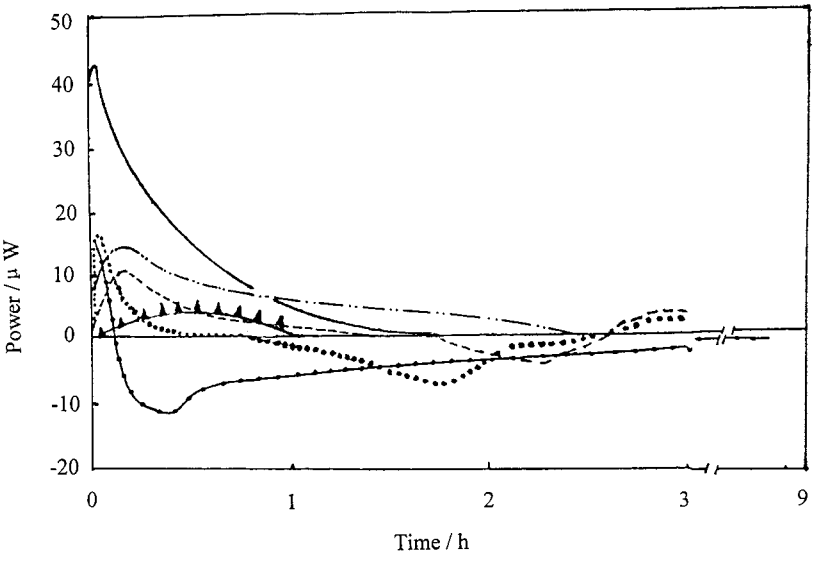


Fig. 2. The power–time curves of actin polymerization in the presence of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP and *trans*-DCDP at 310.15 K. (–·–·–) actin, (···) *cis*-DCDP, (–·–·–·–) *cis*-DBDP, (–▲–▲–▲–) *cis*-DIDP, (——) *cis*-DADP and (——) *trans*-DCDP.

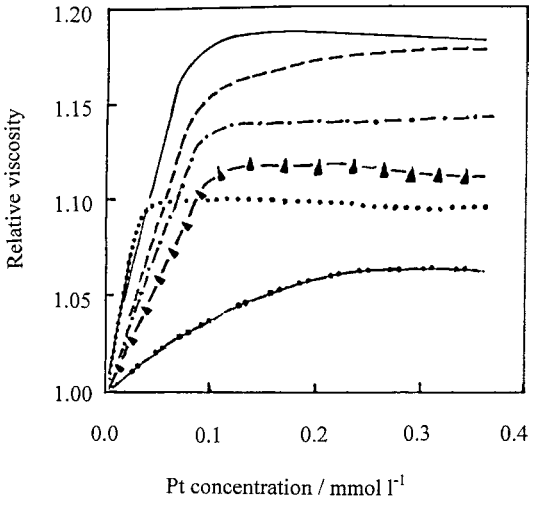


Fig. 3. The Pt concentration dependence of reduced viscosity of actin polymerization in the presence of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *cis*-DADP and *trans*-DCDP at 310.15 K. (—) actin, (–▲–▲–▲–) *cis*-DCDP, (——) *cis*-DBDP, (–·–·–) *cis*-DIDP, (–···–···–) *cis*-DADP and (··· ···) *trans*-DCDP.

concentration dependence of reduced viscosity of actin polymerization in the presence of cisplatin or its analog complexes via platinum concentration. It is reciprocal of the concentration of monomer([A]eq), for $[A_{(n)}] \approx [A_{(n+1)}]$ when polymerization is at equilibrium. The value of ΔG^0 can be derived from the following equation:

$$\Delta G^0 = -RT \ln K_p = RT \ln[A]_{eq} \quad (3)$$

The value of ΔS^0 is calculated from the energy–entropy relation for changes at the temperature 310.15 K and normal atmosphere,

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

4. Discussion

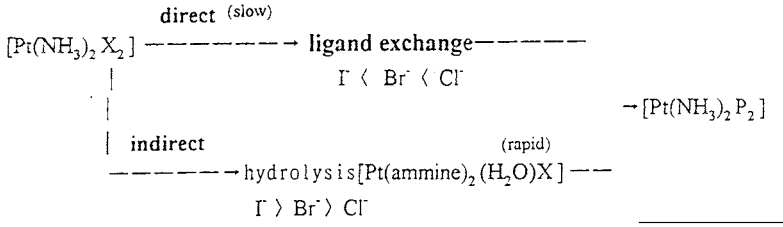
Although there are ample evidences that the primary target of platinum anti-tumor drugs is DNA [7], the contributions of non-DNA targets cannot be underestimated that the existence of specific members protein receptors mediate cisplatin transport [8]. Cisplatin induces the change of membrane conformation [9] and affects the transport system of amino acid on membrane [10]. In addition, the action of platinum complexes on the actin, including the binding to the monomer actin (G-actin) and inducing the conformation change and perturbating to the self-association of the monomer, is shown to be important in the cell damaging process [2,11]. Comparative research of effects of cisplatin or its analogs on actin polymerization, as a part of research on the structure–anticancer activity relationship, seems to be necessary.

From Table 1, the enthalpy changes of actin polymerization in the presence of cisplatin or its analog complexes are evidently different from that in the absence of the compounds, and decrease with the order of *cis*-DCDP, *cis*-DBDP, *cis*-DIDP, *trans*-DCDP, *cis*-DADP. These results show that different substitution group in the molecule of *cis*-$[Pt(NH_3)_2X_2]$ contribute distinct effects on actin polymerization. The different chemical stereo structures for $Pt(NH_3)_2Cl_2$ make distinct effects on the actin polymerization too. The free energy changes values for actin polymerization in the presence of cisplatin or its analog complexes increased compared with that of G-actin to F-actin in absence of the compounds. It reflected that the cisplatin or its analog complexes will perturb the process of actin monomer polymerization by the related reactivties. The reaction mechanism between $Pt(NH_3)_2Cl_2$($X = H_2O, Cl^-, Br^-, I^-$) and the protein-(actin) may go through two ways [12], direct Ligand exchange and via hydrolysis.

Table 1
Thermodynamic parameters of actin polymerization in the presence of cisplatin or its analog complexes at 310.15 K[a]

Thermodynamic parameters	Actin	Actin (*cis*-DCDP)	Actin (*trans*-DCDP)	Actin (*cis*-DBDP)	Actin (*cis*-DIDP)	Actin (*cis*-DADP)
ΔH^0(kJ mol^{-1})	49.3	6.36	−35.0	3.72	−30.0	−102
ΔG^0(kJ mol^{-1})	−25.7	−5.94	−12.3	−6.47	−6.30	−3.58
ΔS^0(J mol^{-1} K^{-1})	242	39.7	−73.2	32.9	−76.4	−317

[a] Platinum concentration (0.1 mmol/mg protein solution).



In general, platinum has a preferential binding to *S*-donor groups which is the main reaction with protein resulting in the modification of Cys or Met. Since in most cases, the –SH to –S–S plays an important role in determining the protein conformation and protein function, it can be expected that platinum binding will cause conformation changes and then make an alteration of the consequent events such as the normal association of G-actin and its elongation (to F-actin). If $Pt(NH_3)_2Cl_2$ react via hydrolysis, it will have a stronger damage to actin because hydrolysis products of cisplatin analogs give a rapid attack and stronger reaction to actin. There are different reaction rate in the above two reaction ways: exchange rate is $I^- < Br^- < Cl^-$ [13], but hydrolysis rate is $I^- > Br^- > Cl^-$. The reaction pathway for cisplatin analogs with actin is determined by the competition between exchange rate and hydrolysis rate. Obviously, *cis*-DIDP tends to react via hydrolysis with actin, which is reflected by enthalpy change value of actin polymerization (Fig. 2). From Fig. 2, the endothermic heat effect were decreased definitely after the addition of *cis*-DCDP or *cis*-DBDP in which endothermic process of G-actin polymerization in coexist system of G-actin and F-actin was inhibited.

Acknowledgements

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