研究快报

钌(Ⅱ)多吡啶配合物与 DNA 相互作用的等温滴定量热研究

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Studies on Interaction of Ruthenium(Ⅱ) Polypyridyl Complex with DNA Using Isothermal Titration Calorimetry

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Abstract: The interaction of ruthenium(Ⅱ) polypyridyl complex with DNA has been studied by isothermal titration calorimetry (ITC). The results show that complex $[Ru(phen)_2PMIP]^{2+}$ {phen=1,10-phenanthroline, PMIP=2-(4-methylphenyl)imidazo[4,5-*f*]1,10-phenanthroline} interacts with calf thymus DNA (CT DNA) in terms of a model for a single set of identical sites through intercalation. The results are in agreement with our previous observations from spectroscopic methods and viscosity measurements. In addition, the results further show that the driving force for DNA binding with the complex is mainly driven by the enthalpy changes, and the contribution from the entropy changes to this driving force is negligible.

Key words: Ruthenium(Ⅱ) polypyridyl complexes; CT DNA; ITC; Interaction

0 Introduction

Over the past decade, the interaction of ruthenium(Ⅱ) polypyridyl complexes with DNA has attracted much attention and has been extensively studied. The use of these complexes as probes of DNA structure and sites has proven to be quite fruitful[1~5]. Despite a considerable amount of literature reports, the nature and the mechanism of DNA binding to these complexes have still not been answered clearly. Therefore, it calls for extensive and further studies using new methods.

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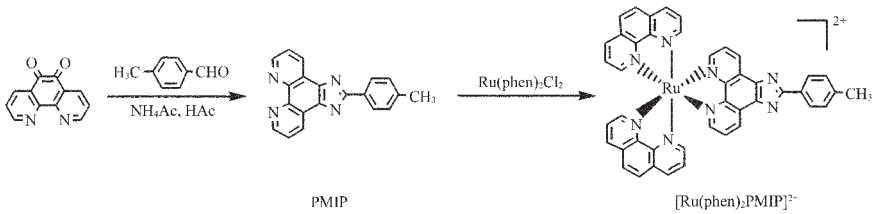
Isothermal titration calorimetry (ITC) is a valuable tool for investigating the thermodynamics of the reaction of two solutions with different compositions[6~8]. The thermodynamic information is necessary for a thorough understanding of the nature and mechanism of the interactions of ruthenium(Ⅱ) polypyridyl complexes with DNA, however, to the best of our knowledge, there have been no reports on the thermodynamics of these interactions using ITC method.

Herein, we report our investigations on the interaction of Ru(Ⅱ) complex $[Ru(phen)_2PMIP]^{2+}$ {phen=1, 10-phenanthroline, PMIP=2-(4-methylphenyl)imidazo [4,5-*f*]1,10-phenanthroline} with calf thymus DNA (CT DNA) using ITC. The detailed thermodynamic data are given for DNA binding with the complex, and the driving force for the binding is discussed.

1 Experimental

1.1 Materials

CT DNA was purchased from Sigma-Aldrich Co.. Other reagents and solvents used were made in China and of analytical grade. Complex $[Ru(phen)_2PMIP]^{2+}$ was prepared according to our previous publication[9], and the synthetic route of the complex is shown in scheme 1.



Scheme 1　Synthetic routes of $[Ru(phen)_2PMIP]^{2+}$

1.2 Measurements

A doubly distilled deionized water and a buffer solution containing 30 mmol·L^{-1} HEPES and 0.1 mmol·L^{-1} EDTA, and with pH of 7.2 adjusted by sodium hydroxide were used for all experiments. DNA solution gave UV absorbance at 260 and 280 nm with a ratio of about 1.8~1.9:1, indicating that it was sufficiently free of protein[10]. CT DNA was dissolved in the buffer before use with an appropriate concentration of about 0.4 mmol·L^{-1}, stored at 4 ℃ and used within 2 days. The concentration per nucleotide of CT DNA solution was determined by absorption spectroscopy using the molar absorption coefficient of 6 600 L·mol^{-1}·cm^{-1} [11] at 260 nm.

ITC measurements were carried out at 25.0 ℃ using a VP-ITC titration calorimetry (MicroCal, Northampton, MA). The calorimeter was calibrated with electrically generated heat pulses as recommended by the manufacturer. All solutions were thoroughly degassed before use by stirring under vacuum. The sample cell was loaded with 1.43 cm^3 of DNA solution and the reference cell contained doubly distilled deionized water. Titration was carried out using a 0.250 cm^3 syringe filled with the complex solution, with stirring at 300 rpm. A titration experiment consisted of 28 consecutive injections of 0.010 0 cm^3 volume and 20 s duration each, with a 5 min interval between injections. To calibrate the heat effects of dilution and mixing, a control experiment was performed by injecting the complex solution into the buffer alone. The heat released by dilution of CT DNA was negligible. Calorimetric data for DNA binding of the complex were analyzed using MicroCal ORIGIN software supplied with the instrument. The intrinsic molar enthalpy change ($\Delta_b H_m^{\ominus}$), the binding stoichiometry (n) and binding constant (K_b) for the binding process were obtained from the best fit of the calorimetric data. The standard molar binding free energy ($\Delta_b G_m^{\ominus}$) and standard molar binding entropy ($\Delta_b S_m^{\ominus}$) for the binding reaction were calculated by the fundamental equations of thermodynamics: (1) $\Delta_b G_m^{\ominus} = -RT\ln K_b$; (2) $\Delta_b S_m^{\ominus} = (\Delta_b H_m^{\ominus} - \Delta_b G_m^{\ominus})/T$.

2 Results and discussion

ITC profile for DNA binding of complex $[Ru(phen)_2PMIP]^{2+}$ is shown in Fig.1. A shows representatively raw ITC curves resulting from the injections of the complex into DNA solution. The areas under these

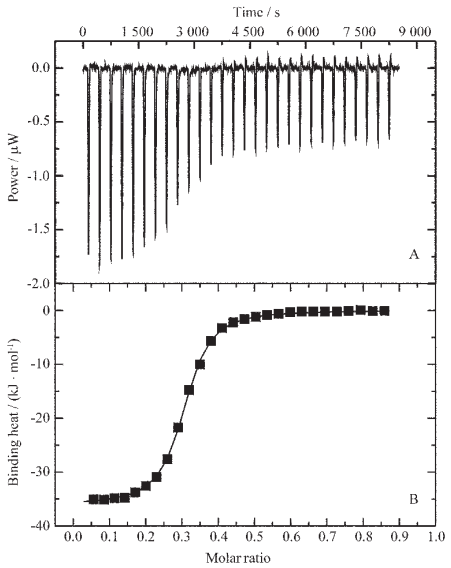


Fig.1 Isothermal titration calorimetry profile for DNA binding of complex $[Ru(phen)_2(PMIP)]^{2+}$ in HEPES buffer at 25.0 ℃
A represents the raw data for sequential 10 μL injections of $[Ru(phen)_2(PMIP)]^{2+}$ (0.10 mmol·L^{-1}) into CT DNA (0.025 mmol·L^{-1}).
B shows the plot of the heat evolved (kJ) per mole of the complex added against the molar ratio of $[Ru(phen)_2(PMIP)]^{2+}$ to CT DNA.
The data (■) were fitted to a model for a single set of identical sites and the solid line represents the best fit.

heat burst curves were determined by integration to yield the associated injection heats. These injection heats were corrected by subtraction of the corresponding dilution heats. With the injection of the complex, the peaks of exothermic decrease gradually and approached to those of simple dilution of the complex into the buffer (figure is not given), indicating that the binding reaction was almost completed at the last injection. B shows the resulting corrected injection heats plotted as a function of the ratio of [Ru]/[DNA], in which the data points reflect the experimental injection heats, while the solid line, reflects the calculated fit of the data with a model for a single set of identical sites[12]. The model employed in the fit was the only model that yielded a reasonable fit of the experimental data.

Experiments were repeated thrice and the data set from each experiment was regressed independently. The thermodynamic parameters K_b, $\Delta_b H_m^{\ominus}$, $\Delta_b S_m^{\ominus}$, $\Delta_b G_m^{\ominus}$ and binding stoichiometry (n) obtained from our ITC experiments are summarized in Table 1.

Table 1 Thermodynamic parameters for the binding of complex $[Ru(phen)_2(PMIP)]^{2+}$ to CT DNA measured by ITC at 25.0 ℃

Nucleic acid	$K_b\times10^6$ / (L·mol^{-1})	$\Delta_b G_m^{\ominus}$ / (kJ·mol^{-1})	$\Delta_b H_m^{\ominus}$ / (kJ·mol^{-1})	$\Delta_b S_m^{\ominus}$ / (J·mol^{-1}·K^{-1})	n
CT DNA	8.87±0.32	−39.66±0.08	−36.07±0.12	12.05±0.71	0.297 6±0.000 7

Data are expressed as mean ±S.D.

Inspection of these thermodynamic data in Table 1 and comparing K_b and n with those reported in previous publications, we can find the following features: (1) The binding constant (K_b) obtained from our ITC experiments is comparable to those (~10^6 L·mol^{-1}) observed for DNA binding of intercalated drug molecules by the same method [13]. The binding stoichiometry (n) of 0.297 7±0.000 6 indicates that each complex binds to 3~4 DNA bases, which is similar to that obtained from our previous spectroscopic method[9], and also similar to the binding stoichiometry of some other Ru(Ⅱ) polypyridine complexes to DNA[14,15]. (2) The binding constants obtained from ITC experiment (8.9×10^6 L·mol^{-1}), from absorption method by Wolfe et al.[16] (5.7×10^4 L·mol^{-1} [9]) and from fluorescence with McGhee-von Hippel method[17] (4.7×10^5 L·mol^{-1} [9]) are different. This is not without precedent. For instance, for DNA binding of complex $[Ru(phen)_3]^{2+}$ (phen=1,10-phenanthroline), the binding constant determined from equilibrium dialysis experiments is 6.2×10^3 L·mol^{-1} [18], but for DNA binding of its enantiomers, the binding constants obtained from fluorescence with McGhee-von Hippel method are 4.9×10^4 for its Δ form and 2.8×10^4 L·mol^{-1} for the Λ form[19], and 1.8~3.0×10^6 L·mol^{-1}, 0.3~4.2×10^6 L·mol^{-1} for the Δ, Λ isomers in different concentrations with the intrinsic method suggested by Rodger et al.[14]. These differences mentioned above should be caused by the different measurements and the different calculation methods, showing that we can compare DNA binding

strength of one complex with that of the others using binding constant only obtained from the same method. (3) DNA binding of complex $[Ru(phen)_2\ PMIP]^{2+}$ accompanied a large favorable enthalpy decrease and a less favorable entropy enhancement, indicating that the event is driven by the change in enthalpy, and the contribution of entropy is negligible. This can be explained by the fact that DNA binding mode of the complex is intercalation, which has been validated by our previous spectroscopic studies and viscosity measurements[9,20]. Once complex $[Ru(phen)_2\ PMIP]^{2+}$ intercalated into the adjacent base pairs of CT DNA, a variety of noncovalent molecular interactions including stacking interactions of the aromatic ligand in the complex with the base pairs of DNA, specific hydrogen bonds and van der Waals interactions between the complex and DNA may occur[21]. Each of these weak interactions leads to negative enthalpy change [22], and that the apparent enthalpy decrease should be the summation of all of these weak interactions between the complex and DNA[13]. On the other hand, the less favourable entropy should result from the relaxation of supercoiled CT DNA induced by the intercalation of the complex[23].

In conclusion, from ITC studies, we obtain the overall thermodynamic parameters and the driving force for DNA binding to complex $[Ru(phen)_2PMIP]^{2+}$ and can understand the binding process in more detail, showing that ITC is an effective method for studying the interactions of metal complexes with DNA. Information obtained from this study will be helpful to the development of new therapeutic reagents and DNA molecular probes.

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