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Electrochemical and Spectrometric Studies of Double-Strand Calf Thymus Gland DNA Denatured by Al(III) at Neutral pH

Fuping ZHANG, Qing CAO, Jiongjia CHENG, Caihua ZHANG, Na AN, and Shuping BI†

School of Chemistry & Chemical Engineering, State Key Laboratory of Coordination Chemistry of China & MOE Key Laboratory for Life Science, Nanjing University, Nanjing 210093, China

The interaction between double-strand calf thymus gland DNA (ds-DNA) and Al(III) was studied by using differential pulse voltammetry (DPV) at a hanging mercury drop electrode (HMDE), Raman spectrometry and circular dichroism (CD) spectra. It was shown that at neutral pH ds-DNA did not produce any cathodic peak at the HMDE in the potential window from –550 to –2000 mV *vs*. SCE. However, in the presence of Al(III), a cathodic peak was generated at about –1660 mV, which is ascribed to a reduction of adenine and cytosine residues of single denatured DNA (sd-DNA). It was concluded that ds-DNA was completely denatured to sd-DNA by Al(III) at a neutral pH. The apparent denaturing kinetic velocity constants of ds-DNA by Al(III) were derived from linear increases of the cathodic peak currents with time. When $[Al(III)]\times[OH^-]^3 \geq 2 \times 10^{-26}$, the precipitation of $Al(OH)_3$ was observed and identified by the Raman spectrum, and inductively coupled plasma atomic emission spectrometry (ICP-AES). CD spectra showed that the B-type of structure conformations of ds-DNA and related sd-DNA did not change with the increment of Al(III) from 5.0×10^{-7} to 1.0×10^{-5} M, but the corresponding absorption strengths increased. The related physiological significances and possible applications of the observations were considered.

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Introduction

Aluminum (Al) comprises 8% of the Earth's crust, and is widely used in modern society, for instance, common utensils and materials for civil life. Its abundant spread in the environment makes it impossible for people to avoid exposure or contact. Alzheimer's disease (AD) accounts for about 60 – 75% of elderly dementia patients, and Parkinson's disease (PD) imperils 16% of persons over 80 years-old. Al is believed to be a cofactor or an aggravating factor in the pathologies of both AD and PD, although there are still many contradictory data among epidemiological studies, analytical measurements, and clinical examinations.[1-3] The causative role of AD was confirmed to be related to familial autosomal problems, such as the beta amyloid (Aβ) pathway, tau presenilins and ApoE.[4] Al was identified to have a special effect in enhancing Aβ toxicity.[5] Meanwhile, there are 10 to 20% PD cases that are attributed to genetic mutations.[6] Therefore, to answer the question as to whether Al is involved in stimulating abnormal genes is very important.

It is well known that DNA modulates the division of cells and encodes heritage and genetic information for the replication and transcription of proteins and enzymes. The binding of metal ions, such as Cu(I), Ag(I), Au(I), Ca(II), Mg(II), Ba(II), Sr(II), Zn(II), Cd(II), Cu(II), Co(II), Ni(II), Mn(II), Pt(II), Pd(II), Pb(II), Hg(II), La(III), Ru(III), Tb(III), Dy(III), Ce(III), Cr(III) and Al(III), to different sources of DNA was widely studied and applied to chemical analyses and polymer syntheses.[7-16] Recently, Al was shown to induce DNA damage and endoreduplication and to have clastogenic effects in human lymphocytes incubation.[17-19] The Al-DNA adduct was identified in clinic samples.[20] Three Al-DNA complexes were proposed before two decades when ds-DNA was denatured at pH 5.0 – 7.0 with the mole ratios of Al:DNA from 0 to 0.7 and at pH 6.5 – 7.5, when the mole ratio was higher than 0.8, DNA precipitation was also observed.[21] However, Al did not perturb the CD spectra of ds-DNA solutions at room temperature.[21] Oppositely, from variations of the CD spectra of $(CCG)_{12}$ in the presence of Al at pH 7.4, Al was suggested to induce a conformation transformation of B-DNA to Z-DNA, which is believed to be a possible factor concerning neurological disorders.[22] Furthermore, at pH 6.0 and 7.0, ds-DNA precipitated by Al completely but without binding to it, as was observed by another independent research group.[23] Also, precipitations were observed from both the addition of ds-DNA to Al solutions at pH 4.0, and the reversed addition at pH 4.6 without any precipitation appearing.[24] Apparently, in Refs. 21, 23 and 24, all of the $[Al(III)]\times[OH^-]^3$ were much higher than K_{sp} (1.3×10^{-33}) of $Al(OH)_3$, and the precipitations or aggravations were not separated, and were analyzed further. Up to now, there has been no additional evidence for testifying about the aforementioned three Al-DNA complexes. Therefore, some extensive research is needed concerning these subjects.

Evaluating the interaction between DNA and metal ions, or some small molecules using electrochemical and spectrometric procedures is very important in molecular biological studies.[25,26] In this paper, the interaction between ds-DNA and Al(III) at neutral pH was studied by monitoring the DPV cathodic responses of ds-DNA at HMDE and coupled with CD spectra in the presence of Al(III). The results showed that Al(III) completely denatured ds-DNA with a one-order apparent kinetic

† To whom correspondence should be addressed.
E-mail: bisp@nju.edu.cn

velocity of 0.61 – 1.2 mg/L min^{-1} ds-DNA. The precipitation of $Al(OH)_3$ was observed and identified by ICP-AES and Raman spectra when $[Al(III)]\times[OH^-]^3$ was over 2×10^{-26} (10^7 times surplus to K_{sp} of $Al(OH)_3$). Also, an extensive discussion about the mechanism of the interaction is included.

Experimental

Apparatus and reagents

A three-electrode system was used, which consisted of a HMDE (0.01623 cm^2) work electrode, a platinum foil counter electrode, and a saturated calomel reference electrode (SCE). All electrochemical experiments were performed with a BAS-100 electrochemical system (BAS Inc., IN). The settings of the DPV mode were: scan rate, 20 mV/s; pulse amplitude, 50 mV; pulse width, 60 ms. Raman spectra were recorded on JY HR800 Raman spectrum instrument (France). ICP-AES results were provided by the Analytic Center of Nanjing University. CD spectra were recorded on a J-810 (s) spectra instrument manufactured by JASCO. The pH values were regulated by a PHSJ-4A pH meter (Shanghai Science Meter Limit Company, China), and the temperature was controlled with a 501 super constant-temperature water circulatory flume (Sichuan Experimental Apparatus Manufactory, China).

ds-DNA was bought from Sigma and used directly without further purification. Its 1000 mg/L stock solution was prepared by dissolving a proper amount of ds-DNA in a 0.2 M Atkins–Pantin buffer solution (pH 7.83) under N_2. The related mole concentrations were detected by a UV-vis 3100 meter (Shimadzu, Japan) at 259.6 nm with ε = 6600 M/cm. For example, 50, 100 and 200 mg/L of ds-DNA correspond to 1.21×10^{-4}, 2.50×10^{-4} and 4.71×10^{-4} M, respectively. An Al(III) stock solution (0.01 M) was prepared by dissolving super-purity Al powder (99.99%) in a 1:10 HCl solution and kept at pH < 2. It was diluted to the proper volume when used. Buffer solutions were prepared by mixing 2-amido, 2-methyl, 1,3-dihydroxy propane with appropriately hydrochloric acid (Tris–HCl). The electrolyte salt was 0.1 M KCl. All of the chemicals were of analytical reagent grade unless stated otherwise. All of the diluted solutions were prepared by diluting related stock solutions with double-distilled water. Necessary polyethylene vessels were used. All labware was soaked in 10% HNO_3 for at least 24 h before being carefully washed with water and double-distilled water in turn. All of the experiments were carried out at air-conditioned room temperature.

Procedures

In analytical tests, 15 ml of sample solutions were transferred into an electrolytic cell and deoxygenated by bubbling with nitrogen for 5 min and during measurements over their surface. After resting for 2 min, the HMDE was dipped into the solution and along with subsequent applying the DPV mode. Then, the current response was recorded. Following every spiking of Al(III), 2 min of stirring plus 2 min resting were processed. The HMDE was refreshed every time and the corresponding operations were repeated.

Results and Discussion

Electroanalytical studies on the denaturation of ds-DNA by Al(III) at neutral pH

Figure 1(a) shows that in the scanning potential window, there were no current peaks in buffer solutions (pH 7.3) containing

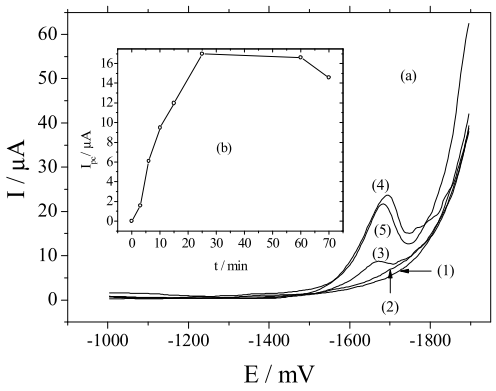

Fig. 1 (a) DPV responses of both ds-DNA in the presence of Al(III) and sd-DNA at pH 7.3. (1) Buffer solution plus 4×10^{-5} M Al(III), (2) buffer solution plus 50 mg/L ds-DNA, (3) 3 min after 4×10^{-5} M of Al(III) added finally to (2), (4) 25 min after 4×10^{-5} M of Al(III) added finally to (2), (5) buffer solution plus 50 mg/L ds-DNA denatured at 95°C for 40 min followed by a cooling in ice water. (b) The dependence of the cathode peak currents of 50 mg/L ds-DNA on the time in the presence of 4×10^{-5} M of Al(III) at pH 7.3. The currents shown in Fig. 1(b) and curves (3) and (4) have been deducted from the background response of curve (2). Curve (5) has been deducted from the background response of the buffer solution.

Al(III) and ds-DNA separately. However, after 3 min of adding Al(III) to ds-DNA, a cathode peak at –1660 mV appeared. With time, its potential shifted slightly more negatively, and the related peak current gradually increased (Fig. 1(b)). After 25 min it reached a maximal current of 17.2 μA at –1688 mV, and then decreased. In a solution of 50 mg/L of ds-DNA, denatured thermally, a cathode peak at –1680 mV with a current of 16.1 μA was also generated (Fig. 1(a)).

These peaks are the trait of sd-DNA, which differentiates from ds-DNA, and are ascribed to the reduction of adenine and cytosine residues of DNA.[27-32] However, a cathode peak of the reduction of guanine, which was believed to be at about –1.85 V, was not observed.[33,34]

The maximum cathode peak current of ds-DNA in the presence of Al(III) was 6.6% higher than that of the thermal denatured sd-DNA (Fig. 1(a)). This means that Al(III) is able to denature ds-DNA to sd-DNA completely, and is also capable of catalyzing the reduction of adenine and cytosine. This finding is quite coincidence with the observation that Al(III) totally unwound supercoiled pUC18 DNA immediately.[35] Figure 1(b) shows that in the period of the first 25 min after the addition of Al(III) in a solution of ds-DNA, the cathode peak currents varied linearly with the waiting time. In other words, this means that the concentrations of adenine and cytosine on the surface of a mercury electrode also changed linearly with the waiting time. Accordingly, it is concluded that the peak currents vary linearly with the reducible concentrations of adenine and cytosine in a bulk solution, too. Because the reducible concentrations of adenine and cytosine are proportional to the concentrations of sd-DNA ($C_{sd\text{-}DNA}$), a linear equation of $C_{sd\text{-}DNA}$ (μA) = 0.743t (min) is derived with a coefficient of R = 0.979. Obviously, when the cathode peak current of thermally denatured sd-DNA is taken as a reference, the apparent kinetic velocity constant (k_a) for the ds-DNA denatured by Al(III) can be deduced, and given in Table 1.

Figure 2 shows the DPV responses of ds-DNA in the presence of Al(III) at pH 7.3. When the concentrations of both ds-DNA and Al(III) increased comparably, it seemed very peculiar that

Table 1 Apparent kinetic velocity constants of ds-DNA denatured by Al(III)

$C_{ds\text{-}DNA}$/ mg L^{-1}	Al(III)/ 10^{-5} M	pH	TS/ min	k (μA)/ min^{-1}	k_a (mg/L)/ min^{-1a}	R
50	3.84	7.3	25	0.759	1.18	0.984
	4.00			0.743	1.16	0.979
200	1.20	7.0	37	0.391	0.607	0.917
	2.00		42	0.542	0.841	0.877

TS denotes the time scale for the determination, k, the kinetic velocity of the production of sd-DNA from ds-DNA denatured by Al(III).
a. In the deduction, all of the I_p subtracted the background and the I_p of 50 mg/L ds-DNA denatured thermally was selected as the reference.

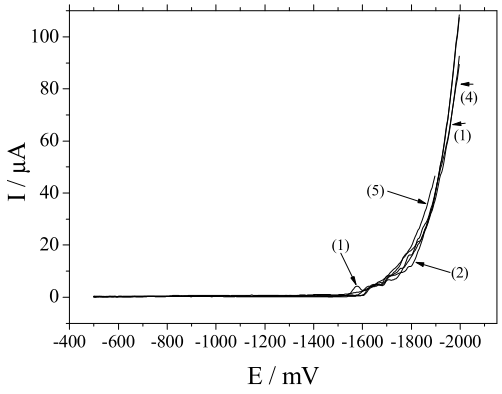

Fig. 2 DPV responses of ds-DNA in the presence of Al(III) at pH 7.3. (1) The buffer solution plus 7.7×10^{-5} M Al(III), (2) the buffer solution plus 100 mg/L ds-DNA, (3) the buffer solution plus 100 mg/L ds-DNA after the addition of 7.7×10^{-5} M Al(III) after 18 min, (4) the buffer solution plus 200 mg/L ds-DNA, (5) the buffer solution plus 200 mg/L ds-DNA after the addition of 7.7×10^{-5} M Al(III) after 60 min.

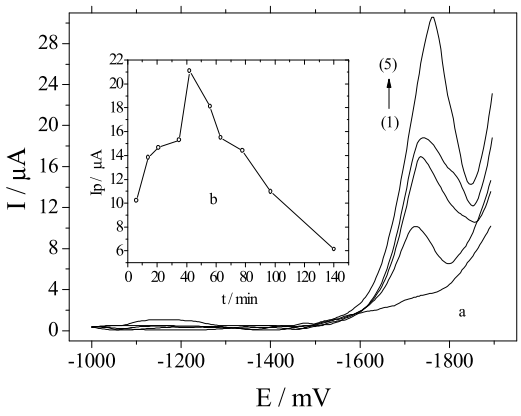

Fig. 3 (a) DPV responses of ds-DNA in the presence of Al(III) at pH 7.0. (1) Buffer solution plus 200 mg/L ds-DNA, (2) 8 min later after the addition of 1.2×10^{-5} M Al(III) to (1), (3) 37 min later after the addition of 1.2×10^{-5} M Al(III) to (1), (4) 14 min later after the addition of 2×10^{-5} M Al(III) to the buffer solution plus 200 mg/L ds-DNA, (5) 42 min later after the addition of 2×10^{-5} M Al(III) to the buffer solution plus 200 mg/L ds-DNA. (b) The dependence of the cathode peak currents of 200 mg/L ds-DNA on the time in the presence of 2×10^{-5} M of Al(III) at pH 7.0.

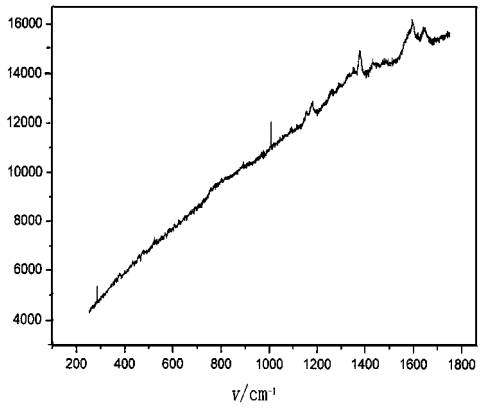

Fig. 4 Raman spectrum of the floccule filtered from a solution of 200 mg/L ds-DNA after the addition of 2.0×10^{-5} M Al(III) at pH 7.0.

the cathode peak of the reduction of adenine and cytosine did not appear. In fact, when the addition of Al(III) even reached to 3.1×10^{-4} M, there were no observable cathode peak appeared either (not shown in the figure). This might be due to the special role of the different Al(III) species for the denaturing of ds-DNA.

Figure 3(a) (2, 3) shows the DPV responses when the pH value was controlled at 7.0 and the concentration of Al(III) was decreased down to 1.2×10^{-5} M. The reduction peaks of adenine and cytosine were observed at about –1724 mV. The related peak currents increased with the waiting time, and reached to the highest values 37 min after the addition of Al(III) to the ds-DNA solution. The related apparent kinetic velocity constants of ds-DNA denatured by Al(III) reasoned by the above-mentioned method are presented in Table 1.

$Al(OH)_3$ precipitation analyzed by ICP-AES and Raman spectra

Nevertheless, when the concentration of Al(III) rose to 2.0×10^{-5} M (Fig. 3(a) (4, 5) and Fig. 3(b)), the phase variation was quite different from that of Al(III) = 1.2×10^{-5} M. In the first 42 min, the cathodic peak currents continued to increase. Since then, an observable precipitable floccule grew up from the solution, and the cathodic peak currents decreased almost linearly with the following waiting time. Two days later, the cathodic peak disappeared. It is obvious that at a given pH the concentration of Al(III) played a crucial role to determine the cathodic response of ds-DNA in the solution. The floccule was filtered and examined by the Raman spectrum (Fig. 4), and residual solution was analyzed by ICP-AES. The results showed that the concentrations of Al(III) in the blank and the residual solutions were lower than 3.7×10^{-6} and 1.44×10^{-5} M, respectively. Also, the concentrations of phosphorus in the ds-DNA solution without the addition of Al(III) and that in the residual solution were 4.8×10^{-4} and 5.7×10^{-4} M, respectively. Namely, 46.5% of the Al(III) added to the solution was removed without any loss of the phosphorus element. These results demonstrated that the floccule was $Al(OH)_3$. Therefore, the reason for the lack of a cathodic peak in Fig. 2 was a shortage of "active Al(III)" due to the formation of $Al(OH)_3$. However, there was still about 1.07×10^{-5} M Al(III) in the residual solution. This indicated that 53.5% of Al(III) added may have been tightly bonded and contributed nothing to the DPV responses mentioned above.

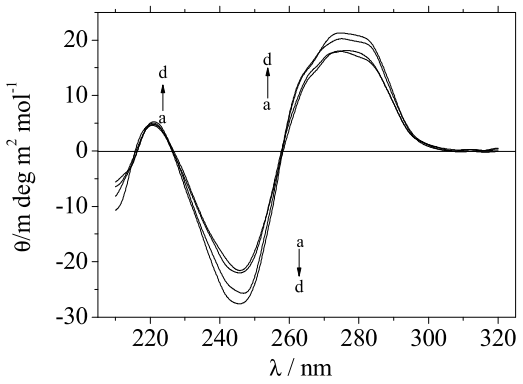

Fig. 5 Effect of the Al(III) on CD spectra of ds-DNA at pH 7.0. (a) Buffer solution plus 100 mg/L ds-DNA, (b) addition of 5.0×10^{-7} M of Al(III) to (a), (c) addition of 5.0×10^{-6} M of Al(III) to (a), (d) addition of 1.0×10^{-5} M of Al(III) to (a).

Compared with K_{sp} of $Al(OH)_3$, all of the $[Al(III)]\times[OH^-]^3$ shown in Figs. 1 to 3 were much larger than it, but the deposition of $Al(OH)_3$ was observed only in the cases of Fig. 3(a) (4, 5) and Fig. 3(b) ($[Al(III)]\times[OH^-]^3 = 2 \times 10^{-26}$). All of the above results prove three effects: (1) the capability of Al(III) to denature ds-DNA completely; (2) some parts of Al(III) were consumed in the denaturing reaction (roughly less than 53.5% calculated from the addition of Al(III) and the results of ICP-AES); (3) the competition for Al(III) between ds-DNA and OH^- was balanced by both the kinetic and thermodynamic forces. Some portion (about 46.5%) of Al(III) was bonded by ds-DNA with a kinetic preference, which was eventually coordinated to OH^- with a thermodynamic advantage. The other portion was tightly united by ds-DNA and incapable to be removed by OH^- at the given pH. In general, metal ions such as Cu(II), Co(II), Mn(II) and Mg(II) bind to ds-DNA at both phosphate groups and base residues sites, and Al(III) binds to it at the former site.[36-39] Al(III) binding to ds-DNA at base residues sites was previously proposed by Karlik *et al.*,[21] and testified to be the case by ^{27}Al NMR.[24] Our studies have shown that about 53.5% of Al(III) is probably binding to ds-DNA at phosphate groups sites, and the other 46.5% of Al(III) is kinetically united at base residues sites. The special affinity between Al(III) and the amino group of dopamine was observed recently by our research group, which supports the following conclusion.[40] The latter portion of Al(III) unwinds the double helix of ds-DNA through the disconnection of Watson–Crick hydrogen bonds between the base pairs of ds-DNA. The former part of Al(III) was incapable of being removed by OH^- at neutral pH, while the latter could be withdrawn by OH^- thermodynamically. Therefore, even if $[Al(III)]\times[OH^-]^3$ is much greater ($\leq 10^7$) than K_{sp} of $Al(OH)_3$, $Al(OH)_3$ precipitation could not form.

Studies on CD spectra of ds-DNA at pH 7.0 in the presence of Al(III)

Figure 5 depicts the effects of Al(III) on the CD spectra of ds-DNA. In the absence of Al(III), one negative peak at 245.6 nm and two positive peaks at 221 and 274.2 nm (broad) were observed for the B-form of ds-DNA.[22,41] The 221 nm peak is due to the formation of a hydrogen bond.[37] The peaks at 245.6 and 274.2 nm are ascribed to the base pairs of ds-DNA.[42,43] After the addition of Al(III) from 5.0×10^{-7} to 1.0×10^{-5} M, the broad 274.2 nm peak inclined to shift to be more positive, and seemed to split to a shoulder peak at 281 nm. The lack of new peak indicates there showed no structure conformation transformation of ds-DNA in the presence of Al(III).

These observations are quite different from those of $(CCG)_{12}$,[22] but are similar to other reports concerning ds-DNA in the presence of Al(III).[21,36] However, the absorption strengths at 245.6 and 274.2 nm gradually increased with an increase of Al(III). The increment of the CD optical absorption of phosphorylated neurofilament with 17 amino acid sequences in the presence of Ca(II) was assigned to an increase in the production of an unordered structure component.[44] Accordingly, the observed increments of the CD spectra presented here are ascribed to the greater liberation of the same chiral species in the denaturing process of ds-DNA by Al(III). These results are in agreement with those of an electrochemical analysis, that showed that Al(III) denatures ds-DNA to single-strand sd-DNA.

Conclusions

From the above studies, we have concluded that:

(1) Al binds to ds-DNA or sd-DNA at both phosphate groups and base residues sites. The Al bound at base residue sites is able to completely denature ds-DNA by unwinding the double helix without disturbing its structure conformation at the physical pH. This would lead to potential applications for the examination, repair and refreshment of damaged sd-DNA *in vitro* in the presence of Al(III), and provided a more convenient and soft method for the denaturation of ds-DNA to sd-DNA *in vitro* than the heating method. However, the denaturation of ds-DNA to sd-DNA by Al(III) will increase the chances of both mismatching between base pairs in the following renaturation and attacking by some chemical components.

(2) The apparent kinetic velocity for the denaturation of ds-DNA by Al is one order of the concentration of ds-DNA. When the initial concentration of Al is high enough ($[Al(III)]\times[OH^-]^3 \geq 2.0 \times 10^{-26}$), this part of Al can be withdrawn by OH^- to form $Al(OH)_3$.

Acknowledgements

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