Electrochemical and Spectroscopic Studies on the Interaction between Macrocyclic Nickel (II) Complex and DNA

S. S. ZHANG, S. Y. NIU, G. F. JIE AND K. JIAO*
College of Chemistry and Molecular Engineering,
Qingdao University of Science and Technology
Qingdao, Shandong 266042, P. R. China

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Summary: The macrocyclic nickel complex (MCNi) was prepared and identified by IR, UV/ Vis spectroscopy. The interaction between MCNi and salmon sperm DNA was studied by cyclic voltammetry and UV/ Vis spectroscopy, which confirmed that MCNi could interact with DNA by electrostatic interaction and form a 1:1 MCNi-DNA complex with a binding constant of 363.8 mol^{-1}.l. The sufficient reacting time of this interaction was determined to be about eight minutes. In addition, pH values and DNA concentration had great effect on MCNi-DNA interaction.

Introduction

Deoxyribonucleic acid (DNA) is the most important germ plasm of most organisms. It plays an important role in the process of storing, copying and transmitting germ messages. There are many articles on the interaction between small molecules and DNA since 1960s. These researches have contributed to the understanding of the way of interaction between small molecules and DNA. The binding of small molecules, especially transition metal complexes to DNA and molecular identification are important research subjects in life science. It is generally accepted that there are three kinds of binding models for small molecules to DNA, which refer to intercalative binding, groove binding and electrostatic binding. What is more, these researches are helpful to expound the action mechanism of anticancer drugs. In recent years, it has been reported that many metal complexes have anticarcinogenesis. Among these compounds, people have paid much attention to the platinic coordination complex, such as $Fe[EDTA]^{2-}$, $Cu(phen)_2^{2+}$, RuNi binuclear complex, dicyclopentadienyliron etc. They have the ability of splitting DNA and distinguishing DNA [1-4].

Furthermore, it was reported that macrocyclic complexes have activity of chemistry nuclease [5], which make them have anti-AIDS activity [6], so the researches on the interaction between macrocyclic complex and DNA are helpful to expound the action mechanism of anti-AIDS drugs. In addition, the interaction between MCNi and DNA has not been reported in literature. Therefore, it is significant to study the interaction between MCNi and DNA. In this paper, we have conducted experiments on the interaction between MCNi and DNA by electrochemical and spectroscopic methods with pure MCNi prepared according to the literature [7]. The experimental results have proved that MCNi can interact with DNA by electrostatic interaction. This conclusion would surely bring detailed insight into the action mechanism of macrocyclic complexes and provide useful message for designing new and efficient drugs.

Results and Discussion

Structural characterization of pure MCNi

The IR absorption bands of the product of MCNi are identified as follows: The absorption band at 2922 cm^{-1} is the characteristic band of stretching vibration of N-H. The absorption band at 1635 cm^{-1} is the stretching vibration of C = N; and that at 1512 cm^{-1} is the bending vibration of N-H. The absorption band at 1384 cm^{-1} is the deformation vibration of gem-dimethyl. The absorption band at 2362 cm^{-1} which is the characteristic band of the quaternary amine of ligand disappears in those of MCNi.

There are two absorption peaks in the UV/ Vis spectrum of the pure product. The wavelengths were at 260.0 nm and 511.0 nm respectively. The peak at 260.0 nm was caused by $\pi \rightarrow \pi^*$ electron transition of macracyclic, while the peak at 511.0 nm was the characteristic band caused by d→d electron transition of MCNi. On account of the above spectroscopic

*To whom all correspondence should be addressed.

characterization of pure MCNi and literature [8], the stucture of MCNi can be identified as follows:

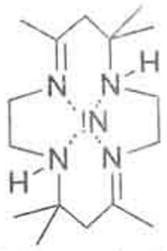

Fig.1 Stucture of Macrocyclic Nickel Complex

Electrochemical studies of the interaction between MCNi and DNA

MCNi has a pair of well-defined redox peaks on the cyclic voltammogram in the base solution of 0.1 mol·l^{-1} Na_2HPO_4-NaH_2PO_4, 0.2 mol·l^{-1} B-R, 0.05 mol·l^{-1} $(CH_2OH)_3CNH_2$-HCl or 0.1 mol·l^{-1} NaOAc-HOAc respectively. Among them, the peaks in 0.1 mol·l^{-1} NaOAc-HOAc are the best. Therefore, 0.1 mol·l^{-1} NaOAc-HOAc was selected as the supporting electrolyte.

Effect of scan rate on the oxidation peak current of MCNi

The relationship curve between I_{pa} and $v^{1/2}$ was showed in Fig. 2. From the figure, we can see that I_{pa} is linear to $v^{1/2}$, which indicates that the electrode process of MCNi is controlled by the diffusion of MCNi.

Effect of reacting time on the oxidation peak current of MCNi

Fig. 3 showed the relationship curve of the oxidation peak current of MCNi and the reacting time of MCNi and DNA after they were mixed. The peak current of MCNi decreases with the increase of the reacting time and reaches a constant value after about eight minutes, which indicates that the reaction of MCNi with DNA has reached an equilibrium state. Consequently, the sufficient reacting time of the reaction of DNA and MCNi is about eight minutes within the dosages of the experiment.

Effect of pH on the oxidation peak current of MCNi

Fig. 4 showed the relationship curve of the oxidation peak current of MCNi and pH. During the experiment, the oxidation peak current increases firstly and then reaches a maximum when pH is 4.15.

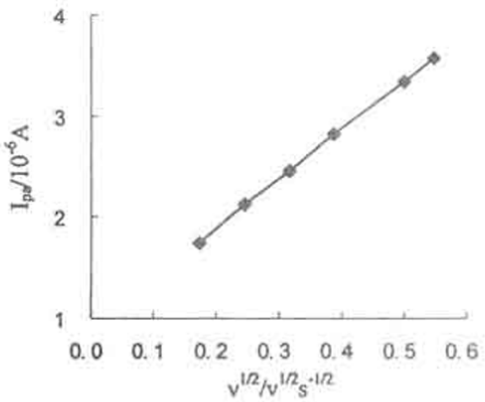

Fig. 2: The relationship curve of $I_{pa}$and $v^{1/2}$ of MCNi C_{MCNi}: 4.17×10^{-5} mol·L^{-1}

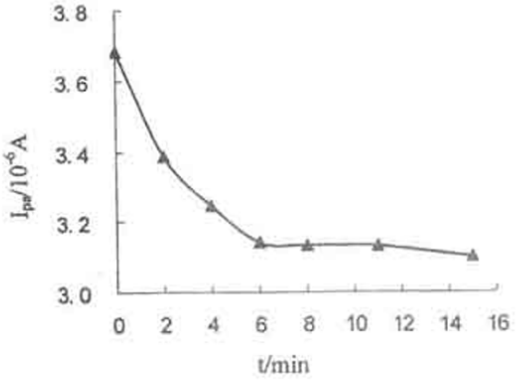

Fig. 3: The relationship curve between I_{pa} and interacting time of MCNi and DNA system. C_{MCNi}: 6.95×10^{-5} mol·l^{-1}; C_{DNA}: 2.81×10^{-4} mol·l

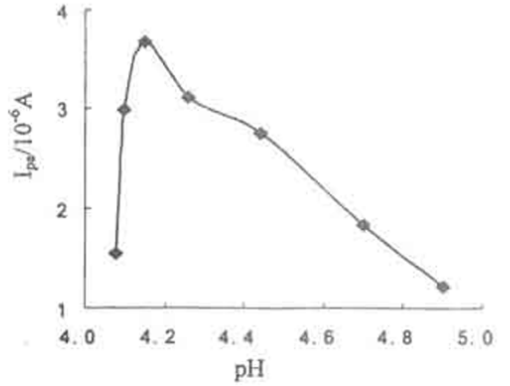

Fig. 4: The relationship curve between I_{pa} and pH of MCNi and DNA system. C_{MCNi}: 6.95×10^{-5} mol·l^{-1}; C_{DNA}: 2.81×10^{-4} mol·l.

After that, it decreases slowly. Consequently, 4.15 was chosen as the best pH of the reaction.

Effect of DNA on the cyclic voltammogram and the peak current of MCNi

Fig. 5 showed the cyclic voltammograms of MCNi before and after adding DNA. The curve 1 displayed the result obtained in the solution of MCNi without DNA. The oxidation peak potential(E_{pa}) was 0.583 V, the reduction peak potential(E_{pc}) was 0.517 V, the formal potential, $E^{o'}$, taken as the average of E_{pc} and E_{pa} was 0.550 V. After adding DNA, the curve 2 showed that the peak current decreased obviously, $E^{o'}$ shifted negatively to 0.489 V, and also no new oxidation-reduction peaks appeared. So MCNi interacting with DNA formed electrochemically non-active complex and caused an equilibrium concentration or the diffusion coefficient of MCNi to be decreased, which resulted in a decrease of the peak current. It is generally accepted that there are mainly three non-covalent action modes between small molecules and DNA: intercalation, groove binding and electrostatic action. Bard has reported [9] that when small molecules interact with DNA, if $E^{o'}$ shifts negatively, the interaction mode is electrostatic interaction. On the contrary, if $E^{o'}$shifts positively, the interaction mode is intercalation. According to the molecule structure of MCNi and the literature [10], the above experimental result can be interpreted as follows: MCNi is positively charged, whereas the sugar- phosphate backbone in DNA is negatively charged, so MCNi interacts with DNA via an electrostatic interaction, which was also verified by the following spectroscopic studies.

Maintaining MCNi concentration, while increasing DNA concentration, the relationship curve of the oxidation peak current of MCNi and DNA concentration was obtained, showed in Fig. 6. With the addition of DNA, the peak current of MCNi decreased obviously. When the concentration of DNA increased to a certain degree, the peak current reached a constant value. Then the conclusion could be drawn that the interaction between MCNi and DNA was saturated.

The binding ratio and the binding constant of the MCNi-DNA complex

It is assumed that DNA and MCNi only produce a single complex DNA-nMCNi according to the reference [11]:

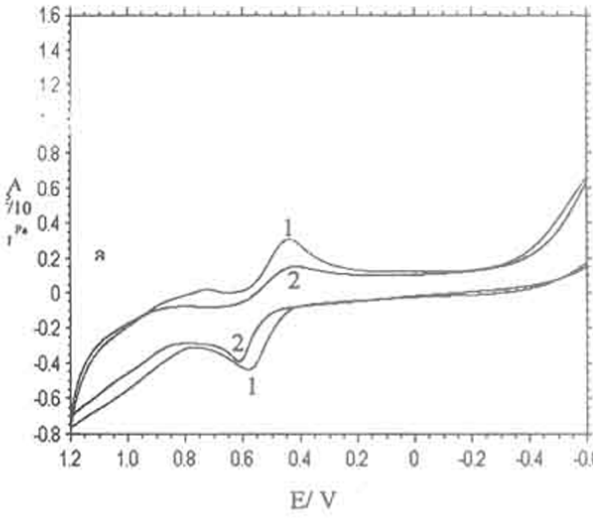

Fig. 5: The cyclic voltammograms of the interaction between MCNi and DNA. C_{MCNi}: 6.95×10^{-5} mol·l^{-1}; (1) C_{DNA}: 0; (2) C_{DNA}: 2.81×10^{-4} mol·l^{-1}.

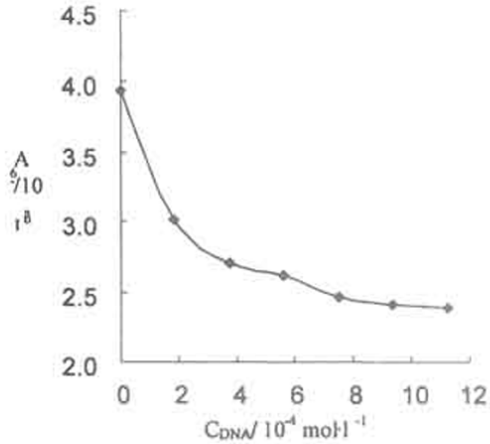

Fig. 6: The relationship curve of I_{pa} and C_{DNA}. C_{MCNi}: 8.34×10^{-5} mol·l^{-1}

DNA + n MCNi $\rightleftharpoons$ DNA-n MCNi (n=1,2,3, ...or 1,1/2,1/3,)

The equilibrium constant can be expressed as follows:

$$\beta = \frac{[\text{DNA} - \text{nMCNi}]}{[\text{DNA}][\text{MCNi}]^{n}} \quad (1)$$

And the following equations can be deduced:

$$\Delta I_{max} = K \cdot C_{DNA} \quad (2)$$

$$\Delta I = K'[\text{DNA-nMCNi}] \quad (3)$$

$$[\text{DNA}] + [\text{DNA-nMCNi}] = C_{DNA} \quad (4)$$

$$\Delta I_{max} - \Delta I = K'(C_{DNA} - [\text{DNA-nMCNi}]) \quad (5)$$

$$\Delta I_{max} - \Delta I = K'[\text{DNA}] \quad (6)$$

Introducing Eqs. (3) and (6) into Eq. (1), leads to :

$$\frac{1}{\Delta I} = \frac{1}{\Delta I_{max}} + \frac{1}{\beta \Delta I_{max}[\text{MCNi}]^n} \quad (7)$$

With different n, there are different relationship curves of ΔI^{-1} and $[MCNi]^{-n}$. According to equation (7), the relationship curve of ΔI^{-1} and $[MCNi]^{-n}$, with the suitable n, should be a straight line if there is only one complex was formed when DNA and MCNi were mixed together. From the slope and intercept of the best line, the binding constant β can be calculated.

The relationship curve of the oxidation peak current of MCNi solution before and after adding DNA was showed in Fig. 7. By different ΔI^{-1} and $[MCNi]^{-n}$ calculated from Fig. 7, the relationship curve of ΔI^{-1} and $[MCNi]^{-1}$ was obtained. As for n=1, the curve is a straight line(r = 0.996), showed in Fig. 8. While for n = 1/3 and 1/2, the curve bends up and down respectively. From the slope and intercept of ΔI^{-1} and $[MCNi]^{-1}$, the binding constant β can be calculated to be 363.8 $l \cdot mol^{-1}$, which is corresponding to the equation DNA + MCNi $\rightleftharpoons$ DNA-MCNi.

UV/Vis spectroscopic studies of the interaction between MCNi and DNA

The UV spectra of DNA solution in the absence or presence of MCNi were showed in Fig. 9. After adding MCNi, the phenomena of hypochromic effect and red shift appeared, which indicates that MCNi could interact with DNA via an electrostatic interaction. The result is consistent with the above electrochemical studies. According to the literature [12], electrostatic interaction between MCNi and DNA caused linear charge density in the backbone strain of DNA to be decreased, the strain winding of DNA increased, as well as the structure of DNA contracted. Therefore, the chromophoric group

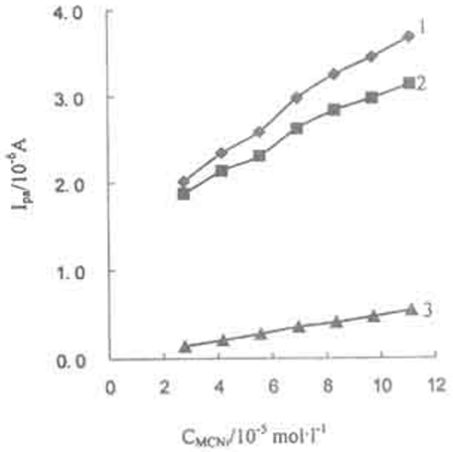

Fig. 7: The relationship curve of I_{pc} and ΔI_{pa} vs. [MCNi] (1)C_{DNA}: 0 (2)C_{DNA}: 3.74×10^{-4} mol·l^{-1} (3)I_{p1}-I_{p2}

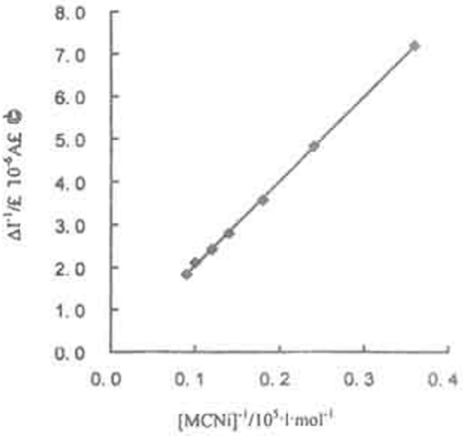

Fig. 8: The relationship curve of ΔI^{-1} vs. $[MCNi]^{-1}$

invaginates and the absorption dipole moment varies, which resulted in the above result of UV spectrum of DNA solution.

In addition, we have examined the binding interactions of EB with DNA in the presence of MCNi, in the hope of providing information about the similarities or differences in the nature of the binding modes of these complexes to DNA. Absorption spectra of EB showed that EB had an absorption peak at 478 nm in the buffer solution. There were phenomena of hypochromic effect and red shift in the presence of DNA. This phenomenon resulted from the intercalation between EB and DNA. It is

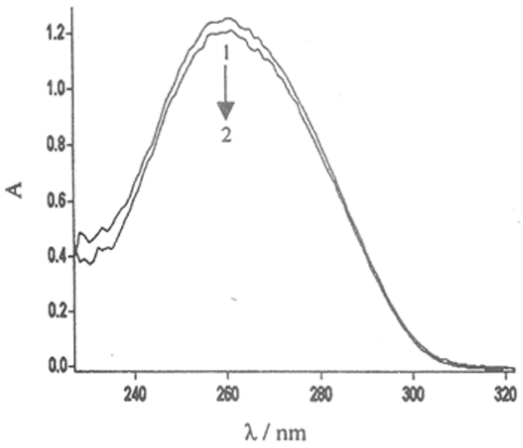

Fig. 9: UV spectra of DNA solution C_{DNA}: 3.12×10^{-4} mol·l^{-1}; C_{MCNi}: (1)0; (2)4.63×10^{-5} mol·l^{-1}

generally recognized that the strong mode of binding of EB to double-stranded nucleic acids results in the intercalation of the planar phenanthridinium ring between adjacent base pairs on the double helix [13-14]. After the addition of MCNi, the absorption peak of EB did not increased, which has proved that the interaction between MCNi and DNA is different from the intercalation between EB and DNA. Based on the above experimental results, the conclusion can be drawn that MCNi could interact with DNA via an electrostatic interaction.

Experimental

Instruments and reagents

CHI 832 electrochemical analyzer was produced by Shanghai Chenhua Instrument Company of China; the three-electrode system was composed of a glassy carbon electrode (GCE) as working electrode, a Ag/AgCl as the reference electrode and a platinum electrode as auxiliary electrode; Cary 50 UV/Vis spectrophotometer was produced by Varian Company of Australian. 510P FT-IR spectrometer was produced by Nicolet Company of the United States; pHS-25 pH meter was produced by Shanghai Leici Instrument Factory of China.

Salmon sperm DNA was purchased from Huashun Biologic Engineering Company of Shanghai. Its concentration was 10 mg·ml^{-1}, used without further purification. 6.95×10^{-3} mol·l^{-1} MCNi was prepared by dissolving 0.0395 g of MCNi in 5 mL doubly deionized water. Ethidium Bromide (EB), purchased from Shanghai Huashun Biochemistry Technology Company, was diluted to the needed concentration, 2.4×10^{-4} mol·l^{-1}. 0.1 mol·l^{-1} NaOAc-HOAc solution, pH 4.15, was used as buffer solution. The other reagents were all analytical reagents prepared with doubly deionized water.

Preparation and characterization of MCNi

A mixture of 13 ml anhydrous ethylenediamine, 10 mL anhydrous alcohol, 36 ml hydriodic acid and 30 mL acetone were added slowly into a round-bottom flask, which was put into a container of frozen water and had kept there for about fourteen hours. The solid appeared was separated by decompressing filtration, followed by washing with acetone and then white crystal of macrocyclic ligand was obtained.

0.01 mol macrocyclic ligand and 0.01 mol nickel nitrate were dissolved in 40 ml anhydrous alcohol completely. After refluxed for an hour, the mixture was filtered. The filtrate was heated in hot water until solid began to form, followed by being cooled in frozen water for several hours. The solid appeared was separated by decompressing filtration and recrystallized from anhydrous alcohol, then the pure macrocyclic nickel complex was obtained.

In addition, UV/ Vis, FT-IR spectra of MCNi was obtained with MCNi solution on the instruments as mentioned above.

Electrochemical studies of the interaction between MCNi and DNA

60 µl of 6.95×10^{-3} mol·l^{-1} MCNi was added to 5 ml of 0.1 mol·l^{-1} NaOAc-HOAc buffer solution. The cyclic voltammogram of MCNi was recorded on CHI 832 electrochemical analyzer with the three-electrode system above. Then different quantities of salmon sperm DNA were added to the solution followed by recording the cyclic voltammograms figure. Instrument parameters are initial potential: -0.6 V, high potential: 1.2 V, low potential: -0.6 V, scan rate: 0.06 V·s^{-1}, sample interval: 0.001 V and quiet time: 2 s.

UV/ Vis studies of the interaction between MCNi and DNA

20 µl of 4.68×10^{-2} mol·l^{-1} DNA was added to each tube of the two colorimetric tubes. And then, 20 µl of 6.95×10^{-3} mol·l^{-1} of MCNi was added to one of

the two tubes. They were then diluted to the desired scale and shaken up. After that, the solutions were kept for eight minutes to react completely.

The UV/ Vis spectra of these samples were recorded on Cary 50 UV-Vis spectrophotometer in 1 cm quartz cuvettes. The range of the scanning wavelengths is from 200 nm to 800 nm.

Conclusions

The interaction between MCNi and DNA was studied by cyclic voltammetry and UV/Vis spectroscopy. In the presence of DNA, the oxidative peak current of MCNi decreased and the formal potential shifted negatively. The absorbance of DNA at its absorption peaks decreased after adding MCNi solution. The conclusion can be drawn that MCNi could interact with DNA by electrostatic interaction and form a 1:1 MCNi-DNA complex with a binding constant of 363.8 mol^{-1}·l.

Acknowledgements

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