Study on interaction between Dy(III)(NR)$_3$ complex and herring sperm DNA by spectroscopy

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The action mode between Dy(III)(NR)$_3$ and herring sperm DNA is studied by ultraviolet-visible (UV-vis) and fluorescence spectra as well as electrochemistry. Double-reciprocal method studies show that the binding stoichiometry between Dy(III)(NR)$_3$ and DNA is 1 : 1, the binding constants at different temperatures are $1.5 \times 10^2$ L/mol at 25°C and $2.09 \times 10^2$ L/mol at 35°C, and the corresponding thermodynamic parameters are $\Delta H_m^\circ = 2.48 \times 10^4$ J/mol, $\Delta S_m^\circ = -2.34 \times 10^4$ J/mol, $\Delta G_m^\circ = 161.7$ J/(mol·K), $\Delta S_m^\circ$ is the driving force in this reaction. Combined with Scatchard method and melting parameters and binding constants are also obtained.

The complex was prepared by concentrating stoichiometric dysprosium chloride and NR in absolute ethanol, recirculated on a water bath at 80°C for 14 h. The sample was dried in an oven for 5 h and remaining the volume of about 10 mL. After standing for several days, brownish crystals of Dy(III)(NR)$_3$Cl$_3$·H$_2$O were yielded. Infrared (IR) spectra data display clearly the symmetrical stretching band of NH at 3460 cm$^{-1}$, the NH bending band at 1625 cm$^{-1}$, and the stretching band of CH in benzene ring at 860 cm$^{-1}$, and the stretching band of CN at 1170 and 1080 cm$^{-1}$. Elemental analysis shows the composition of C43.33 H4.71 N14.25 Dy14.38.

All of the samples were dissolved in tris-HCl buffer solution with the concentration of 0.1 mol/L. Acidometer was used to examine the pH value of the buffer until it gained prominence because of their relevance in the development of new reagents for medicine. On one hand, we can select new nucleic acid fluorescence probe during the study. On the other hand, some anticancer drugs take cancer DNA as head target by ways of breaking its structure and interfering its gene regulation and expression, showing anticancer activity.

The noncovalent binding of some ligands may be followed by chemical reaction or ligand-induced cleavage of the nucleic acid. It is generally accepted that there are three binding modes of small molecules to the DNA double helix: intercalation binding, groove binding, and electrostatic binding. Intercalation, a strongly favorable binding mode that can influence the function of DNA by rupturing DNA directly or restraining its replication and transcription, involves the π-stacking of a ligand between adjacent base pairs of DNA. This π-stacking interaction requires the intercalating ligand to be a flat, extended aromatic compound, which is annulated with heterocyclic rings, e.g., pyridine and pyrazine. Neutral red (NR), a planar phenazine dye, is sensitive to the size, shape, chirality, and hydrophobic characteristics of the complex. Compared with transition metals, rare metals are more effective to cleave the phosphate diester bond. As a result, the study of their metal complex Dy(III)(NR)$_3$ provides useful insights into drug design and we can understand the binding mechanism of these drugs.

Spectrophotometric measurements are known as powerful tools for investigating the interaction between small molecules and DNA. Although DNA has a natural fluorescence, it is too weak to be used directly to measure DNA. Compared with a common fluorimetric probe, ethidium bromide (EB) and acridine orange (AO) offer lower toxicity, higher stability, and convenience of use. In this letter, AO is selected as the probe. The interaction between Dy(III)(NR)$_3$ complex and herring sperm DNA is confirmed by spectrophotometer and electrochemistry measurements. A series of thermodynamic parameters and binding constants are also obtained.

The herring sperm DNA (purchased from Sigma Biological Co.) was used without further purification. The DNA was dissolved in doubly distilled deionized water with 50-mmol/L NaCl and dialyzed for 48 h against a buffer solution at 4°C. The purity of the DNA was checked by monitoring the ratio of the 260-nm absorbance to 280-nm absorbance ($A_{260}/A_{280}$). If the ratio was 1.83 indicated that DNA was fully free of protein. The DNA concentrations per nucleotide were determined from the light absorbance at 260 nm by using the molar extinction coefficient of 6600 L/(mol·cm). The solution of DNA was stored at 4°C for a short time and then used.

All of the samples were dissolved in tris-HCl buffer (The concentration of tris was 0.1 mol/L by adding HCl solution with the concentration of 0.1 mol/L. Acidometer was used to examine the pH value of the buffer until it was adjusted to 7.4). The DNA concentrations per nucleotide were determined from the light absorbance at 260 nm by using the molar extinction coefficient of 6600 L/(mol·cm). The solution of DNA was stored at 4°C for a short time and then used.

The DNA attracted much interest due to its crucial role in gene expression, gene transcription, mutagenesis, carcinogenesis, and cell death, etc. In recent years, studies on the interaction of small molecules with DNA have gained prominence because of their relevance in the development of new reagents for medicine. On one hand, we can select new nucleic acid fluorescence probe during the study. On the other hand, some anticancer drugs take cancer DNA as head target by ways of breaking its structure and interfering its gene regulation and expression, showing anticancer activity.
reached 7.00). NR was purchased from Kelong Chemical plant in Chengdu. Dy(III)Cl₂O₃ was purchased from Beifang Fangzheng rare metal lab company (99.99%). Dy(III)Cl₂O₃ was dissolved in dense HCl, and then HCl was vaporized slowly to get Dy(III)Cl₃ solutions in different concentrations. AO was purchased from Shanghai-China medicine chemical plant. Other reagents were at least analytical grade, and were used without further purification.

The absorption spectra were recorded on an UV-210 spectrophotometer. The fluorescence spectra were recorded on a FL-4500 spectrofluorophotometer. The electrical conductivity measurements were performed on a PARSTAT 2273 electrochemical workstation with a three-electrode system: the working electrode – a glassy carbon, the reference electrode – calomel, and the counter electrode – a platinum wire. The pH value was recorded on a pHS-2C acidometer. In fluorescence mode, both excitation and emission bandwidths were set at 5 nm, λₑx = 365 nm. All of the spectroscopic work was carried out at pH 7.00 remained by a tris–HCl buffer.

The glassy carbon electrode surface was polished firstly with Al₂O₃ polishing powder, and then cleaned ultrasonically for 5 min in doubly distilled water. The continuous voltammetric scan was carried out with a scan rate of 0.05 V/s.

The absorption and fluorescence spectra titrated with DNA were conducted by keeping the solution of a certain concentration which was dissolved in tris–HCl buffer solution (pH 7.00), putting 3.00 mL in 1-cm comparison dish, and varying another solution concentration by adding it 10 µL each time. The volume effect was so small that could be ignored, and the tris–HCl buffer solution with nothing added worked as the reference solution.

Ultraviolet-visible (UV-vis) absorption spectra show that the absorption of NR from 350 to 650 nm decreases with Dy(III)Cl₃ solution added. In order to determine the stoichiometry for the formation of dysprosium complex, the mole ratio method experiment was done at the absorption peak of 465 nm. The mole ratio plots of NR with Dy(III)Cl₃ are shown in Fig. 1. The binding ratio of the complex was got as $c_{3} = 3$ : 1. According to the Lambert-Beer law $A = εbc$, where $A$ is the absorbance of the complex, $ε$ is the apparent mol absorption coefficient of the complex, $b$ is the thickness of the comparison dish, and $c$ is the concentration of Dy(III)(NR)$_3$. The apparent mol absorption coefficient of Dy(III)(NR)$_3$ is calculated to be $3.13 \times 10^3$ L/(mol-cm).

Absorption spectra of Dy(III)(NR)$_3$-DNA (concentration ratio $c_{Dy(III)(NR)_3}/c_{DNA} = 1$) admixture upon adding acridine orange are shown in Fig. 2. The result also agrees with the above viewpoint. AO can intercalate into the base pairs of double helix DNA uniquely which have been studied previously with a feature of a planar phenazine ring[18]. It was employed as molecule probe. In the UV region, the Dy(III)(NR)$_3$-DNA system exhibits an intense absorption bands around 547 nm. After adding AO solution, the absorption peak of the admixture at 547 nm decreased with blue shift, and a new peak at 503 nm turned out and increased gradually. An isochromatic point occurred at 518 nm. These changes with intercalation have been observed in several instances[19,20].

The mole ratio method was also used at the single wavelength of 547 nm in order to discuss the binding ratio. The binding ratio is $n_{Dy(III)(NR)_3}^{DNA} = 1$ : 1. According to the Lambert-Beer law, the apparent mol absorption coefficient $ε = 3.99 \times 10^4$ L/(mol-cm) is deduced.

Fig. 1. Mole ratio plots of NR-Dy(III). pH = 7.00, $λ = 465$ nm, $c_{NR} = 2.50 \times 10^{-5}$ mol/L.

Fig. 2. Absorption spectra of Dy(III)(NR)$_3$-DNA admixture in different concentrations of AO. $c_{Dy(III)(NR)_3} = 2.40 \times 10^{-6}$ mol/L, $c_{AO} = 9.00 \times 10^{-5}$ mol/L. 10 µL per scan; 1–16 : 0–150 µL.

Fig. 3. Double-reciprocal plots of Dy(III)(NR)$_3$-DNA. pH = 7.00, $λ = 547$ nm, $c_{Dy(III)(NR)_3} = 3.00 \times 10^{-6}$ mol/L.
The absorption relationship between the complex and DNA is expressed by the double-reciprocal equation:

\[ \frac{1}{A_0 - A} = \frac{1}{A_0} + \frac{1}{(K \times A_0 \times c_{\text{DNA}})}, \]

where \( A_0 \) is the absorbance of Dy(III)(NR)\(_3\) in the absence of DNA, \( A \) is the absorbance of Dy(III)(NR)\(_3\) in the presence of DNA, \( K \) is the binding constant between Dy(III)(NR)\(_3\) and DNA, and \( c_{\text{DNA}} \) is the concentration of DNA.

The double-reciprocal plots of \( 1/(A_0 - A) \) versus \( 1/c_{\text{DNA}} \) at 25 and 35 °C are linear, and the association binding constants are calculated from the ratio of the intercept on the vertical (Fig. 3): \( K_{25}^{\Theta} = 1.51 \times 10^9 \text{ L/mol}, K_{35}^{\Theta} = 2.09 \times 10^9 \text{ L/mol}. \)

The relation equation of \( K^{\Theta}, \Delta_rH_m^{\Theta}, \) and \( T \) is

\[ \ln K_r^{\Theta} / K_1^{\Theta} = -\Delta_rH_m^{\Theta} (1/T_2 - 1/T_1) / R, \]

where \( K_r^{\Theta} \) is the standard binding constant of Dy(III)(NR)\(_3\) and DNA at 25 °C, \( K_1^{\Theta} \) is the standard binding constant of Dy(III)(NR)\(_3\) and DNA at 35 °C, \( T_1 \) is 298.15 K (25 °C), \( T_2 \) is 308.15 K (35 °C), \( \Delta_rH_m^{\Theta} \) is the standard molar reaction enthalpy. Then \( \Delta_rH_m^{\Theta} = 2.48 \times 10^4 \text{ J/mol} \) is deduced. The positive result shows that it is an endothermic reaction that temperature enhancement redounding to reaction processes.

The relation equation of \( K^{\Theta}, \Delta_rG_m^{\Theta}, \) and \( T \) is

\[ \Delta_rG_m^{\Theta} = -RT \ln K^{\Theta}, \]

where \( \Delta_rG_m^{\Theta} \) refers to the standard molar reaction Gibbs free energy, \( T \) is 298 K (~25 °C), \( K^{\Theta} \) refers to the standard binding constant of Dy(III)(NR)\(_3\) and DNA at 25 °C. Then \( \Delta_rG_m^{\Theta} = -2.34 \times 10^4 \text{ J/mol} \) is deduced. The negative result shows the spontaneous interaction tendency between Dy(III)(NR)\(_3\) and DNA.

According to the Gibbs-Helmholtz equation:

\[ \Delta_rG_m^{\Theta} = \Delta_rH_m^{\Theta} - T \Delta_rS_m^{\Theta}, \]

where \( \Delta_rS_m^{\Theta} \) refers to the standard molar reaction phantom, \( T \) is 298 K (~25 °C), \( \Delta_rS_m = 161.7 \text{ J mol}^{-1} \text{ K}^{-1} \) is deduced. The result suggests that \( \Delta_rS_m^{\Theta} \) is the driving force in this reaction. Generally, the positive \( \Delta_rH_m^{\Theta} \) and \( \Delta_rS_m^{\Theta} \) values indicate that hydrophobic interaction plays a major role in the binding of a small molecule and a macromolecule, which means that there is the groove binding mode in the Dy(III)(NR)\(_3\)-DNA system.

The fluorescence measurements were also carried out with AO as a probe. When AO intercalates into DNA, it has a characteristic fluorescence peak at about 522 nm, and the fluorescence intensity increases. Figure 4 shows the fluorescence of DNA-AO in different concentrations of Dy(III)(NR)\(_3\). It can be seen that the fluorescence of DNA-AO is efficiently quenched by adding the Dy(III)(NR)\(_3\) with small wavelength shift. A new fluorescence peak at 617 nm turns out and increases with a small shift to longer wavelength. Isosbestic point is achieved at 575 nm. This phenomena proves that AO is replaced with Dy(III)(NR)\(_3\), so the characteristic peak of complex DNA-AO is quenched and the characteristic peak of complex DNA-Dy(III)(NR)\(_3\) increases.

The fluorescence spectra of Dy(III)(NR)\(_3\)-DNA admixture are shown in Fig. 5. It can be seen that the fluorescence peak at 423 nm increases and the peak at 522 nm decreases after adding AO. Isosbestic point is achieved at 493 nm. Comparing these changes in the emission spectra of Figs. 4 and 5, we can find that the reaction competition of DNA between AO and Dy(III)(NR)\(_3\) is remarkable, and considered with the intercalation binding between AO and DNA, intercalation effect between Dy(III)(NR)\(_3\) and DNA is basically confirmed.

The influence of Dy (III)(NR)\(_3\) on AO-DNA is studied by fluorescence Scatchard analysis according to Scatchard equation. Scatchard expression equations the binding of AO-DNA in the presence of Dy(III)(NR)\(_3\):

\[ r_{AO}/c_{AO} = K(n - r_{AO}), \]

where \( r_{AO} \) refers to the molecular amount of bound AO to total nucleotide concentration, \( c_{AO} \) is the concentrations of free AO, \( n \) is the number of binding sites of DNA, \( K \) is the intrinsic binding constant of Dy(III)(NR)\(_3\) to AO-DNA.

With the concentration of Dy(III)(NR)\(_3\) increasing, it regards as a dis-intercalation binding mode if the value...
The observed melting temperature of DNA in presence of Dy(III)(NR)$_3$ is 77 °C. The change in $T_m$ of DNA after the addition of Dy(III)(NR)$_3$ means that the binding modes of Dy(III)(NR)$_3$ with DNA are intercalated.

In conclusion, the interaction between Dy(III)(NR)$_3$ and herring sperm DNA has been studied in tris-HCl buffer of pH = 7.00 by several spectroscopic and electrostatic methods. Overall, the present findings have demonstrated that the interaction mode between Dy(III)(NR)$_3$ and herring sperm DNA are intercalation

| Curve | $c_{NR-Dy(III)/c_{DNA}}$ | Concentration of NaCl (%) | Scatchard | $K$ (L/mol) | $n$
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<td>(a)</td>
<td>0.00</td>
<td>5.00</td>
<td>$0.32\times10^3$ - $0.19\times10^6x$</td>
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<td>(b)</td>
<td>0.16</td>
<td>5.00</td>
<td>$1.18\times10^5$ - $1.11\times10^6x$</td>
<td>$1.11\times10^6$</td>
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<tr>
<td>(c)</td>
<td>0.32</td>
<td>5.00</td>
<td>$1.60\times10^5$ - $0.55\times10^6x$</td>
<td>$0.55\times10^6$</td>
</tr>
<tr>
<td>(d)</td>
<td>0.48</td>
<td>5.00</td>
<td>$5.47\times10^4$ - $0.76\times10^5x$</td>
<td>$0.76\times10^5$</td>
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<tr>
<td>(e)</td>
<td>0.64</td>
<td>5.00</td>
<td>$5.15\times10^4$ - $0.94\times10^6x$</td>
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The peak potential are changed again $E_{1/2}$ $= 0.194$ and $–0.94$ V, respectively. With the addition of DNA, no new peak is formed, but a decrease in intensity currents for both reduction and oxidation occurs and reveals a positive shift in $E_{1/2}$ $= 0.629$ V, which again suggests that Dy(III)(NR)$_3$ has intercalated into the DNA.$^{[30]}$

Intercalation binding can increase the stability of helix of DNA, and cause the denatured temperature $T_m$ of DNA to increase.$^{[31]}$ But the non-intercalation binding causes no obvious increase in $T_m^{[32]}$. The value of $T_m$ for Dy(III)(NR)$_3$ is determined by monitoring the maximum fluorescence of the system as a function of temperature from 45 to 95 °C. For each monitored transition, $T_m$ of the assay solution is determined as the transition midpoint of the melting curve.$^{[33]}$

![Fig. 6. Cyclic voltammograms of Dy(III)(NR)$_3$ complex in different concentrations of DNA.](image)

Fig. 6. Cyclic voltammograms of Dy(III)(NR)$_3$ complex in different concentrations of DNA. $c_{Dy(III)(NR)_3} = 1.00\times10^{-4}$ mol/L, $c_{DNA} = 5.00\times10^{-4}$ mol/L. 25 µL per scan; 1–6: 0–125 µL.

![Fig. 7. Melting curve of Dy(III)(NR)$_3$-DNA.](image)

Fig. 7. Melting curve of Dy(III)(NR)$_3$-DNA. $c_{Dy(III)(NR)_3}$-DNA = $1.24\times10^{-5}$ mol/L.
fashion and groove binding force. NR is a specific intercalator to DNA. It coordinates to Dy(III) with the coordination ratio of 3 : 1, and the structure of the complex Dy(III)(NR)₃ is octahedron. The outspread planar ring parts of the complex intercalate into DNA base pairs as NR. These data provide important biological information related to structure-activity relationship, and help us to understand the nature of Dy(III)(NR)₃-DNA interactions. The binding constants of Dy(III)(NR)₃ with DNA are $K_{25^\circ C} = 1.15 \times 10^5 \text{L/mol}$, $K_{35^\circ C} = 2.09 \times 10^5 \text{L/mol}$, respectively, and the corresponding thermodynamic parameters are $\Delta_r H_{\Theta m} = 2.48 \times 10^4 \text{J/mol}$, $\Delta_r G_{\Theta m} = -2.34 \times 10^4 \text{J/mol}$, $\Delta_r S_{\Theta m} = 161.7 \text{J/(mol·K)}$. $\Delta_r S_{\Theta m}$ is the driving force in this reaction.

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References