Study on the interactions between cisplatin and cadherin

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Abstract: Cisplatin is an important platinum drug in the chemotherapy of many malignant tumors in clinical application. It is well accepted that its main target is DNA molecule. Recent studies have shown that platinum drugs may also act on some important functional proteins in the human body and have complex effects on their efficacy. E-cadherin is newly discovered glycoproteins associated with the occurrence and development of some tumor. In this study, the interaction between cisplatin and E-cadherin was explored by fluorescence spectroscopy and atomic force microscopy. The fluorescence spectroscopy results indicated that cisplatin produced fluorescence quenching effect on the E-cadherin, and the binding constant K_b was 3.20×10^6 (25℃), 1.36×10^6(31℃) and 8.22×10^5 L mol^{-1} (37℃). The thermodynamic parameters $\Delta H<0$, $\Delta S<0$, $\Delta G<0$, reveal that the fluorescence quenching effect of cisplatin on E-cadherin is static quenching, which mainly depends on hydrogen bond and van der Waals. The results of atomic force microscopy revealed that there was a staggered interaction between the intermolecular domains of E-cadherin to form a long ball chain structure. The addition of cisplatin could significantly affect the staggered effect of the E-cadherin molecules. The results of this study reveal that E-cadherin is a very important target for the cisplatin.

Keywords: Cisplatin; E-cadherin; Fluorescence spectrometry; Atomic force microscopy

1 Introduction

No matter in developed or developing countries, the high mortality rate of various types of cancer makes it one of the most important diseases endangering human health. Chemotherapy is one of the main methods in cancer treatment. At present, the widely used anticancer drugs include natural and synthetic alkaloids, taxanes and platinum anticancer complexes. Among them, platinum complexes

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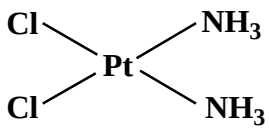
play an important role in the treatment of various cancers. It is estimated that about 50% of patients use platinum drugs in the chemotherapy of various cancers [1].The annual sales of platinum anticancer drugs in the world has now reached the order of $2 billion [2]. Platinum anticancer drugs were first discovered by American scientists Rosenberg and others in the 1960s. Rosenberg et al. observed that *E. coli* changed from sausage like to filament like structure after a period of current was applied to *E. coli* in ammonium chloride buffer with a platinum electrode, which was due to the inhibition of its cell division[2]. Subsequently, it was found that this phenomenon was mainly caused by the hydrolysate produced on the platinum electrode. In 1969, Rosenberg et al finally discovered that cisplatin (CIS dichlorodiaminoplatinum, cisplatin, see structural formula 1) has strong anticancer activity [3]. This important discovery opens up a new field of anticancer research of metal complexes. Based on the structure-activity relationship of cisplatin against cancer, scientists try to synthesize new high-efficiency and low toxicity anticancer metal complexes.



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Chemical structure of Cisplatin (CIS dichlorodiaminoplatinum)

Recent studies have shown that many small drug molecules can interact with proteins in human body and ultimately affect their pharmacological activities [4].The main target of cisplatin is DNA molecule. Recently, it has been found that cisplatin can interact with some proteins to affect their final activity [1]. For example, cisplatin can interact with ubiquitin proteins and can be affected by coexisting glutathione[5]. Cisplatin DNA complex can interact specifically with high mobility protein (HMG domain protein). This specific interaction can have a significant impact on the recognition, clearance and repair of platinum drugs [6].

Recently, researchers found that a large class of transmembrane glycoproteins named E-cadherin are important signs of the occurrence and development of some cancers [7-9]. Cadherins are a kind of calcium ion dependent adhesion glycoproteins. Each cadherin molecule plays an important role in cell-cell adhesion after binding 12 Ca^{2+}. The research report published in Nature confirmed that E-cadherin has an important relationship with the occurrence and development of cancer [8, 9]. In addition, recent

studies have shown that the increased concentration of E-cadherin in serum is closely related to tumorigenesis, invasion and metastasis. In particular, the concentration of soluble cadherin in serum was found to be significantly higher in patients with gastric cancer than in healthy controls, which may be due to the degradation of E-cadherin on the surface of tumor cells and its release into the blood. Therefore, the concentration of soluble cadherin in serum can be used as an important indicator for the prevention, diagnosis and treatment of gastric cancer [10, 11].

Cisplatin can interact with human serum albumin in blood, which can significantly influence its pharmacological activity[12]. This result implied that cisplatin may interacted with other protein in serum such as E-cadherin. To the best our knowledge, there is no systematic study and report on the interactions of cisplatin and cadherin. In this study, we used fluorescence spectroscopy and atomic force microscopy to analyze the interaction between cisplatin and cadherin.

2 Experimental

2.1 Materials and instruments

Cisplatin (CIS dichlorodiaminoplatinum (II), cisplatin) was purchased from Kunming Guiyan Pharmaceutical Co., Ltd. E-cadherin human (recombinant) was purchased from sigma Aldrich (Shanghai) Co., Ltd. The original concentration of cadherin was 0.5mg/ml, and the concentration used in the experiment was diluted with phosphate buffer solution. The highly oriented pyrolytic graphite sheet (HOPG) used as the substrate in atomic force microscope observation is the product of Mikro Masch company in Estonia. The pure water used in the experiment was purified by milli-Q system, and the resistivity reached more than 18m·Ω. The fluorescence spectrophotometer is F-7000 produced by Hitachi, Japan. The temperature-controlled water bath device equipped with this spectrometer can measure the fluorescence spectrum at different temperatures. The atomic force microscope instrument used in the experiment was molecular imaging Picoplus II system (molecular imaging, USA). The specification of imaging scanning head is a one hundred μm type, which was corrected by standard sample before use. The probe used for scanning is manufactured by Mikromasch, model NSC35, the radius of curvature of the AFM tip is less than 10 nm, the force constant is 7.5n/m, the scanning speed is less than 1lines/s, the resonance frequency of imaging is 130 kHz, and the scanning is carried out in tapping mode under room temperature conditions.

2.2 Experimental method

2.2.1 Fluorescence spectrometry

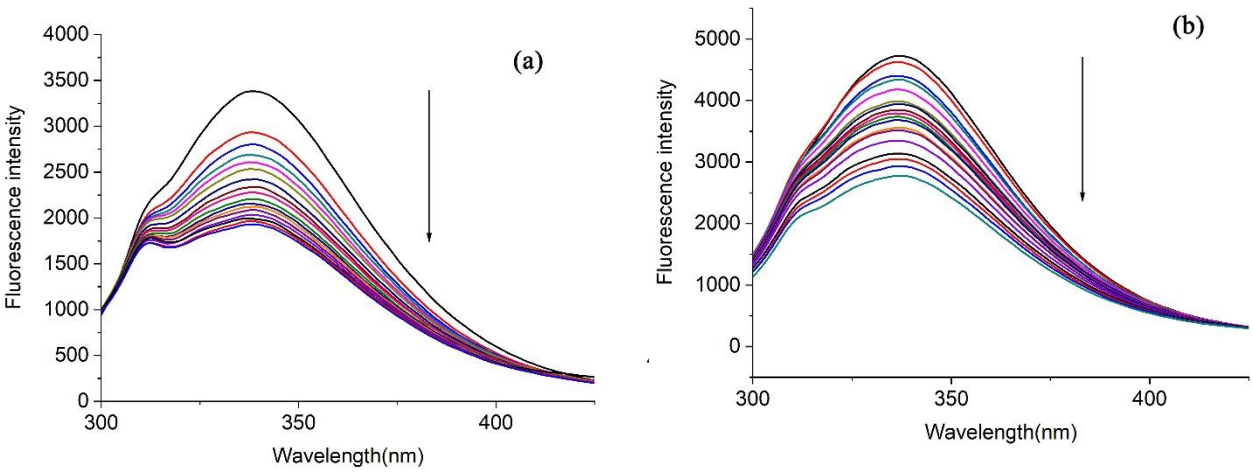
3 mg of pure cisplatin powder was weighed, and dissolved in 10 ml of ultrapure water. The cisplatin storage solution has a concentration of 1mmol/L, and store it at 4 ℃ before use. This 1 mmol / L cisplatin storage solution was diluted with ultrapure water for three gradients to a concentration of 1 μmol/L. 10 μL of cadherin (0.5mg/ml) solution was put into a 1ml centrifuge tube, and added 410 μL phosphoric acid buffer solution (0.2 mol / L, pH = 7.4) to obtain a concentration of 0.2 μmol / L dilute solution of cadherin. In this cadherin solution, 1 μmol/L cisplatin solution was gradually added, keeping the system in a constant temperature water bath in a centrifuge tube for 5 min at the corresponding temperature. The solution was transferred into a micro quartz cuvette, and measured by fluorescence spectrophotometer using 280 nm as the excitation wavelength, recording its fluorescence spectrum at the range of 290-500 nm.

2.2.2 preparation and characterization of cisplatin-cadherin samples

0.2 μM cadherin solution interacting with cisplatin was diluted ten times with 0.2M phosphate buffer solution and used to prepare atomic force microscope samples. The final concentration of cadherin was 0.02 μM for preparing AFM sample. 50 μL of the final solution was added dropwise to the newly cleaved high orientation pyrolytic graphite (HOPG) surface. The droplets removed after 10 min of adsorption on the HOPG, and the surface are soaked in deionized water for three minutes, and then gently rinsed with ultrapure water more than 10 times to remove the loosely adsorbed cadherin. Finally, the samples are dried with inert argon gas, and stored in a desiccator before imaging with an atomic force microscope.

3. Results and discussion

3.1 Fluorescence quenching of cadherin by cisplatin



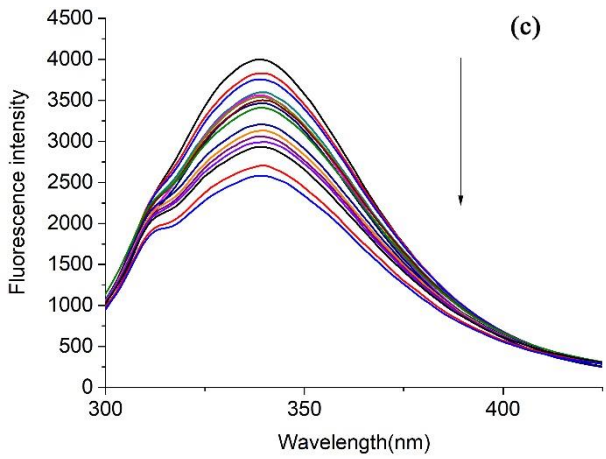


Fig. 1 shows the fluorescence spectrum after gradually adding cisplatin to E-cadherin solution under different temperature conditions. （a）25℃，（b）31℃（c）37℃

Fig. 1 shows the effect of cisplatin, a typical platinum drug, on the fluorescence spectrum of cadherin under different temperature conditions (25 ℃, 31 ℃ and 37 ℃). After the excitation of cadherin at 280 nm, the maximum fluorescence emission peak appeared at 338.6 nm. This fluorescence can be attributed to the tryptophan residues in cadherin structure. With the gradual addition of cisplatin solution, the intensity of its maximum fluorescence emission peak gradually decreased, as shown in Fig. 1 (a) (b) (c). The results show that cisplatin can effectively quench the fluorescence emission of cadherin, indicating that there is a strong interaction between them.

The stern Volmer equation can be used to analyze the fluorescence quenching process [13, 14]

$$\frac{F_0}{F} = 1 + K_{sv}\,[Q] = 1 + K_q \tau_0 [Q]$$

In this formula, F_0 is the fluorescence intensity of the solution when cisplatin is not added, F is the fluorescence intensity of the cadherin solution after cisplatin is added, [Q] is the concentration of cisplatin, K_{SV} is the quenching constant, and K_q is the quenching rate constant. τ_0 is the average lifetime of fluorescent molecules in the absence of quenchers.

The quenching constants Ksv calculated using stern Volmer equation under different temperature conditions are listed in Table 1. It can be seen from table 1 that the quenching constant Ksv decreases with the increase of temperature, indicating that the quenching of cadherin by cisplatin may be static quenching. In addition, K_q was calculated from the K_{SV} values is much larger than the maximum

dynamic quenching rate constant (2.0× 10^{10} L / (mol · s)[15], based on the fluorescent lifetime of biomacromolecules is generally in the order of 10^{-8} s [16]. This further reveal that the quenching of cadherin by cisplatin is a static quenching process, and a complex may be formed between cisplatin and cadherin. In the static interaction, the relationship between the quenching fluorescence intensity and the quencher can be utilized to the expression of the binding constant between the fluorescence quenching molecules. The binding constant (K_b) and the number of binding sites (n) of cisplatin on cadherin can be analyzed by the following static quenching equation [17].

$$\log\left(\frac{F_0 - F}{F}\right) = \log K_b + n\log[Q]$$

Where F_0 and F are the fluorescence intensity of the solution without and with cisplatin, respectively, and the intercept is $\log k_b$. Table 1 lists the calculated values of K_b and n under different temperature conditions. It can be seen from the values in Table 1 that K_b decreases significantly with the increase of temperature, indicating that the stability of the complex of cadherin and cisplatin decreases significantly with the increase of temperature. The n value at room temperature is approximate 1, indicating that cisplatin has a binding site on cadherin.

3.2 Thermodynamic properties of interaction between cisplatin and cadherin

Table 1 Quenching constant and thermodynamic parameters of Cis-Cad（Cis is Cisplatin; Cad is cadherin）

	Ksv **(L mol^{-1})**	**K_b** **(L mol^{-1})**	n	**△H** J·mo l^{-1}	**△S** J·mo l^{-1}	**△G** J·mo l^{-1}·K^{-1}
Cis+Cad(25℃)	2.07×10^7	3.20×10^6	0. 889			-1.71 ×10^4
Cis+Cad(31℃)	1.66×10^7	1.36 ×10^6	0. 865	-3.92×10^4	-73.96	-1.67 ×10^4
Cis+Cad(37℃)	1.17×10^7	8.22×10^5	0. 86			-1.63×10^4

In the current research system, the temperature change is relatively small, and the enthalpy change △H of the binding reaction of cisplatin and cadherin can be approximately considered as a constant. Then the thermodynamic parameters free energy change △G, enthalpy change △H and entropy

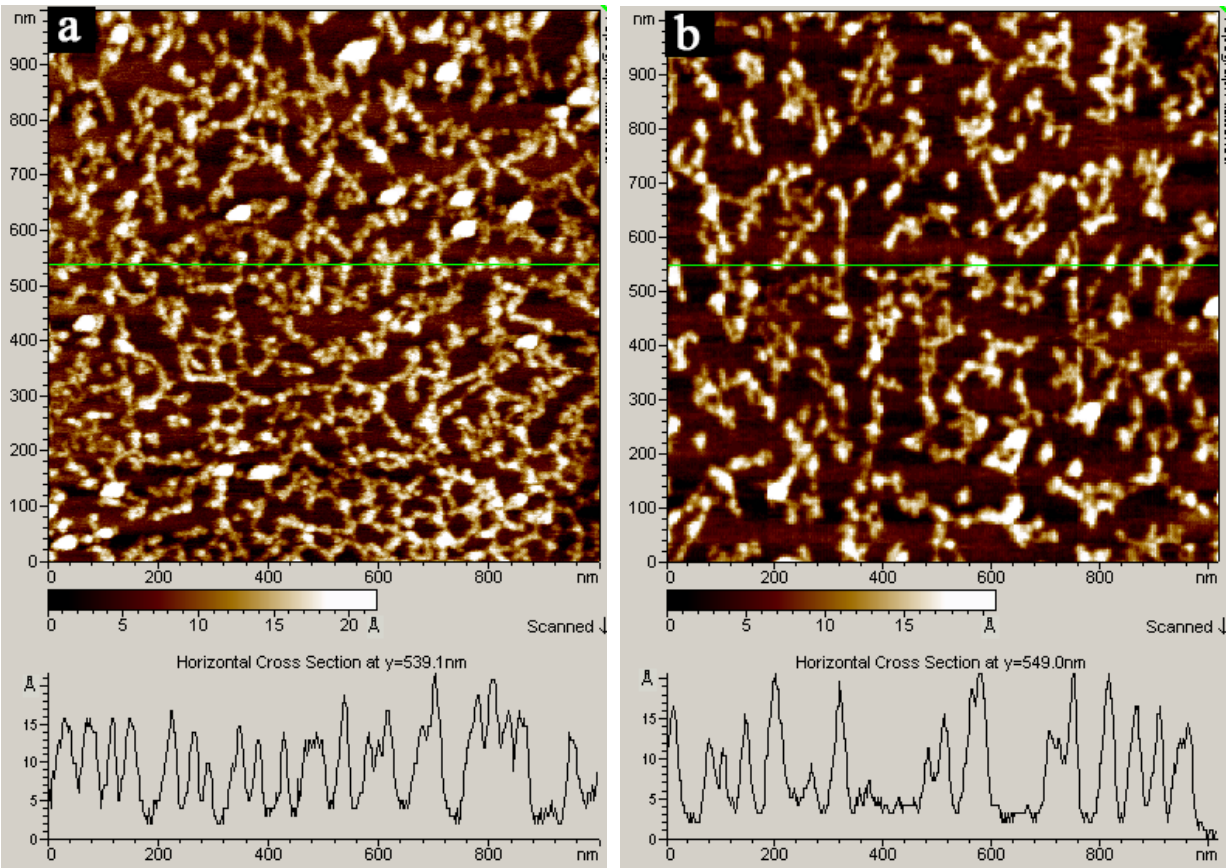
change Δ S can be obtained by the following free energy formula and van't Hoff equation. Table 1 lists the calculated △H, Δ S and △G in the experiment,.

$$\triangle G = -R\,T\,\ln K$$

$$\triangle G = \triangle H - T\triangle S$$

The negative value of △G indicates that the binding of cisplatin and cadherin is a spontaneous process. △H < 0, indicating that the formation of the complex is an exothermic process. The thermodynamic parameters of the interaction between small molecules and proteins can be used to judge the type of force between them. According to the research on the relationship between thermodynamic data and various forces in the process of binding small molecules to proteins by Ross et al.[18], in the present experiment, △H and ΔS <0, indicating that the binding process is dominated by hydrogen bonding and Van der Waals force.

3.3 Analysis of atomic force microscope (AFM) imaging results



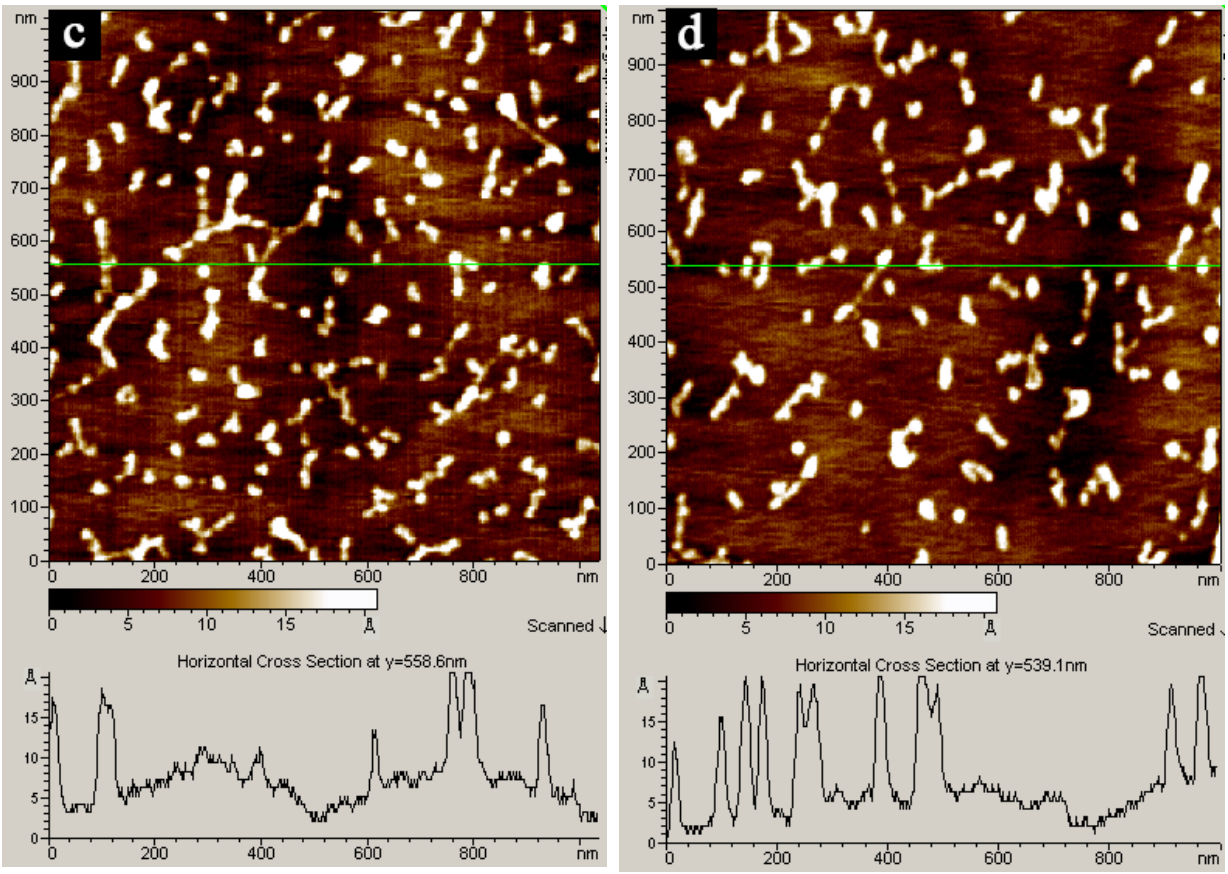


Fig. 2 The representative AFM image of cadherin（a）, Cis-Cad at 25℃ (b), Cis -Cad at 31℃ (c) and Cis -Cad at 37℃ (d) on HOPG surface.

AFM has the advantages of simple sample preparation and intuitive imaging in the research of single molecules. In recent years, atomic force microscopy can be used to study proteins and their assembly structures at the single molecule level [19-25]. Analyzing the adsorption behavior and assembly structure of biomacromolecules at the single molecule level can be of great significance for the study and cognition of their physiological activities. The high resolution structure and assembly structure of human serum albumin adsorbed on the surface of HOPG can be observed by atomic force microscopy [22]. In this study, on the basis of fluorescence spectroscopy, the structure of cadherin and the effect of cisplatin on cadherin assembly were further studied by atomic force microscopy.

Fig. 2 shows a typical AFM image before and after the interaction of cadherin and cisplatin. It can be seen that the morphology of cadherin changed significantly after the interaction with cisplatin. In Fig. 2 (a), cadherin that does not interact with cisplatin appears as a spherical chain structure and a cross chain structure. After interaction with cisplatin, the length of the chain structure formed between

cadherin molecules is significantly reduced, as shown in Fig. 2 (b). In comparing the atomic force microscope images under different temperature conditions (Fig. 2 (b), (c) and (d)), the results show that the interaction temperature has a significant effect on the assembly structure of cadherin molecules. With the increasing of the temperature, the chain structure formed between cadherin molecules is further reduced. In Fig. 2 (d), it is judged that there are some cadherin oligomers according to their morphology.

The high-resolution structure of cadherin outside the cell membrane has been reported recently [26-29]. The results of crystal structure study show that each cadherin molecule outside the cell membrane is composed of five repeated domains connected head-to-end（**EC domain 1-5**）[30]. Each domain is a cylindrical structure with a three-dimensional dimension of 4.5 nm × 2.5 nm × 2.5 nm. Because there is a staggered interaction when every two domains are connected, the whole cadherin molecule appears as a chain molecule with a certain arc, and the total length of its head to tail is 20.7 nm. There is a hydrophobic cavity at the N-terminal (EC1 domain) of cadherin molecule, and there is also a tryptophan residue at its distal side. Liquid phase NMR and crystal structure studies show that stable dimers can be formed between two cadherin molecules, in which tryptophan residues of each cadherin molecule can enter the hydrophobic cavity of the other molecule for chain exchange to form a stable structure[30].The results of high-resolution electron microscopy show that cadherin molecules can also form more complex structures. For example, three cadherin molecules can form a cross structure, and four cadherin molecules can form a four chain structure [26]. In addition to the N-terminal EC1 domain, the force spectrum experimental study shows that there may also be cross domain interaction between cadherin molecules, such as the interaction between EC1 domain and other domains of another molecule [27].

The chain structure and the crossed chain structure observed by the atomic force microscope in Fig. 2 (a) can be explained as follows. The spherical chain structure in Fig. 2 (a) may be formed by the interaction between the EC1 domain of the cadherin molecule and other domains of the cadherin molecule due to the cross interaction derived from chain exchange. The cross structure may be formed by the chain exchange of tryptophan residues at the N-terminal (EC1 domain) of three cadherin molecules, which indicates that there is an interdisciplinary interaction between domains among

cadherin molecules. In Fig. 2 (b), when the cadherin molecule interacts with cisplatin, the length of the chain structure significantly decreases, mainly because cisplatin interacts with the tryptophan residue at the N-terminal (EC1 domain) of the cadherin molecule, resulting in fluorescence quenching. As confirmed by the fluorescence results, the interaction between the EC1 domain of cadherin and other intermolecular domains of cadherin may be affected after the cisplatin binding, which is not conducive to the formation of a long chain structure. As the interaction temperature increases to 31 ℃ and 37 ℃, as shown in the atomic force micrographs in Fig. 2 (c) and (d), the chain structure further decreases, mainly because higher temperature is more unfavorable for the cross interaction between the cadherin EC1 domain and other cadherin intermolecular domains. In Fig. 2 (d), it is judged that there are some cadherin oligomers according to their morphology. The average height of the measured cadherin molecule polymer is around of 2 nm, which is close to the calculated value of the crystal structure research results (the diameter of cadherin molecule is about 2.5 nm). The formation of cadherin oligomers among molecules is due to the chain exchange of tryptophan in the N-terminal EC1 domain among multiple cadherin molecules, which is consistent with the results of NMR and crystal structure studies on the formation of cadherin oligomers.

4 Conclusion

In this study, the interaction between cisplatin and cadherin was analyzed by fluorescence spectroscopy and atomic force microscopy. The results of fluorescence spectroscopy show that cisplatin can significantly quench the fluorescence emitted by cadherin after excitation. It is inferred that this is a static quenching process by calculating the quenching constant and its trend with temperature. The entropy, enthalpy and free energy values of the interaction between cisplatin and cadherin indicate that their binding is a spontaneous process dominated by hydrogen bonds and Van der Waals forces. High resolution atomic force microscopy (HRAFM) showed that long length of the spherical chain structure formed between cadherin molecules. After the interaction with cisplatin, these structures decreased significantly, and became more significant with the increase of temperature. At higher temperature, the formation of a oligomers structure between cadherin molecules was observed. The results of atomic force microscopy revealed that there was a cross interaction between the domains of cadherin molecules to form a spherical chain structure, and the addition of cisplatin can significantly affect this cross action and thus affect the assembly structure of cadherin molecules. The results of this study reveal that cadherin is a very important target that affects the effective activity of cisplatin.

References

[1] Klein, A. V., and Hambley, T. W. (2009) Platinum Drug Distribution in Cancer Cells and Tumors, *Chem. Rev. 109*, 4911-4920.

[2] Alderden, R. A., Hall, M. D., and Hambley, T. W. (2006) The Discovery and Development of Cisplatin, *J. Chem. Educ. 83*, 728-734.

[3] Rosenberg, B., Camp, L. V., Trosko, J. E., and Mansour, V. H. (1969) Platinum compound. a new class of potent antitumour agents, *Nature 222*, 385-386.

[4] Genhu, L., Liting, L., Xiaojun, D., Yinmao, W., and Bolin, G. (2010) Investigation on the Competition Interaction of Ferulic Acid and Paeonol with Human Serum Albumin by High-performance Affinity Chromatography, *Acta Chim Sinica 68*, 55-61.

[5] Peleg-Shulman, and Gibson, D. (2001) Cisplatin-Protein Adducts Are Efficiently Removed by Glutathione but Not by 5¢-Guanosine Monophosphate, *J. Am. Chem. Soc. 123*, 3171-3172.

[6] Dunham, S. U., and Lippard, S. J. (1997) DNA sequence context and protein composition modulate HMG-domain protein recognition of cisplatin-modified DNA, *Biochemistry 36*, 11428-11436.

[7] Nagar, B., Overduin, M., Ikura, M., and Rini, J. M. (1996) Structural basis of calcium-induced E-cadherin rigidification and dimerization, *Nature 380*, 360-364.

[8] Guilford, P., Hopkins, J., Harraway, J., McLeod, M., McLeod, N., Harawira, P., Taite, H., Scoular, R., Miller, A., and Reeve, A. E. (1998) E-cadherin germline mutations in familial gastric cancer, *Nature 392*, 402-405

[9] Perl, A.-K., Wilgenbus, P., Dahl, U., Semb, H., and Christofori, G. (1998) A causal role for E-cadherin in the transition from adenoma to carcinoma, *Nature 392*, 190-193

[10] Katayama, M., Hirai, S., Kamihagi, K., Nakagawa, K., Yasumoto, M., and Kato, I. (1994) Soluble E-cadherin fragments increased in circulation of cancer patients, *Br. J. Cancer 69*, 580-585.

[11] Tsalikidis, C., Papachristou, F., Pitiakoudis, M., Asimakopoulos, B., Trypsianis, G., Bolanaki, E., Syrigos, K. N., and Simopoulos, C. (2013) Soluble E-cadherin as a diagnostic and prognostic marker in gastric carcinoma, *Folia Medica 55*, 26-32.

[12] Ivanov, A. I., Christodoulou, J., Parkinson, J. A., Barnham, K. J., Tucker, A., Woodrow, J., and Sadler, P. J. (1998) Cisplatin Binding Sites on Human Albumin, *Journal of Biological Chemistry 273*, 14721-14730.

[13] Yong-hui, S., Hua, L., Jia-juan, S., and Dong-hu, X. (2010) Studies on the interaction between anticancer drug hydroxycamptothecin and bovineserum albumin by fluorescence spectroscopy, *Chin J Pharm Anal 30*, 59-62.

[14] Nanda, R. K., Sarkar, N., and Banerjee, R. (2007) Probing the interaction of ellagic acid with human serum albumin: A fluorescence spectroscopic study, *Journal of Photochemistry and Photobiology A: Chemistry 192*, 152–158.

[15] Sun, G.-P., Wan, X., Xu, S.-P., Wang, H., Liu, S.-H., and Wang, Z.-G. (2008) Antiproliferation and apoptosis induction of paeonol in human esophageal cancer cell lines, *Diseases of the Esophagus 21*, 723-729.

[16] Zhong, L., Jie, H., Yeli, H., Bo, C., Zhangyouhong, and Guolin, Z. (2008) Interaction of Anticancer Drug Resveratrol and Human Serum Albumin by Fluorescence Spectroscopy, *J .Wuhan Univ 54*, 457 - 461.

[17] Feng, X., Jin, R., Qu, Y., and He, X. (1996) Studies on the Ions' Effect on the Binding interaction Between HP and BSA, *Chemical Journal of Chinese Universities 17*, 866-869.

[18] Ross, P. D., and Subramanian, S. (1981) Thermodynamics of protein association reactions: forces contributing to stability, *Biochemistry 20*, 3096-3102.

[19] Aiguo, W., Gang, W., and Zhuang, L. (**2004**) Biological Samples Investigated by Atomic Force Microscopy, *Chinese Journal of Analy tical Chemistry 32*, 1421-1425.

[20] Liu, Z. G., Zu, Y. G., Fu, Y. J., Zhang, Z. H., and Meng, R. H. (2008) Assembling and Imaging of His-Tag Green Fluorescent Protein on Mica Surfaces Studied by Atomic Force Microscopy and Fluorescence Microscopy, *Microscopy Research and Technique 71*, 802-809.

[21] Liu, Z. G., Meng, R. H., Zu, Y. G., Li, Q. Y., and Yao, L. P. (2009) Imaging and Studying Human Topoisomerase I on Mica Surfaces in Air and in Liquid by Atomic Force Microscopy, *Scanning 31*, 160-166.

[22] Peng, X., Fu, H. R., Liu, R. S., Zhao, L., Zu, Y. G., Xu, F. J., and Liu, Z. G. (2015) Adsorption of Human Serum Albumin Onto Highly Orientated Pyrolytic Graphite Surface Studied by Atomic Force Microscopy, *Scanning 37*, 158-164.

[23] Li, L. P., Liu, R. S., Xu, F. J., Zu, Y. G., and Liu, Z. G. (2015) Study on the interactions between anti-cancer drug oxaliplatin and DNA by atomic force microscopy, *Micron 76*, 46-51.

[24] Liu, Z. G., Liu, R. S., Zhou, Z., Zu, Y. G., and Xu, F. J. (2015) Structural changes of linear DNA molecules induced by cisplatin, *Biochemical and Biophysical Research Communications 457*, 688-692.

[25] Xu, F.-J., Liu, R.-S., Biao, L.-H., Zu, Y.-G., and Liu, Z.-G. (2016) Study on the Interactions Between Anti-Cancer Drug Oxaliplatin and DNA, *Progress in Biochemistry and Biophysics 43*, 684-690.

[26] He, W., Cowin, P., and Stokes, D. L. (2003) untangling Desmosomal Knots with Electron Tomography, *Science 302*, 109-113.

[27] Pokutta, S., and I.Weis, W. (2007) Structure and Mechanism of Cadherins and Catenins in Cell-Cell Contacts, *Annu. Rev. Cell Dev. Biol. 23*, 237-261.

[28] Roya, F. V., and Berx, G. (2008) The cell-cell adhesion molecule E-cadherin, *Cell. Mol. Life Sci. 65*, 3756 - 3788.

[29] Li, Y., Altorelli, N. L., Bahna, F., Honig, B., Shapiro, L., and III, A. G. P. (2013) Mechanism of E-cadherin dimerization probed by NMR relaxation dispersion, *PNAS 110*, 16462–16467.

[30] Boggon, T. J., Murray, J., Chappuis-Flament, S., Wong, E., Gumbiner, B. M., and Shapiro, L. (2002) C-Cadherin Ectodomain Structure and Implications for Cell Adhesion Mechanisms, *Science 296*, 1308-1313.