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Thermochimica Acta 359 (2000) 55–60

thermochimica acta

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Urease conformational change induced by transplatin A comparison on the interaction of urease with transplatin and cisplatin

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Received 3 January 2000; received in revised form 8 March 2000; accepted 17 March 2000

Abstract

The interaction between transplatin and urease was studied by microcalorimetry, DSC, circular dichroism (CD) spectrum and fluorescence spectrum. Transplatin can render urease inactive as is indicated by the experimental results of microcalorimetry. Thermal denaturation experiments of urease by DSC show that transplatin generally reduces urease thermal stability since urease T_m decreases dramatically. From the features of urease CD and fluorescence spectra, transplatin leads to urease conformational change at lower concentrations than in case of cisplatin. The interaction of transplatin with urease is stronger than that of cisplatin and the binding site of urease with transplatin may be different from that with cisplatin.

Keywords: Transplatin; Urease; Cisplatin; Conformational stability

1. Introduction

Cis-diamminodichloroplatinum(II) (Cisplatin, CDDP) is an anticancer drug which interferes with DNA replication and transcription by binding DNA via intrastrand cross-links to ApG or GpG sequence in DNA [1,2]. Its use as an antitumor drug is limited by its toxicity, which is due to its capability of binding proteins in vivo mainly [3–5]. Investigations of cisplatin with bionucleophiles other than DNA are of no lesser biological importance, because these interactions play central roles in modulating the activity of the platinum-based antitumor drugs.

We have investigated the interaction between cisplatin and urease, a nickle metalloenzyme. Research found that urease activity is implicated in many diseases, such as infection-induced urinary stones, catheter encrustation, peptic ulcers and, possibly, stomach cancer [6,7]. So investigation on the interaction between urease and small molecule of drug is very important for biomedical research. Our work reveals that cisplatin renders not only urease inactive, but also introduces further enzymatic conformational changes [8].

The isomer of cisplatin, trans-diamminodichloroplatinum (II) (transplatin, TDDP) shows no anticancer activity, though its damage to DNA exists [9]. Research found transplatin displayed a different binding affinity relative to cisplatin on interactions with some proteins [10,11]. In our present work, the interaction between transplatin and urease was reported and its binding affinity with urease was compared with cisplatin. This will help us understand the toxic side

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0040-6031/00/$ – see front matter
PII: S0040-6031(00)00501-3

effects of the two isomers much more clearly and supply information for the interactions of cisplatin with proteins.

2. Experimental

2.1. Materials

Transplatin was synthesized at Beijing Medical University and stored in total darkness. Crystalline urease (*Canavalia ensiformis*, M.W. 480KD) was from Boehringer Mannheim (Germany) and stored at 253 K until used. Fresh enzymatic solution was prepared by dialysis in HEPES buffer solution (pH 7.0) for a day and its concentration was measured spectrometrically [12], using the extinction coefficient $E_{280}^{1\%}$=6.2 cm^{-1}.

Cisplatin was from Qi-Lu Pharmaceutical Factory, China. Urea (superpurity) was from Serva and HEPES from Merck. Other chemicals were commercial products of analytical grade. All solutions were prepared in 5 mmol l^{-1} HEPES buffer (pH 7.0) using double distilled water. The mixing solution of drug with urease was kept at 277 K for 24 h before being used for the experimental measurements.

2.2. Apparatus and procedure

The influence of transplatin on enzymatic activity was measured by enzymatic catalysis reaction of urea hydrolysis [13], using a Calvet MS-80 microcalorimeter (Setaram, France) [14]. The microcalorimeter is of the heat conduction type. An aliquot (60 mmol l^{-1}) of urea solution (2.5 ml) was added to the stainless-steel cell, and 0.5 ml urease solution or mixing solution of urease and transplatin was added to the glass cup in this experiment. In the control cell, urea was replaced by buffer. The reaction was started by turning the microcalorimeter up and down five times ($5\times180^{\circ}$), after the microcalorimeter had achieved thermal equilibrium at 298.2 K. All concentrations in this part referred to the reactants in the calorimetric vessel before mixing.

Measurements of thermal denaturation of urease were performed by a new high-sensitive differential scanning microcalorimetry (Micro DSC III, Setaram, France) with a Compaq computer recording thermograph of heat flow via temperature. Temperature correction and baseline correction had been done before experiments. The experimental temperature range was from 298.2 to 383.2 K, at a scan rate of 1 K min^{-1}. Urease concentration in this experiment was 0.5 mg ml^{-1}.

Fluorescence of urease was measured by F-4500 Hitachi Fluorescence spectrophotometer (Japan), with a computer recording the emission spectrum automatically and processing under windows. The excitation wavelength was 280 nm. Emission spectrum was recorded from 300 to 450 nm. Urease concentration was 0.12 mg ml^{-1}.

Circular dichroism (CD) spectra were recorded by a Jasco J-500 automatic-recording spectropolarimeter (Japan) in a quartz cell with a path length of 0.2 cm. Urease concentration was 0.12 mg ml^{-1}. Scanning wavelength was from 200 to 250 nm under the condition of sensitivity 2 mdegrees.

All the measurements were performed at least twice.

3. Results

3.1. Measurements of enzymatic activity

The biochemical significance of urease is that it catalyzes the hydrolysis of urea to form ammonia and carbamate at a rate which is 10^{14} times faster than uncatalyzed reaction [15]. In our experiment, enzymatic activity was experimentally determined by calorimetry, using the substrate's enzymatic catalysis reaction [13]. The method for determination is the same as our previous work on the interaction of cisplatin with urease, and urease activity was expressed in terms of relative activity [8]. Urea concentration was so chosen as to make catalysis reaction rate approach the maximum and ensure enzymatic active sites saturated by the substrate, according to the request of Michaelis constant K_m of enzymatic catalysis reaction in HEPES buffer solution (pH 7.0) [13].

The effect of transplatin on enzymatic activity was measured under urease concentration of 0.12 mg ml^{-1} and 0.3 mg ml^{-1}, respectively. As transplatin was added to the solution of urease, the enzymatic activity gradually decreased to zero with increasing transplatin concentration (Fig. 1). The decreasing rate of enzy-

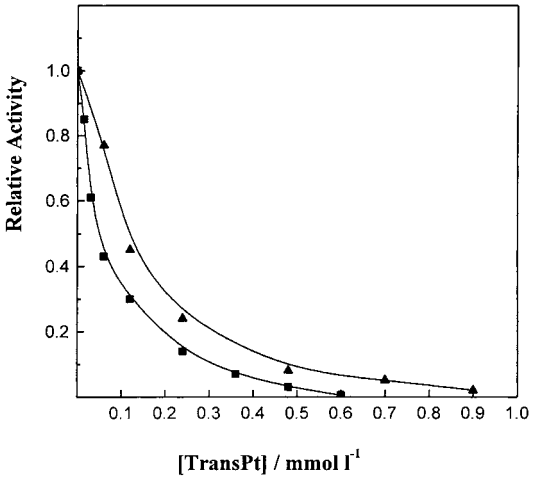

Fig. 1. The effect of transplatin on enzymatic activity (urease): (■), 0.12 mg ml^{-1}; and (▲), 0.3 mg ml^{-1}.

matic activity induced by higher concentrations of transplatin was slower than that by lower concentrations of transplatin. In comparison, the enzymatic activity decreased linearly with increasing cisplatin concentration, while transplatin rendered urease inactive more quickly and exhibited a different decreasing curve (Fig. 2). Urease in solution of 0.12 mg ml^{-1} showed zero enzymatic activity at 1.7 mmol l^{-1} cisplatin [8], while urease in a solution of the same concentration showed zero enzymatic activity at

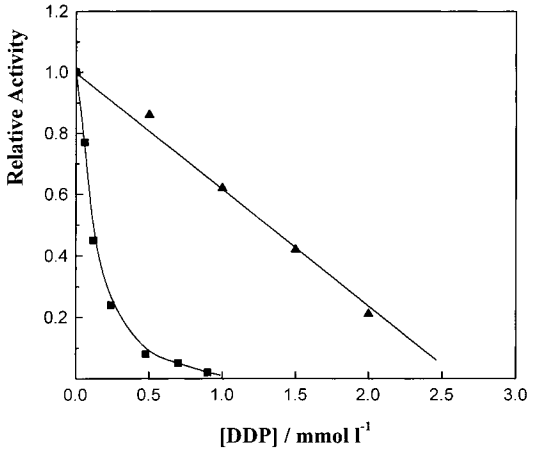

Fig. 2. The different effect of DDP isomer on enzymatic activity (urease): 0.3 mg ml^{-1}; (■), transplatin; and (▲), cisplatin; cited from Ref. [8].

0.6 mmol l^{-1} transplatin. The difference in urease inactivation might arise from the different binding sites in urease, and transplatin displays a stronger binding ability to urease.

3.2. Thermal denaturation of urease

DSC scan was started from 298.2 to 383.2 K and returned from 383.2 to 298.2 K. Urease denaturation enthalpy change (ΔH) was obtained by integration of thermal peak, and the temperature of onset point, but not the peak top, was taken as denaturation temperature (T_m) in order to avoid inaccuracy resulting from thermal lag. Urease itself showed an irreversible denaturation process with an exothermic peak at 361.4 K, possibly due to protein aggregation. Addition of transplatin from 0 to 0.36 mmol l^{-1} decreased urease thermal denaturation enthalpy change within a smaller range, but lowered denaturation temperature dramatically from 361.4 to 333.6 K (Fig. 3, Table 1). It disclosed that urease thermal stability quickly reduced due to binding by transplatin. Unlike transplatin, cisplatin decreased urease denaturation enthalpy change quickly, but almost did not decrease denatura-

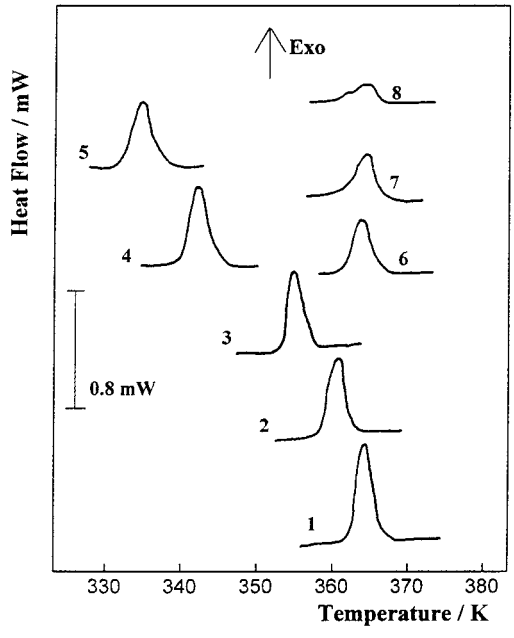

Fig. 3. The DSC curve of urease and effects of transplatin and cisplatin (sample number see Table 1).

Table 1
The effect of transplatin on thermal denaturation of urease by DSC

Sample	Transplatin (mmol l^{-1})	ΔH (mJ)	T_m (K)
1	0	−88.3	361.4
2	0.06	−85.6	358.7
3	0.12	−83.7	352.9
4	0.24	−78.6	340.4
5	0.36	−73.1	333.6
6	0.10[a]	−74.8	361.4
7	0.20[a]	−62.4	361.5
8	0.50[a]	−40.1	362.2

[a] Cisplatin.

tion temperature even at the concentration of 0.5 mmol l^{-1}. This might imply that the interactions between urease and these two isomers differ in binding modes, thereby inducing dissimilar changes in thermal stability.

3.3. Measurement of urease fluorescence

Protein fluorescence mainly arises from Tyr and Trp residues. The emission spectrum under excitation wavelength 280 nm is generally from the contribution of Tyr and Trp residues [16]. Protein fluorescence quenching by small molecules can indicate protein structural information [17]. In this part of experiments, 280 nm was used as excitation wavelength to measure urease fluorescence. Limited by the solubility of transplatin in buffer, the influence of transplatin on urease fluorescence was investigated for transplatin concentration from 0 to 1.7 mmol l^{-1}. From Fig. 4 one could see urease fluorescence was quenched by transplatin. The quenching curve was obtained by the Stern–Volmer plot through (F_0/F) vs. the concentration of transplatin. It showed a linear relation between F_0/F and the concentration of transplatin. 0.6 mmol l^{-1} transplatin resulted in 65% quenching of urease fluorescence intensity, while this solution corresponded to zero enzymatic activity (see Fig. 1). Quenching by cisplatin was measured under the same condition as that of transplatin. Cisplatin manifested weak quenching power against the same concentration of transplatin. However, the quenching extent at zero enzymatic activity by cisplatin of 1.7 mmol l^{-1} is larger than that by transplatin of 0.6 mmol l^{-1}. Fluorescence spectrum of urease indicates that cisplatin and transplatin induce further conformational change, and the effect of transplatin on urease microenvironment around Trp and Tyr residues and then on the overall conformation of urease is greater than that of cisplatin.

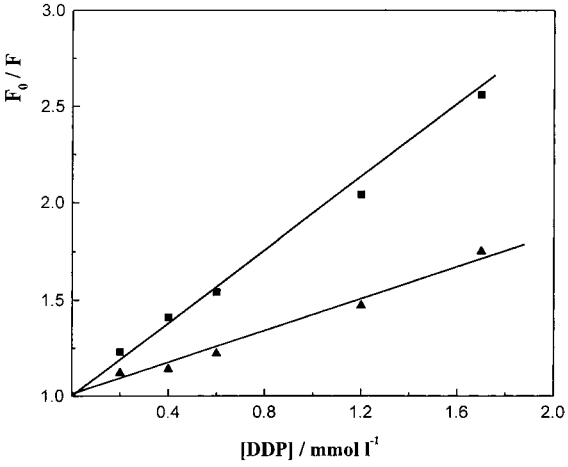

Fig. 4. The quenching curve of urease fluorescence by transplatin and cisplatin: (■), transplatin; and (▲), cisplatin.

3.4. Measurements of urease CD spectra

The secondary structure of proteins can be expressed by the circular dichroism (CD) spectrum in the far-ultraviolet region [18]. Urease contains a greater component of α-helix and β-sheet, as its CD spectrum showed a negative absorption peak at 208 nm and a shoulder at 220 nm [19] (Fig. 5). With the addition of transplatin the negative absorption peak reduced, which meant the secondary structure of urease was changed, the content of α-helix and β-sheet decreased, and a partial unfolding of this protein produced due to binding with transplatin. Urease CD spectral changes due to transplatin and cisplatin are basically the same, since CD spectrum represents a more macro-structural information. But transplatin gives rise to changes in urease CD spectrum at lower concentrations.

4. Discussion

Urease (EC 3.5.1.5) was the first enzyme isolated as a crystalline protein [20]. It is also the first example of

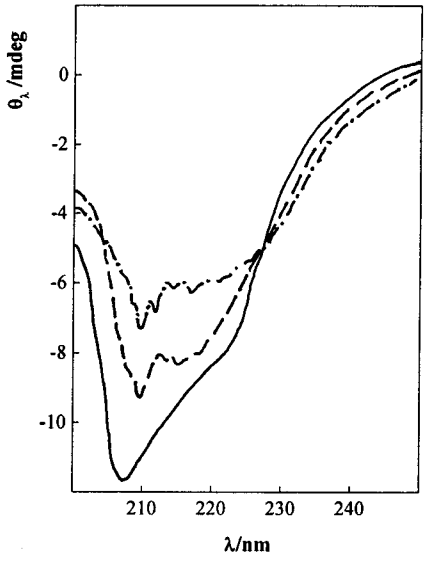

Fig. 5. The effect of transplatin on urease CD spectrum: (—) 0; (–·–), 0.6 mmol l^{-1}; and (——) 1.5 mmol l^{-1}.

a nickel metalloenzyme [21]. It is used to identify artificial kidney [26], which depends on its catalysis ability to hydrolyze urea. Recent investigations found that its activity is implicated in many diseases [6,7]. This paper reports a study on the interaction between transplatin and urease, and makes a comparison on the interaction of urease with transplatin and cisplatin.

The study of microcalorimetry indicates that enzymatic activity is decreased by transplatin. Compared with cisplatin, transplatin possesses a stronger binding affinity with urease. When the concentrations of transplatin and cisplatin in a mixing solution are 0.6 mmol ml^{-1} and 1.7 mmol ml^{-1}, respectively, the 0.12 mg ml^{-1} urease activity is reduced to zero. Furthermore, the variations of enzymatic relative activity with the concentration of transplatin and cisplatin are quite different (Fig. 2). Enzymatic relative activity decreases with the concentration of cisplatin linearly, however, with the concentration of transplatin it decreases in a curve with larger slopes. This implies a stronger and sophisticated interaction between urease and transplatin.

DSC occupies a central role in the study of the conformational stability of protein molecules, giving such information as the enthalpies of thermal denaturation processes, the influence of molecular interactions on the stability of proteins, and the correlation between thermodynamic and structural features of proteins [22]. Thermal denaturation of urease produced an exothermic peak in DSC experiment, since urease is a large molecular protein and temperature elevation makes it aggregate before its tertiary structural change starts, which often shows an endothermic process. Lower concentration transplatin decreases urease denaturation temperature dramatically, from 361.4 to 333.6 K under experimental condition; however, the variation of urease thermal denaturation enthalpy change is not significant. It indicates that transplatin very rapidly decreases enzymatic thermal stability, as shown by the obvious decrease in T_m values. In course of time, cisplatin leads to a large decrease in urease thermal denaturation enthalpy, though no remarkable T_m effects at similar concentrations with transplatin.

Though transplatin displays different influences on urease activity and thermal stability from cisplatin, the mode that transplatin induces urease CD and fluorescence spectral change is quite similar to cisplatin, as seen from the features of spectra. It implies that, at least, they possess partly similar binding sites in urease, since they are isomers. Transplatin reduces urease CD negative absorption peak and quenches urease fluorescence, which represent urease conformational change. Inspection of these characteristics with results of microcalorimetry and DSC reveals that transplatin leads to not only enzymatic inactivation, but to a further conformational change by higher binding affinity with urease than in case of cisplatin. For transplatin, before enzymatic activity reduces to zero, the urease CD spectral change is smaller (Fig. 5); while urease fluorescence quenching has been down to 65% when the relative activity was zero.

It has been reported that platinum binds to protein by Pt–S and Pt–N bonds [23,24]. Inspecting the interaction of actin with transplatin and cisplatin, Zeng et al. [5] suggested that the different effects of cisplatin and transplatin on proteins conformation and action arise from different types of reaction of drugs with protein. In comparison with cisplatin, there are various Pt–S and Pt–N binding sites in protein complex with transplatin. Therefore, transplatin strongly influences the conformation of protein [25]. In our present work on urease conformational change by transplatin and cisplatin, transplatin still shows a greater binding affinity with urease than cisplatin.

In summary, both transplatin and cisplatin give rise to urease inactivation and enzymatic conformational change. Their differences exhibited in the decreasing curve of enzymatic activity, DSC thermal denaturation and spectra characteristics suggest their different binding sites and binding process on the interactions with urease. These will excite further interests in the study of the interaction between them and other proteins, and serve for drug toxicity mechanism exploration.

Acknowledgements

We thank Dr. Zeng for so generously supplying transplatin. This work was supported by the National Natural Science Foundation of China, Grant No. 29633020.

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