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DSC STUDIES OF THE EFFECTS OF CISPLATIN AND TRANSPLATIN ON G-ACTIN

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Abstract

The effects of cisplatin and its *trans* isomer transplatin on the thermal denaturation of G-actin were studied with a Micro DSC-III differential scanning calorimeter. The denaturation enthalpy of G-actin was found to be 12 J g^{-1}, and the denaturation temperature was 328 K. The thermal denaturation curve showed that increasing cisplatin concentration decreased the enthalpy change. However, after the ratio of cisplatin to G-actin attained 8:1 (mol:mol), the denaturation enthalpy no longer decreased. Transplatin decreased the enthalpy change more rapidly. In contrast with cisplatin, the denaturation peak at 328 K disappeared, and a strong exothermic peak appeared at 341 K when the ratio of transplatin to G-actin was 8:1 (mol:mol). The enthalpy change was 75 J g^{-1}, which is far in excess of the range of weak interactions. This strong exothermic phenomenon probably reflects the agglutination of protein. The effects of cisplatin and transplatin on the number of the free thiol groups of G-actin are discussed.

Keywords: actin, cisplatin, DSC, thiol, transplatin

Introduction

Actin, a cytoskeleton protein, is widely present in eukaryotic cells, and plays important roles in biological process, such as muscle contraction, cell motility, cell division, cytoplasmic streaming, etc. [1]. This protein can exist in a globular monomeric form (G-actin) or a filamentous polymeric form (F-actin). A biological balance of these two forms exists in vivo, which is largely regulated by ATP, the ionic strength, actin-binding protein, etc. G-actin (*MW* 42 kD) consists of a single chain of 374~377 amino acid residues, including 5 cysteine residues. These 5 cysteine residues display different activities [2]. *Cys*-10, –285 and –374 can be alkylated under native conditions, *Cys*-257 can be alkylated under denaturing conditions, and *Cys*-217 cannot be alkylated under any conditions. These differences are probably related to the spatial sites in the protein.

Cisplatin is an antitumour agent. It has been deeply studied. The early work focused on the interaction of cisplatin with DNA, but recent research demonstrated that cisplatin could provoke a complex response in the cell [3]. Miller *et al.* [4] found that cisplatin could cause the crosslinking of actin with DNA in the Chinese hamster ovary cell. Luo *et al.* [5] reported that cisplatin and its analogues could induce the recomposition and dissociation of microfilaments. Further studies [6] of Zeng *et al.*

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showed that cisplatin and transplatin at different concentrations had different effects on the association of actin. Though the thiol group was considered to be the probable binding site to cisplatin, the exact mechanism of these effects is not yet known. Transplatin differs from cisplatin only in the conformation, but it has a different physiological activity. Cisplatin exhibits antitumour activity, while transplatin causes toxicity in the cell. The difference in interaction mechanism between cisplatin and transplatin is an interesting topic.

The present paper reports the effects of cisplatin and transplatin on G-actin itself. The denaturation of proteins reflects the destruction of the spatial structure of the protein directly. If the conformation of the protein changes, the denaturation temperature or enthalpy may change correspondingly. Therefore, useful information can be obtained by detection of the protein denaturation process. Differential scanning calorimetry (DSC) has been extensively used to study biological samples. Bertazzon *et al.* [7] studied the denaturation of actin by means of DSC, CD and fluorescence. We have now studied the effects of cisplatin and transplatin on the denaturation of G-actin by DSC. The changes induced in the number of free thiol groups by cisplatin and transplatin are also discussed.

Materials and methods

Actin purification

Actin was isolated and purified from rabbit skeletal muscle according to the method of Pardee and Spudich [8] (buffer A: 2 mM Tris-HCl, 0.2 mM Na_2ATP, 0.5 mM 2-mercaptoethanol, 0.2 mM $CaCl_2$, 0.005% NaN_3, pH 8.0). The purity of the actin was checked by SDS-PAGE; it gave a single 42 kD band. The purified G-actin was lyophilized into G-actin powder and stored at –20°C. The G-actin powder was dissolved in double distilled water prior to use. The protein concentration was determined by UV spectrophotometry [9], with BSA as a standard.

Differential scanning calorimetry

DSC was performed with a MicroDSC-III differential scanning calorimeter (Setaram, France). By means of DSC, the enthalpy changes in the sample over temperature and/or time can be monitored. Such changes include exothermic and endothermic events, and also glass transitions, either during chemical reactions or during state changes. The sensitivity of the calorimeter was 0.2 μW. The onset point, which was observed as the starting of the denaturation, and the area, which was observed as the enthalpy change, were obtained via the special software provided by the manufacturer. The standard batch vessels (volume 1 ml) were used. The temperature scanning range was from 293 to 368 K at a rate of 1 K min^{-1}. A series of cisplatin or transplatin at different concentrations was added to the actin solution. They were kept at 277 K for 1 h. The same procedures were then performed.

Free thiol group determination

The number of thiol groups was obtained by the method of Ellman [10]. DTNB was obtained from Fluka. Absorbances were determined with a UV-751G spectrophotometer (Shanghai Analytical Instrument Plant, China).

Results and discussion

Figure 1 is the curve of the thermal denaturation of G-actin. The detected denaturation enthalpy of G-actin (12 J g^{-1}) and the denaturation temperature (328 K) were in accordance with the results of Bertazzon *et al.* [7]. The addition of cisplatin had no significant effect on the denaturation temperature, but it decreased the enthalpy of denaturation. The variations in the enthalpy of G-actin thermal denaturation with the mole ratio of cisplatin to G-actin are shown in Fig. 2. The decrease in ΔH with increase of the concentration of cisplatin was initially linear, and reached a limit at a

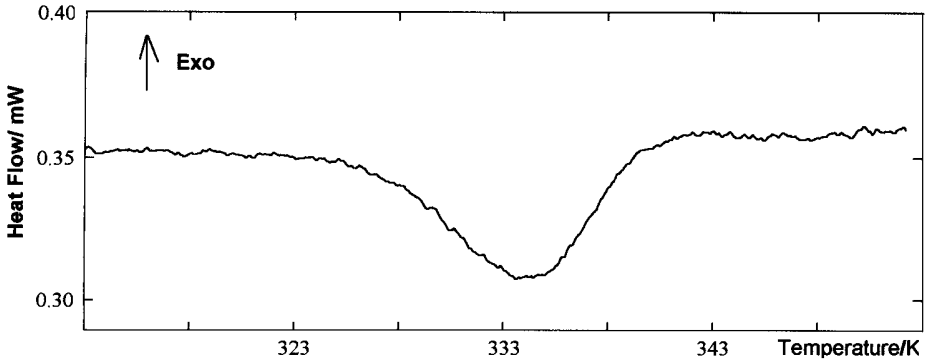

Fig. 1 Denaturation experiments on G-actin (1.5 mg); Onset point: 328 K; Enthalpy change: 12 J g^{-1}

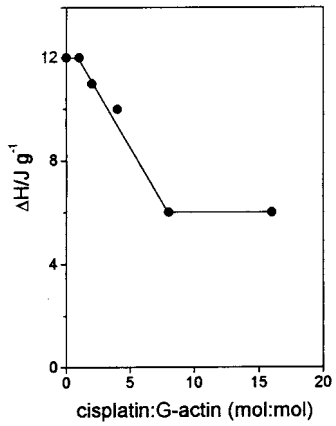

Fig. 2 Enthalpy changes at different mole ratios of cisplatin to G-actin (0.5 mg)

ratio of cisplatin to G-actin equal to 8:1. Saturation appeared to be reached. It was reported by Zeng [11] that the thermal process of the reaction of cisplatin and actin was rapid; the thermoanalytical curve of G-actin displayed only an exothermic peak, and the reaction was completed in a few minutes. As concerns the quantity of heat, it was a combination reaction. It can be suggested that the decrease in the denaturation enthalpy indicated that G-actin underwent multiple-spot binding to cisplatin. With increase of the concentration of cisplatin, the protein structure was progressively destroyed, and the enthalpy change became smaller. However, the enthalpy change was still 6 J g^{-1} at saturation. It may be concluded that cisplatin does not completely destroy the protein structure.

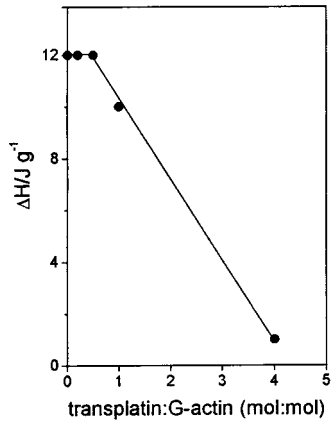

Fig. 3 Enthalpy changes at different mole ratios of transplatin to G-actin (0.5 mg)

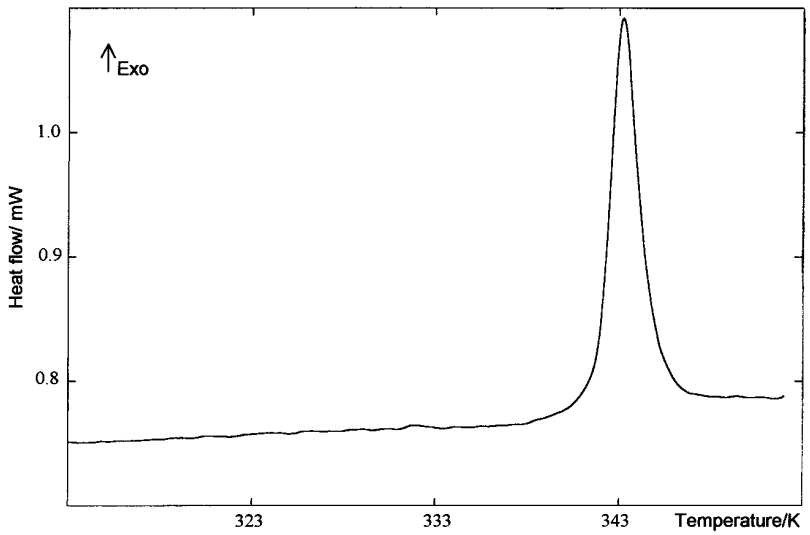

Fig. 4 Denaturation experiments on G-actin (0.5 mg) in the presence of transplatin; Transplatin:G-actin (mol:mol)=8:1; Onset point: 341 K; Enthalpy change: 75 J g^{-1}

Like cisplatin, transplatin had no significant effects on the denaturation temperature, but it decreased the enthalpy change more rapidly (Fig. 3). The enthalpy change was close to zero when the ratio of transplatin to G-actin was 4:1. This indicated that transplatin destroyed the protein structure more extensively than did cisplatin. It almost completely destroyed the protein structure. G-actin also underwent multiple-spot binding to transplatin; the number was lower than that of cisplatin. A significant difference from cisplatin was that the denaturation peak at 328 K disappeared when the ratio of transplatin to G-actin was 8:1 (Fig. 4) and a strong exothermic peak appeared at around 341 K. The enthalpy change was 75 J g^{-1}, which was far beyond the range of weak interactions. This strong exothermic peak probably resulted from the agglutination of protein. (We found an analogous exothermic phenomenon in studies of tubulin.) The different thermal phenomenon evidently reflects a different mechanism of interaction of cisplatin with G-actin from that with transplatin.

The effects of cisplatin and transplatin on the number of free thiol groups in G-actin are shown in Table 1. The number of free thiol groups in G-actin was found to be 1.8 by the method of Ellman [10]. Because of the different sites of the cysteine residues in G-actin, some thiol groups cannot be detected. As shown in Table 1, both cisplatin and transplatin decreased the measurable number of thiol groups. This result revealed that both cisplatin and transplatin are bound to free thiol groups, thereby decreasing the number of free thiol groups. It was also observed that transplatin was less effective than cisplatin under the same conditions. This indicated that transplatin had fewer binding sites for thiol groups, which was in accordance with the previous calorimetric results. Another reason for the weak effects of transplatin on the change in the number of thiol groups may be that transplatin can bind to other groups [6].

Table 1 The effects of cisplatin or transplatin on the detected number of free thiol groups*

Cisplatin:G-actin (mol:mol)	Detected number	Transplatin:G-actin (mol:mol)	Detected number
0	1.8	0	1.8
3:1	1.0	3:1	1.6
6:1	0.6	10:1	1.5
10:1	0.4	20:1	0.9

*Protein concentration: 24 μmol l^{-1}

From the calorimetric results and the detection of the number of thiol groups, it may be concluded that both cisplatin and transplatin can change the conformation of G-actin by binding to thiol groups, but the mechanisms of action of cisplatin and transplatin are different. Transplatin has fewer binding sites on thiol groups, but it has stronger effects on the conformations of G-actin, which is probably related to the different pharmacological or toxicological actions of cisplatin and transplatin.

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