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Electrochemical and Spectroscopic Study of Samarium Ion Interaction with DNA in Different pHs

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In this work, for the first time the electrochemical behavior of the Sm^{3+} and Sm^{3+} ion interaction with short single strand DNA (ssDNA) sequence in two pHs was studied. Then the UV-Vis spectroscopic method was used for supporting these evidences. The interaction between Sm^{3+} and ssDNA have different binding mode in different pHs. The ratio between [Sm^{3+}] and [ssDNA] is dependence to pH and pK_a of bases. In pH 2.7 Sm^{3+} binds to ssDNA mainly by electrostatic attraction. Binding number, n, of 2 of Sm^{3+} per ssDNA and binding constant, k´, of 1.75×10^{-3} M^{-1} were obtained with cyclic voltammetry (CV) and differential pulse voltammetry (DPV) methods, respectively. In this pH, the bases of ssDNA are totally protonated and Sm^{3+} interacts electrostatically with phosphate groups. The UV-Vis study showed similar results. The results in pH 5.5 show that Sm^{3+} can bind to ssDNA with electrostatic and covalent bond. In this pH, beside phosphate groups, the bases can be interacted to Sm^{3+}, too. The binding number 4 of Sm^{3+} per ssDNA was obtained.

Keywords: Samarium, ssDNA, Cyclic voltammetry, Differential pulse voltammetry, Spectroscopy

1. INTRODUCTION

Interaction of DNA with ions and molecules is an important fundamental issue on life sciences [1]. DNAs are negatively charged and interact strongly with metal ions [2]. The binding of cations to DNA can be an interesting field of research due to the importance of these ions in biological media [3-5]. Several techniques have been employed to study the binding of small molecules to DNA (and other polyelectrolytes) including, for example, viscometry [6-8], UV-Vis. spectroscopy [9,10], luminescence [11,12], electrophoresis [13], NMR [14,15], and electroanalytical techniques [16,17]. Electrochemical techniques have been reported to have several advantages in these measurements [18].

The lanthanides are inner transition elements, defined as the 4f-orbital-filling elements, but also generally including La itself, with electron shell [Xe]$4d^1 6s^2$. The biologic importance of the lanthanide ions is because of their similarity to Ca^{2+} ions. All lanthanides show a marked bioinorganic similarity to Ca^{2+} ion, with near equivalence of ionic radii, but with a higher charge density [19-24]. The lanthanides, display Lewis acid properties which make them useful in the hydrolytic cleavage of phosphor-diester bonds of DNA which, otherwise, is extremely resistant to hydrolysis; cleavage of DNA is an essential step in developing gene therapy [25].

For monitoring of lanthanide ions and following up their interaction with DNA molecules, an online and non-destructive method is needed. There are several reports on selective determination and monitoring of lanthanide ions by ion selective electrodes [26-30]. However, since five member of lanthanide are electroactive (Ce, Sm, Eu, Tb, Yb) electrochemical methods based on current determination can be applied for its online monitoring too.

In this work, for the first time electrochemical behavior of Sm^{3+} ions (one of the electroactive member of lanthanides) was studied by cyclic voltammetry (CV) method in pH=2.7 and then the interaction of Sm^{3+} ions with ssDNA was investigation by CV and differential pulse voltammetry (DPV) methods. The constant ratio of complex and binding number of Sm^{3+} ion to ssDNA in this pH was also calculated. Also the pH effect in this interaction was studied by spectroscopy method. UV-Vis. studies show the similar results in pH=2.7. The experiment was also carried out in biologic pH and higher that the interesting and different result was obtained.

2. EXPERIMENTAL PART

2.1. Apparatuses

Electrochemical experiments were performed using AUTO LAB PGSTAT 30 electrochemical analysis system and general propose electrochemical system (GPES) 4.9005 software package (Eco Chemie. Netherlands). The three electrode system consisted of the platinum electrode (surface area of 0.0314 cm^2) as working electrode, Ag/AgCl as the reference electrode and a platinum wire as the auxiliary electrode was used.

A PERKIN-ELMER UV-Vis. spectrophotometer with a 1 cm path cell was used for spectrophotometric determinations. Also a Heidaloh MR 3001K stirrer and an ultravoltammetry pH meter were used in this work.

2.2. Reagents

A 10-mer oligonucleotids were supplied (as lyophilized powder) from MWG-Biotech, with following sequences: 3´-GGAGCTCCTG-5´. Sm_2O_3 was obtained from Merck Co.

2.3. Preparation of samples

The stock solutions of short ssDNA sequence (1×10^{-2} M) were prepared by dissolving powder primer in doubly distilled water and kept frozen in -20℃ temperature. The stock solution of Sm^{3+}

(1×10^{-2} M) was prepared by dissolving 0.348 g of Sm_2O_3 in minimum amount of nitric acid and diluting it with doubly distilled water in 100 ml volumetric flaks. Dilute solution were prepared just before use.

2.4. Preparation of Platinum Electrode

Platinum electrode was first polished successively with 0.3 μm (grain size) alumina powder (Metrohm) and then cleaned ultrasonically in water.

2.5. Procedure

For electrochemical investigation, Sm^{3+} ion solution with pH=2.7 was transferred into a 500 μL electrochemical cell and 0.1 M NaCl added to it. The increasing flexibility of ssDNA with salt has been shown experimentally using diffusion measurements [31]. The CV and DPV detection was carried out. All CV experiments were carried out in a potential ranging from -0.7 to 0.1 V, and the scanning rate was 0.1 Vs^{-1}. The differential pulse voltammograms of the solution were recorded. The initial potential was -0.7 V, the end potential was 0.6 V, the step potential was 0.015 V, the modulation time was 0.02 s, and the interval time was 0.53 s.

For the study of interaction between DNA and Sm^{3+} ion by CV and DPV methods the concentration of Sm^{3+} ion was kept constant and varying concentrations of ssDNA were added to it. The photometric titration of ssDNA with Sm^{3+} ion was conducted by keeping the concentration of ssDNA constant and varying Sm^{3+} ion concentration with 3 mL final volume in pH 2.7 and 5.5. All experiments were carried out at the laboratory temperature (25°C).

3. RESULTS AND DISCUSSION

3.1. Electrochemical study

3.1.1. Electrochemical behavior of Sm^{3+} ion

The electrochemical behavior of Sm^{3+} ion was studied on the platinum electrode in pH, 2.7. The range of potential scan is -0.7 V to 0.1 V. Fig. 1 shows the cyclic voltammograms of Sm^{3+} ion in different concentrations (from 2×10^{-3} to 1×10^{-2} M). As can be seen from this Fig. a pair of redox peaks for Sm^{3+} are appeared at -0.346 V (E_{pc}) and -0.417 V (E_{pa}) respectively, and the peak separation (ΔE) of greater than 59 mV can be obtained at the bare platinum electrode. The plot of i_p versus concentration of Sm^{3+} ion should be linear (inset of Fig. 1).

Fig. 2 shows the cyclic voltammograms of 5×10^{-4} M Sm^{3+} ion in pH=2.7 containing 0.1 M NaCl on the platinum electrode in different scan rates from 1 mVs^{-1} to 1200 mVs^{-1}. The potential rang is from -0.6 V to -0.15 V. This Fig. shows that by increasing of scan rate the redox current peaks increase markedly and also E_{pc} and E_{pa} is independent of scan rate. In reversible wave, i_p (as well as the current at any other point on the wave) is proportional to $\upsilon^{1/2}$ (inset of Fig. 2) with equation (1) and the value of D_0 in Eq(1) can be determined from the slop of i_p versus $\upsilon^{1/2}$ plot.

$$i_p = \left(2.69\times10^5\right) n^{3/2} A D_0^{1/2} \upsilon^{1/2} C_0^* \quad (1)$$

Inset of Fig. 2 shows the plots of i_p versus $\upsilon^{1/2}$. The regression equation is $i_p=6\times10^{-5}\upsilon^{1/2}+7\times10^{-8}$ with correlation coefficient R = 0.998, indicating that the electrochemical process was controlled by the diffusion. If n=1, A=0.0314 cm^2 and C=5×10^{-4} M, then D_0 =4.97×10^{-4} cm^2s^{-1} was obtained.

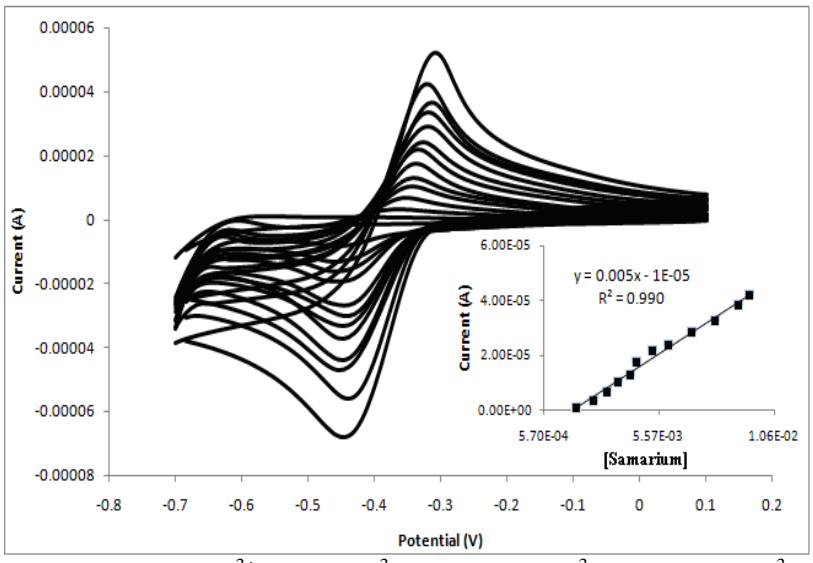

Figure 1. Cyclic voltammograms of Sm^{3+} (a) 2×10^{-3} M, (b) 2.7×10^{-3} M, (c) 3.3×10^{-3} M, (d) 3.8×10^{-3} M, (e) 4.3×10^{-3} M, (f) 4.6×10^{-3} M, (g) 5.3×10^{-3} M, (h) 6.0×10^{-3} M, (i) 6.6×10^{-3} M, (j) 7.0×10^{-3} M, (k) 8.0×10^{-3} M, (l) 9.0×10^{-3} M, (m) 9.5×10^{-3} M, (n) 1.0×10^{-2} M, pH 2.7, 0.1 M NaCl, scan rate, 0.1 Vs^{-1} and potential range -0.7 V to 0.1 V

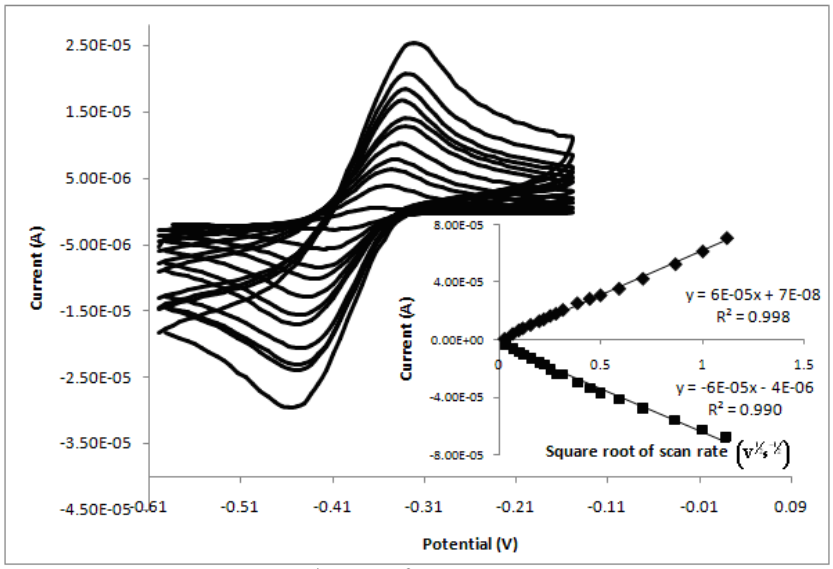

Figure 2. Cyclic voltammograms of 5×10^{-4} M Sm^{3+} in pH, 2.7, 0.1 M NaCl and potential range -0.6 V to -0.15 V. Scan rates are (a) 1 mVs^{-1}, (b) 5 mVs^{-1}, (c) 10 mVs^{-1}, (d) 15 mVs^{-1}, (e) 25 mVs^{-1}, (f) 40 mVs^{-1}, (g) 50 mVs^{-1}, (h) 60 mVs^{-1}, (i) 65 mVs^{-1}, (j) 80 mVs^{-1}, (k) 100 mVs^{-1}, (l) 150 mVs^{-1}
Inset: Plot of i_p *vs.* square root of scan rates in pH, 2.7 and 5×10^{-4} M Sm^{3+}

3.1.2. Electrochemical behavior of ssDNA

We also studied the electrochemical behavior of ssDNA with CV and DPV in this pH and with this condition (potential rang for CV from -0.6 V to -0.15 V and potential rang for DPV from -0.7 V to 0.6 V) on the platinum electrode. We cannot see any peak for ssDNA.

3.1.3. Electrochemical study of the interaction of ssDNA with Sm^{3+} ions

The cyclic voltammograms of Sm^{3+} ion in different concentrations of ssDNA was studied in pH, 2.7. The length of the single strand DNA is changing at low pH. Therefore the slop of the overstretching transition, and the width of the transition, also increases greatly at low pH. The change in length suggests a change in the properties of the single-stranded form. The protonation sites on the nitrogen bases are N3 cytosine (pK_a 4.6) and N1 adenine (pK_a 3.8). Minor protonation of N7 guanine has also been observed. Apparently, the charge reduction of ssDNA makes it harder to stretch. One might suggest that protonation of the bases should have an effect similar to increasing ionic strength, because both factors lead to damping of the electrostatic repulsion in ssDNA. Therefore the flexibility of ssDNA increased with added 0.1 M NaCl [32].

The peak currents (both the i_{pc} and i_{pa}) decrease with increasing concentration of ssDNA while both the E_{pc} and E_{pa} shifted to more negative potentials. The phenomena of the shift of E° and the decrease of peak current implied forming a new association complex. There are three kind of binding modes for small molecules to DNA. Among of those modes, A.J. Bard has reported [33] that if E° shifted to more negative value when small molecules interacted with DNA, the interaction mode was electrostatic binding. On the contrary, if E° shifted to more positive value, the interaction mode was intercalative binding. The cyclic voltammograms of Sm^{3+} ion with different amount of ssDNA shifted to more negative value of E°, therefore the interaction between ssDNA with Sm^{3+} in this pH, 2.7, is only electrostatic binding. In this pH, the bases in ssDNA are protonated and cannot interact with Sm^{3+} ions, but phosphate groups have negative charge, and can interact with Sm^{3+} ions. Thus, Sm^{3+} ions bind to phosphate groups

The phenomena mentioned above were further studied by DPV which were shown in Fig. 3. Curve (a) was the DPV of the 1.6×10^{-4} M Sm^{3+} ion solution and curves (b-d) were the DPV with different amount of DNA was added to Sm^{3+} ion solution. As can be seen the peak currents decreased with increasing concentration of DNA. The binding ratio and binding constant of the DNA-Sm^{3+} complex were studied. It is assumed that the interaction of ssDNA with Sm^{3+} ions only produces a single complex $ssDNA_n$- Sm.

$$Sm^{3+} + nssDNA \longleftrightarrow Sm - ssDNA_n, \quad (2)$$

The equilibrium constant is as follows:

$$K' = \frac{[Sm - ssDNA_n]}{[Sm^{3+}][ssDNA]^n}, \quad (3)$$

And the following equations can be deduced:

$$\Delta I_{max} = KC_{Sm^{3+}}, \quad (4)$$

$$\Delta I = K[Sm - ssDNA_n], \quad (5)$$

$$[Sm^{3+}][Sm - ssDNA_n] = C_{Sm^{3+}}, \quad (6)$$

$$\Delta I_{max} - \Delta I = K[Sm^{3+}], \quad (7)$$

Introducing Eq(5) and (7) into Eq(3), leads to

$$\log\left[\Delta I / (\Delta I_{max} - \Delta I)\right] = \log K' + n \log[ssDNA] \quad (8)$$

If interaction of Sm^{3+} ion with ssDNA forms a single complex, then the plot of $\log\left[\Delta I / (\Delta I_{max} - \Delta I)\right]$ *vs.* $\log[ssDNA]$ would show a linear line with a slope of n (Eq(8)). Fig. 3b indicates a linear relationship which implies that Sm^{3+} can form single complex with ssDNA in different concentration of ssDNA. The value of n=0.49 can be obtained showing that two Sm^{3+} ions bind to each ssDNA. Then binding number, n, was obtained 2 of Sm^{3+} ions per ssDNA. Also value of $K' = 1.75\times10^{-3}\ M^{-1}$ can be obtained.

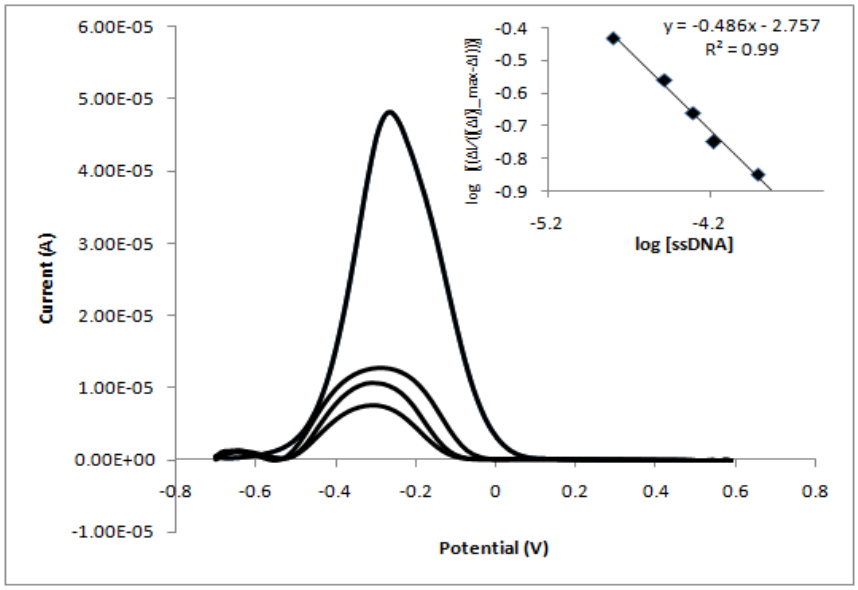

Figure 3. Differential pulse voltammograms of (a) 1.6×10^{-4} M Sm^{3+}, (b) a+1.6×10^{-5} M ssDNA, (c) a+3.33×10^{-5} M ssDNA and (d) a+5×10^{-5} M ssDNA in pH, 2.7, initial potential was -0.7 V, the end potential was 0.6 V, the step potential was 0.015 V, the modulation time was 0.02 s, the interval time was 0.53 s

Inset: Plot of $\log\left[\Delta I / (\Delta I_{max} - \Delta I)\right]$ *vs.* $\log[ssDNA]$ in pH, 2.7 and the same condition

3.1.4. pH effect

Electrochemical behavior of Sm^{3+} in higher pH was studied with CV, but we couldn't obtain cyclic voltammograms of Sm^{3+} in this pH. Because with increasing concentration of OH^-, the insoluble hydroxide complexes of samarium, ($Sm(OH)_3$, $Sm(OH)^{2+}$, $Sm(OH)_2^+$), can be produced [34-38]. The surface of electrode is coated with these compounds and then Sm^{3+} can not receive to the electrode surface. Then, the peaks shifted to higher potentials and the currents of peaks slightly decreased. Therefore we could obtain any remarkable peak in this pH, and used UV-Vis spectroscopy method.

3.2. Electrochemical study

3.2.1. Electrochemical behavior of Sm^{3+}

The UV-Vis spectrum of 1×10^{-6} M ssDNA in pH=2.7 was obtained. In this spectrum there is a peak in 256 nm. The UV-Vis absorbance spectrum of 1.0×10^{-6} M ssDNA and titration of 1.0×10^{-6} M ssDNA with different concentrations of Sm^{3+} in pH=2.7 are displayed in inset of Fig. 4. The UV-Vis spectrum of ssDNA shows an intense absorbance at 256 nm. When Sm^{3+} added to the solution shows a stronger intense absorbance. The interaction of ssDNA with increasing concentration of Sm^{3+} ions produces a small redshift. Then a solution containing 1.0×10^{-6} M ssDNA and varied amount of Sm^{3+} ion from 3.33×10^{-7} M to 2.33×10^{-6} M was studied (Fig. 4). The reaction of Sm^{3+} with ssDNA was linear until approximately two Sm^{3+} ions were added for each ssDNA. After this point no excess samarium was bound.

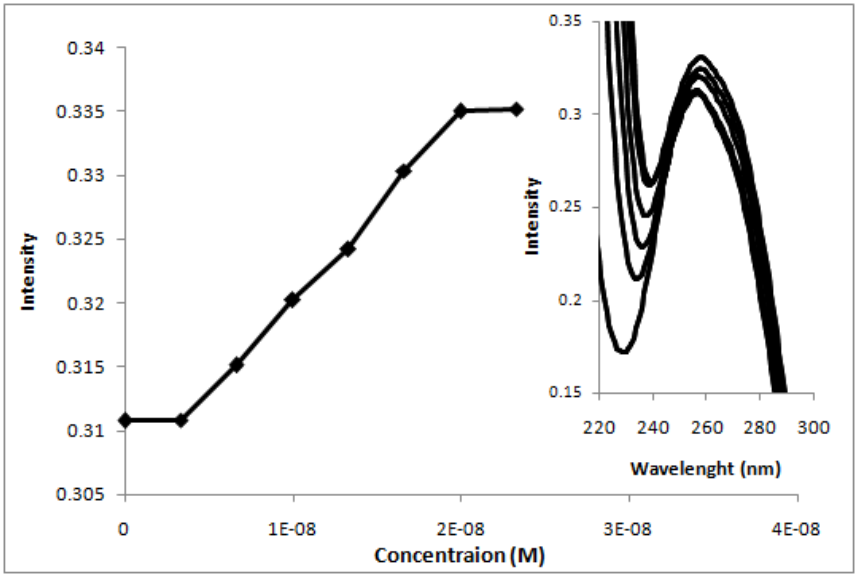

Figure 4. Plot of absorbance *vs.* different concentration of Sm^{3+}, pH=5.5.
Inset: The UV-Vis absorbance spectrum of 1.0×10^{-6} M ssDNA (a) and titration of 1.0×10^{-6} M ssDNA with (b) a+3.33×10^{-7} M Sm^{3+}, (c) a+9.99×10^{-7} M Sm^{3+}, (d) a+1.66×10^{-6} M Sm^{3+}, (e) a+2.33×10^{-6} M Sm^{3+}, (f) a+2.66×10^{-6} M Sm^{3+}

3.2.2. pH effect

UV-Vis spectrum of 5×10^{-6} M ssDNA in pH=5.5 was obtained and different concentrations of Sm^{3+} ion (8.33×10^{-7} M to 2.33×10^{-5} M) was added to ssDNA solution. By increasing pH from 2.7 to 5.5 the ssDNA absorbance shifts to the lower wavelength (253 nm). By adding Sm^{3+} to ssDNA the higher instance absorbance could be seen. Also the reaction of Sm^{3+} with ssDNA was linear until Sm^{3+} concentration received to 2×10^{-5} M of Sm^{3+} ion. Therefore approximately four Sm^{3+} ions were added to each ssDNA and after this point no excess amount of Sm^{3+} ion was bound. These evidences indicate that in this pH, besides binding phosphate linkages [39,40], Sm^{3+} ion directly coordinates electron donor groups on the nucleotide bases [41].

4. CONCLUSIONS

1. The electrochemical behavior of Sm^{3+} interacted with ssDNA was studied in two pHs and then the UV-Vis spectroscopy method was also used for supporting these evidences.
2. The cyclic voltammetry study of Sm^{3+} illustrates a reversible 1e transfer reaction electrode process on platinum. The Sm^{3+} at various scan rates gave a linear correlation between the peak current (i_p) and square root of scan rate, showing that the kinetics of process was diffusion-controlled.
3. Sm^{3+} can bind to ssDNA and forms a stable complex. The ratio between [Sm^{3+}] and [ssDNA] is dependence to pH and pK_a of bases.
4. In pH 2.7 Sm^{3+} binds to ssDNA mainly by electrostatic attraction and binding number, n, was obtained 2 of Sm^{3+} per ssDNA. In this pH the bases of ssDNA are totally protonated and Sm^{3+} interact electrostatically with phosphate groups. The UV-Vis study was obtained similar result.
5. The results in pH 5.5 show that Sm^{3+} can bind to ssDNA with electrostatic and covalent band. In this pH beside phosphate groups, the bases can be interacted to Sm^{3+}, too. The binding number was obtained 4 of Sm^{3+} per ssDNA.

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