Chinese Chemical Letters Vol. 15, No. 4, pp 443-446, 2004
http://www.imm.ac.cn/journal/ccl.html

Studies of Ce(Ⅲ)-ALC-F^- Interacting with Herring Sperm DNA by Electrochemical, Fluorimetric and UV-spectrophotometric Method

Guang Han LU[1]*, Wei LIU, Chuan Yin LIU, Li Ping JIANG, Feng SONG

College of Chemistry, Central China Normal University, Wuhan 430079

Abstract: Ce(Ⅲ)-ALC-F^- complex can react with hsDNA to form an electrochemically non-active supermolecular complex Ce(Ⅲ)-ALC-F^--DNA in the buffer solution of $(CH_2)_6N_4$(pH=4.9), which results in the decrease of the peak current of Ce(Ⅲ)-ALC-F^-. This method can be applied to determine DNA concentration. In addition, by using fluorimetric and UV-spectrophotometric methods with studies of denatured DNA and the effect of NaCl solution , it is also found that the binding mode is intercalation.

Keywords: Ce(Ⅲ)-ALC-F^-, electrochemically non-active supermolecular complex, herring sperm DNA(hsDNA), fluorimetry, UV-spectrophotometry, intercalation.

The interaction of DNA with metal complex has attracted considerable interest and the correlative research has been reported a lot[1], but the research about the interaction of DNA with ternary complex has not been reported so far. Ce(Ⅲ)-alizarin complexone (ALC) -F^- is a ternary complex whose coordination number is 1:1:1[2]. In this paper, the mechanism of the interaction of ternary complex Ce(Ⅲ)-ALC-F^- and herring sperm DNA(hsDNA) is studied for the first time by electrochemical, fluorimetric and UV-spectrophotometric methods.

Procedure

1 mL of 1mol/L $(CH_2)_6N_4$, 0.2 mL of 1×10^{-3}mol/L ALC, 1.2 mL of 5×10^{-4}mol/L Ce(Ⅲ), 1 mL of 1×10^{-4}mol/L fluoride solution, and different concentrations of hsDNA were added into a series of 10 mL beakers. The mixture were allowed to stand for 1 h and then diluted to 10 mL with water, and the DNA concentration was determined by electro-chemical, fluorimetric and UV-spectrophotometric methods.

Results and Discussion

The cyclic voltammetric experiments were carried out on a BAS CV-50W voltammetric analyzer. **Figure 1** shows that ALC has a reduction peak at -0.44V and an oxidation

*E-mail:ghlu@ccnu.edu.cn

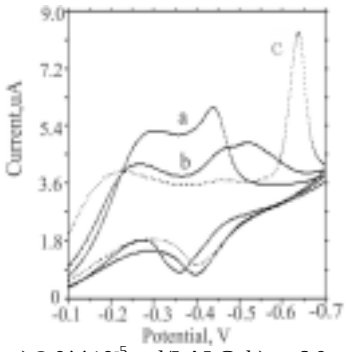
peak at -0.37V(curve a). When Ce^{3+} was dded, Ce(Ⅲ)-ALC showed the reduction peak at -0.52V and the oxidation peak at -0.40V(curve b). When F^- was added, Ce(Ⅲ)-ALC-F^- complex was obtained. This complex showed the reduction peak at -0.64V, and the oxidation peak of Ce(Ⅲ)-ALC at -0.40V, which was caused by the decomposition of Ce(Ⅲ)-ALC-F^{-2}(curve c). **Figure 2** shows that in the presence of DNA, the reduction and oxidation peak current are decreased. The result indicated that Ce(Ⅲ)- ALC-F^- reacted with hsDNA to form an electrochemically non-active supermolecular complex[3-5]. The decrease of the peak current is proportional to the DNA concentration from 0.5 to 5.5 μg/mL, and the correlation coefficient is 0.995. It is found that Ce(Ⅲ)-ALC-F^- reacted with DNA to form a 1:3 complex as determined by molar ratio method(**Figure 3**). The denatured DNA has less influence on the decrease of the peak current, and addition of 0.01~0.25 mol/L NaCl solution has no influence on the reaction of hsDNA with the complex. From above results it can be proposed that the complex intercalated in DNA[5].

A Shimadzu Model UV-265 spectrophotometer was used for spectrophotometric determinations. **Figure 4** shows that with the same concentrations of DNA as the reference, the absorption of Ce(Ⅲ)-ALC-F^- was decreased and the peak position shifted significantly to longer wavelength than that was in the presence of DNA. From this phenomenon, it can be considered that Ce(Ⅲ)-ALC-F^- reacted with DNA in a mode of intercalation[6-8]. The red shift and the decrease of the absorption of Ce(Ⅲ)-ALC-F^- were caused by the influence of the electron clouds of the base of DNA[8,9].

Fluoresence measurements were carried out on a Shimadzu RF-540 fluorescence spectrophotometer. The excitation light was set at 255 nm. It can be seen from **Figure 5** that in the presence of DNA, the fluoresence of Ce(Ⅲ)-ALC-F^- was decreased, but the peak position(λ_{em}=360 nm) does not shift. This phenomenon might be caused by the transfer of the electrons or the energy between Ce(Ⅲ)-ALC-F^- and DNA. All the above results proved that Ce(Ⅲ)-ALC-F^- intercalated into DNA[7,10-12].

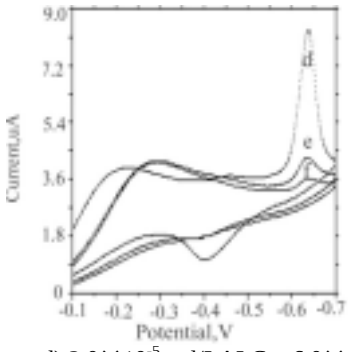
The determination of DNA was not affected by common ions (K^+,Na^+,Cl^- ,SO_4^{2-}) (**Table 1**).

Figure 1 The cyclic voltammograms of a,b,c



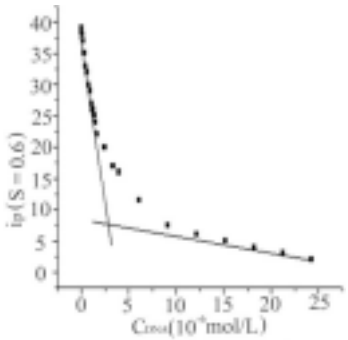
Conditions**:** a):2.0×10^{-5}mol/LALC, b):a+6.0×10^{-5}mol/L Ce(Ⅲ), c**)**:b+1.0×10^{-5} mol/LF^-

Figure 2 The cyclic voltammograms of d,e,f



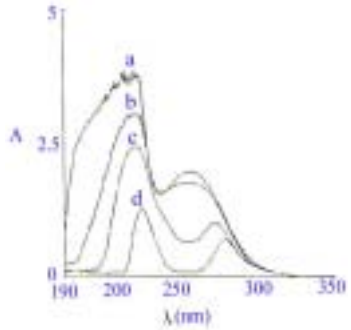
Conditions: d):2.0×10^{-5}mol/LALC + 6.0×10^{-5}mol/L Ce(Ⅲ) + 1.0×10^{-5} mol/LF^-,e): d+10 μg/mLDNA, f**)**:d+20 μg/mLDNA

Figure 3 Determination of coordination number of Ce(Ⅲ) -ALC-F$^-$-DNA complex



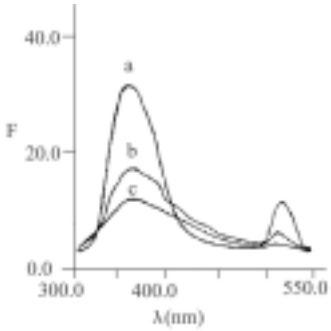
Conditions: 2.0×10^{-5} mol/L ALC+6.0×10^{-5} mol/LCe(Ⅲ)+1.0×10^{-5}mol/L F$^-$

Figure 4 The UV spectra of a,b,c,d



Conditions: a):2.0×10^{-5} mol/L ALC+6.0×10^{-5}mol/L Ce(Ⅲ)+ 1.0×10^{-5} mol/LF$^-$, b):a +50μg/mLDNA, c):a+100μg/mL DNA, d):a+150μg/mL DNA

Figure 5 The fluoresence spectra of a,b,c



The experimental conditions are the same as **Figure 4**

Table 1 The results of the determination of the DNA

sample	DNA(added) (μg/mL)	DNA(found) (μg/mL)	recovery(%)	RSD (n=4) (%)
1	2	1.98	99	1.5
2	4	4.05	101.3	1.3
3	6	5.96	99.3	1.3
4	8	8.03	100.3	1.4

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Received 24 March, 2003