

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 1.15×10^5 L/mol at 25 °C and 2.09×10^5 L/mol at 35 °C, and the corresponding thermodynamic parameters are $\Delta_r H_m^\ominus = 2.48 \times 10^4$ J/mol, $\Delta_r G_m^\ominus = -2.34 \times 10^4$ J/mol, $\Delta_r S_m^\ominus = 161.7$ J/(mol · K), $\Delta_r S_m^\ominus$ is the driving force in this reaction. Combined with Scatchard method and melting method, the results suggest that the interaction mode between Dy(III)(NR)₃ and herring sperm DNA is intercalation fashion and groove fashion.

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DNA attracted much interest due to its crucial role in gene expression, gene transcription, mutagenesis, carcinogenesis, and cell death, etc.^[1] 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^[2–5].

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^[6–8]. 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^[9]. 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^[10–12]. 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^[13,14]. 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 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)₃Cl₃ · H₂O^[11] 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, 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 in percentages^[15,16].

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}). The ratio of 1.83 indicated that DNA was fully free of protein^[17]. 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

reached 7.00). NR was purchased from Kelong Chemical plant in Chengdu. Dy(III)₂O₃ was purchased from Beifang Fangzheng rare metal lab company (99.99%). Dy(III)₂O₃ 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 pH-S-2C acidimeter. In fluorescence mode, both excitation and emission bandwidths were set at 5 nm, $\lambda_{\text{ex}} = 365$ 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 μ L 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 $n_{\text{NR}}:n_{\text{Dy(III)}} = 3:1$. According to the Lambert-Beer law $A = \varepsilon bc$, 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 3.13×10^3 L/(mol·cm).

Absorption spectra of Dy(III)(NR)₃-DNA (concentration ratio $c_{\text{Dy(III)(NR)}_3}/c_{\text{DNA}} = 1$) 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. This finding indicates that AO substitutes for Dy(III)(NR)₃ in the Dy(III)(NR)₃-DNA system. The new peak of

503 nm is due to the compound coming into being. 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 $n_{\text{Dy(III)(NR)}_3}:n_{\text{DNA}} = 1:1$. According to the Lambert-Beer law, the apparent mol absorption coefficient $\varepsilon = 3.99 \times 10^4$ L/(mol·cm) is deduced.

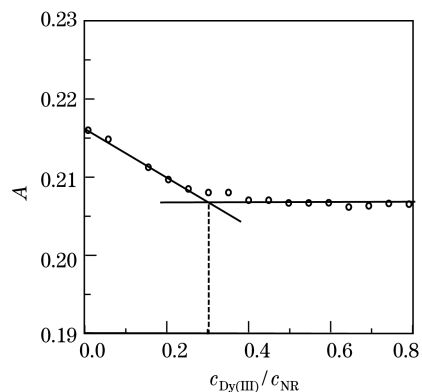


Fig. 1. Mole ratio plots of NR-Dy(III). pH = 7.00, $\lambda = 465$ nm, $c_{\text{NR}} = 2.50 \times 10^{-5}$ mol/L.

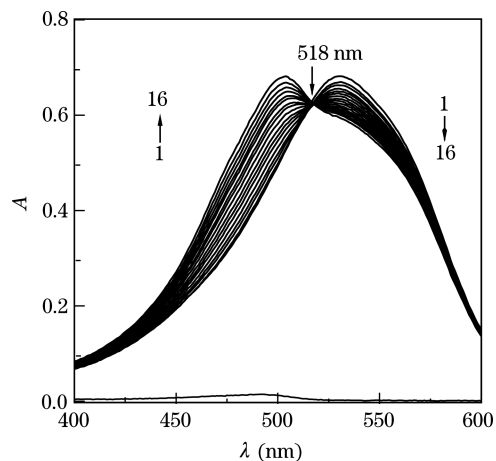


Fig. 2. Absorption spectra of Dy(III)(NR)₃-DNA admixture in different concentrations of AO. $c_{\text{Dy(III)(NR)}_3\text{-DNA}} = 2.40 \times 10^{-6}$ mol/L, $c_{\text{AO}} = 9.00 \times 10^{-5}$ mol/L. 10 μ L per scan; 1–16: 0–150 μ L.

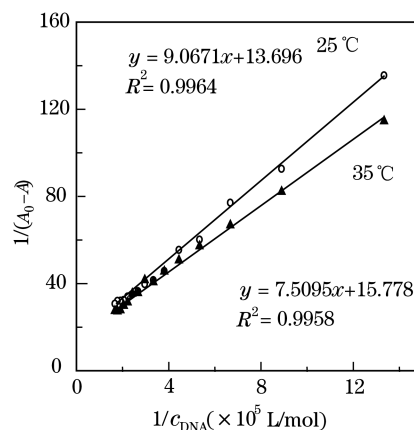


Fig. 3. Double-reciprocal plots of Dy(III)(NR)₃-DNA. pH = 7.00, $\lambda = 547$ nm, $c_{\text{Dy(III)(NR)}_3} = 3.00 \times 10^{-6}$ mol/L.

The absorption relationship between the complex and DNA is expressed by the double-reciprocal equation^[21–23]:

$$1/(A_0 - A) = 1/A_0 + 1/(K \times A_0 \times c_{DNA}), \quad (1)$$

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_{DNA} is the concentration of DNA.

The double-reciprocal plots of $1/(A_0 - A)$ versus $1/c_{DNA}$ 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): $K_{25^\circ C}^\ominus = 1.51 \times 10^5$ L/mol, $K_{35^\circ C}^\ominus = 2.09 \times 10^5$ L/mol.

The relation equation of K^\ominus , $\Delta_r H_m^\ominus$, and T is

$$\ln K_2^\ominus / K_1^\ominus = -\Delta_r H_m^\ominus (1/T_2 - 1/T_1) / R, \quad (2)$$

where K_1^\ominus is the standard binding constant of Dy(III)(NR)₃ and DNA at 25 °C, K_2^\ominus 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), $\Delta_r H_m^\ominus$ is the standard molar reaction enthalpy. Then $\Delta_r H_m^\ominus = 2.48 \times 10^4$ 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^\ominus , $\Delta_r G_m^\ominus$, and T is

$$\Delta_r G_m^\ominus = -RT \ln K^\ominus, \quad (3)$$

where $\Delta_r G_m^\ominus$ refers to the standard molar reaction Gibbs free energy, T is 298 K (~25 °C), K^\ominus refers to the standard binding constant of Dy(III)(NR)₃ and DNA at 25 °C. Then $\Delta_r G_m^\ominus = -2.34 \times 10^4$ 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:

$$\Delta_r G_m^\ominus = \Delta_r H_m^\ominus - T \Delta_r S_m^\ominus, \quad (4)$$

where $\Delta_r S_m^\ominus$ refers to the standard molar reaction phantom, T is 298 K (~25 °C), $\Delta_r S_m^\ominus = 161.7$ J·mol⁻¹·K⁻¹ is deduced. The result suggests that $\Delta_r S_m^\ominus$ is the driving force in this reaction. Generally, the positive $\Delta_r H_m^\ominus$ and $\Delta_r S_m^\ominus$ values indicate that hydrophobic interaction plays a main role in the binding of a small molecule and a macromolecule^[24], 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^[25].

The influence of Dy(III)(NR)₃ on AO-DNA is studied by fluorescence Scatchard analysis according to Scatchard equation^[26,27]. Scatchard equation expresses the binding of AO-DNA in the presence of Dy(III)(NR)₃:

$$r_{AO} / c_{AO} = K(n - r_{AO}), \quad (5)$$

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 val-

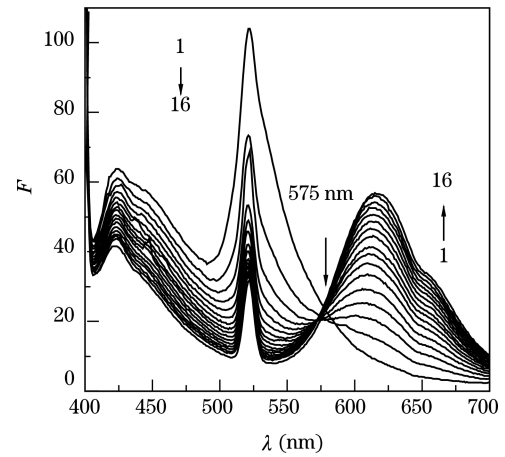


Fig. 4. Emission spectra of DNA-AO admixture in different concentrations of Dy(III)(NR)₃. pH = 7.00, $\lambda_{ex} = 365$ nm, $c_{DNA-AO} = 1.20 \times 10^{-5}$ mol/L, $c_{Dy(III)(NR)_3} = 4.50 \times 10^{-4}$ mol/L. 10 μ L per scan.

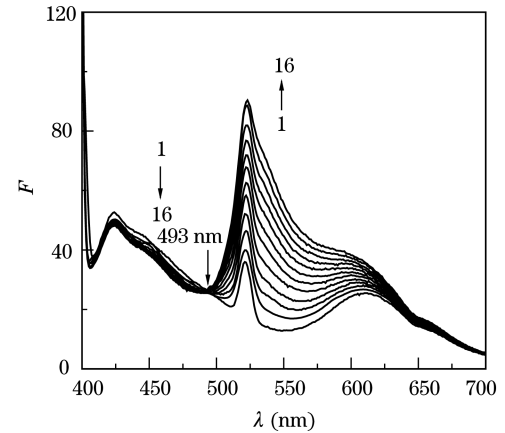


Fig. 5. Emission spectra of Dy(III)(NR)₃-DNA admixture in different concentrations of AO. pH = 7.00, $\lambda_{ex} = 365$ nm, $c_{Dy(III)(NR)_3-DNA} = 5.63 \times 10^{-6}$ mol/L, $c_{AO} = 2.11 \times 10^{-4}$ mol/L. 10 μ L per scan.

Table 1. Date of Scatchard Equation of Interaction between NR-Dy(III) and DNA

Curve	$c_{\text{NR-Dy(III)}/\text{CDNA}}$	Concentration of NaCl (%)	Scatchard	K (L/mol)	n
(a)	0.00	5.00	$0.32 \times 10^4 - 0.19 \times 10^6 x$	0.19×10^6	0.017
		0	$1.18 \times 10^4 - 1.11 \times 10^6 x$	1.11×10^6	0.010
(b)	0.16	5.00	$1.16 \times 10^4 - 0.44 \times 10^6 x$	0.44×10^6	0.026
		0	$2.99 \times 10^4 - 1.39 \times 10^6 x$	1.39×10^6	0.022
(c)	0.32	5.00	$2.09 \times 10^4 - 0.59 \times 10^6 x$	0.59×10^6	0.035
		0	$4.01 \times 10^4 - 1.18 \times 10^6 x$	1.18×10^6	0.034
(d)	0.48	5.00	$5.47 \times 10^4 - 0.76 \times 10^6 x$	0.76×10^6	0.058
		0	$5.15 \times 10^4 - 0.94 \times 10^6 x$	0.94×10^6	0.055
(e)	0.64	5.00	$4.26 \times 10^4 - 0.55 \times 10^6 x$	0.55×10^6	0.077
		0	$6.14 \times 10^4 - 0.94 \times 10^6 x$	0.94×10^6	0.065

ues of K are equal. It regards as an intercalation binding mode if the values of n are equal. It regards as a mix binding mode that contains dis-intercalation and intercalation binding if the values of n and K are both different.

As shown in Table 1, both the slope n and the intercept on the abscissa K are increased with the addition of Dy(III)(NR)₃, indicating that there is competitive inhibition when Dy(III)(NR)₃ is added into AO^[28]. The variation of the parameters n and K suggests a mix interaction herein and both n and K are changed again in presence of NaCl. Generally, if n is reduced in presence of NaCl, the existence of electrostatic interaction is indicated^[29]. But n is increased clearly in Table 1, indicating that the other dis-intercalation binding mode — groove interaction exists in the Dy(III)(NR)₃-DNA system. It should be noticed that the variations of n are much smaller than those of K , which means that the dis-intercalation action is weaker than intercalation action. So we can conclude that the main interactions between Dy(III)(NR)₃ and herring sperm DNA are spectroscopy intercalation and dis-spectroscopy intercalation – groove binding force.

The electrochemical properties of complex Dy(III)-(NR)₃ in the absence and presence of DNA were studied within the sweep range from -1.2 to $+0.0$ V, sweep rate at 0.05 V/s. As shown in Fig. 6, the peak potential difference (ΔE) and the $E_{1/2}$ potential are 0.194 and -0.665 V (average of oxidation and reduction potentials), 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^[13]. Figure. 7 shows the behavior of thermally denatured DNA-Dy(III)(NR)₃ system. The value of T_m for herring sperm DNA is 70 °C.

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

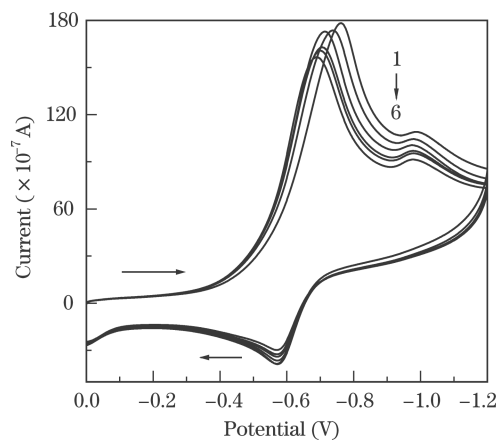


Fig. 6. Cyclic voltammograms of Dy(III)(NR)₃ complex in different concentrations of DNA. $c_{\text{Dy(III)(NR)}_3} = 1.00 \times 10^{-4}$ mol/L, $c_{\text{DNA}} = 5.00 \times 10^{-4}$ mol/L. $25 \mu\text{L}$ per scan; 1–6: 0– $125 \mu\text{L}$.

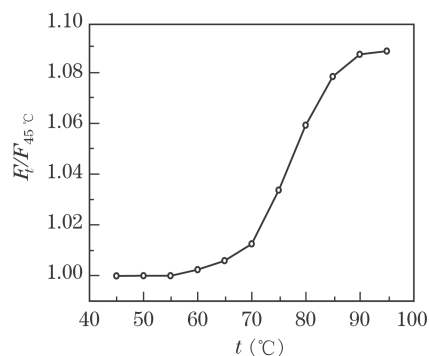


Fig. 7. Melting curve of Dy(III)(NR)₃-DNA. $c_{\text{Dy(III)(NR)}_3\text{-DNA}} = 1.24 \times 10^{-5}$ mol/L.

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 biophysical 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 $K_{25\text{ }^\circ\text{C}} = 1.15 \times 10^5$ L/mol, $K_{35\text{ }^\circ\text{C}} = 2.09 \times 10^5$ L/mol, respectively, and the corresponding thermodynamic parameters are $\Delta_r H_m^\ominus = 2.48 \times 10^4$ J/mol, $\Delta_r G_m^\ominus = -2.34 \times 10^4$ J/mol, $\Delta_r S_m^\ominus = 161.7$ J/(mol·K). $\Delta_r S_m^\ominus$ is the driving force in this reaction.

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