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Conformational transition of DNA in electroreduction studied by in situ UV and CD thin layer spectroelectrochemistry

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Abstract

Electrochemically induced three conformational transitions of calf thymus DNA from $B_{10.4}$ to $Z_{10.2}$-DNA and from $B_{10.2}$ to $B_{10.4}$ and to C-DNA in 10 mM phosphate buffer solution (pH 7.21) at glassy carbon electrode are found and studied by in situ circular dichroism (CD) thin layer spectroelectrochemistry with singular value decomposition least square (SVDLS) analysis. It indicates that the so-called $B_{10.2}$ form and the C-form of DNA may be composed of $B_{10.4}$ and left-A DNA and of $B_{10.4}$ and right-A DNA, respectively. The irreversible electrochemical reduction of adenine and cytosine groups in the DNA molecule is studied by UV-Vis spectroelectrochemistry. Some electrochemical parameters $\alpha n = 0.17$, $E^{0'} = -0.70$ V (vs. Ag/AgCl), and the standard heterogeneous electron transfer rate constant, $k^0 = 1.8 \times 10^{-5}$ cm s^{-1}, are obtained by double logarithmic analysis and non-linear regression.

Keywords: DNA; Conformational transitions; Electroreduction; Singular value decomposition least square analysis; In situ circular dichroism thin layer spectroelectrochemistry

1. Introduction

There are two families of double-helical structures for deoxyribonucleic acid (DNA) known as right-handed and left-handed. The familiar Watson–Crick B-form DNA is a right-handed double helix with 10 base-pairs per turn [1]. A-form DNA is also right-handed, but has 11 basepairs per turn and a slightly different dispositions of base-pairs about the helix axis [2]. Z-form DNA seen so far only in alternatively pyrimidine–purine sequences

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is a novel left-handed structure with 12 base-pairs per turn [3]. The B-form predominates in aqueous solution to the exclusion of A and Z, but in alcoholic or high-salt solution, the situation is reversed. The structures and their structural transitions between right-handed and left-handed and between different right-handed DNA have been studied experimentally and theoretically [4–6].

DNA is reduced at an applied potential more negative than −1.20 V (vs. SCE) accompanied by strong adsorption of DNA molecules on the surface of mercury and graphite carbon electrodes [7,8]. During the electroreduction process, the DNA molecule undergoes a stranded transition from double-stranded (dsDNA) to single-stranded (ssDNA). It was argued that there may exist another conformational change before the stranded transition occurred, which has to be confirmed experimentally. Among several conformation measurements, circular dichroism (CD) is a powerful tool for solution samples [4] and has been used to monitor the electric field orientation or electric dichroism of polypeptides and DNA [6,9–12]. A combination of CD spectrometry with thin layer electrochemistry, called CD thin layer spectroelectrochemistry, can be used to monitor the conformational change in an electrochemical process. How to extract structure information from experimental CD spectroelectrochemical data is another problem in this study. Singular value decomposition (SVD) [13] is a powerful mathematical tool to decompose experimental CD spectra and also considered as a basic step of the other CD analysis methods, which have been reviewed by several authors [6,13–15]. Multiple linear regression (LMR) methods [16] can give spectra and concentrations of components in a sample, but it needs the basic spectrum of each component to be used in the methods. Evolutionary factor analysis is another model-free method to deal with the large data matrix, and has been used to deal with the CD spectra [17] of small molecules, but it needs several steps, and seems not suitable for macromolecules. A combination of SVD with least squares, called singular value decomposition least square (SVDLS) analysis, is a new method for analysis of dynamic CD spectra. In this method, SVD was used to obtain the number of components and basic CD spectrum of each component from dynamic CD spectrum data, and the least square method was used to obtain the concentration distribution of each component. The new method gives not only the number of components and their CD spectra, but also the fraction distribution of each component. In the present paper, in situ CD spectroelectrochemistry with a long optical path length thin-layer cell (LOPTLC) was used to study the conformational change of DNA during electrochemical reduction. By means of SVDLS method, three conformational transitions of DNA from $B_{10.4}$ to $B_{10.2}$, from $B_{10.2}$ to $B_{10.4}$ and C-form in aqueous solution in the thin-layer cell were found as well as the electrochemical parameters.

2. Materials and methods

2.1. Materials and solutions

Calf thymus DNA, purchased from Sigma chemical company (USA), was used as received. A 0.125-mg ml^{-1} solution of DNA in 10 mM phosphate buffer solution (pH 7.21) including 0.2 M KNO_3, which was used to keep the total ionic strength of the solution and to keep the same conditions of electroreduction [8], was prepared and saturated with N_2 prior to use for all experiments in this paper. All the other reagents were analytically pure. All the solutions were prepared with double distilled water.

2.2. Experimental methods

CD thin layer spectroelectrochemistry was carried out in a LOPTLC made in our laboratory [18]. The LOPTLC was constructed with a 1.00-cm optical path length and 0.020-cm thin layer. A piece of glassy carbon (0.8×0.8 cm^2) inserted into one wall of the thin layer served as the working electrode, a platinum wire as an auxiliary electrode and Ag/AgCl (KCl saturated) electrode as reference electrode. All potentials were reported with respect to this reference electrode. If a macromolecule has a lower diffusion coefficient such as 8.3×10^{-8} cm^2 s^{-1} the time for the

macromolecule diffusing from electrode to the opposite end crossing the thin layer can be calculated as $t = 134$ s according to thin layer theory [19]. Therefore, 6 min of time interval is enough for the diffusion of the macromolecule.

An AVIV 62A DS circular dichroism spectrometer (USA) was used for CD spectrum measurement and a PAR-370 electrochemical instrument (USA) was used for electrochemical operation. A common quartz cuvette served as the optical windows. The polarized light passed through the thin layer being parallel to the working electrode, so that only the biomolecule in the solution layer could be monitored during experiments. The optical chamber of the CD spectrometer was deoxygenated with dry nitrogen (99.999% in purity) for 45 min before use and kept the nitrogen atmosphere during experiments. The temperature in the sample compartment was kept at 25.0 ± 0.1°C during the experiment. A clean quartz cuvette was filled with buffer solution or sample solution and deaerated with high purity nitrogen for 15 min. After setting up the electrodes, the quartz cuvette with solution and electrodes was set in the light path of the spectrometer. The CD spectrum of the buffer solution at open circuit served as the background CD spectrum. All CD spectra of sample solutions recorded here were the CD spectra with background subtracted.

2.3. Singular value decomposition least square method

$n \times m$ absorbances are measured at n wavelength for m samples composed of k components. If the absorbances follow Lamber–Beer's law, then each of the absorbances can be expressed as,

$$a_{i,j} = \Sigma E_{i,l} c_{j,l} \qquad (i = 1,2 \ldots n; \quad j = 1,2, \ldots m; \quad l = 1,2, \ldots k) \tag{1}$$

where $E_{i,l}$ are the absorption coefficient of sample l at wavelength i, $c_{j,l}$ are the concentrations of component j in sample l. The absorbances can be expressed as an absorption matrix and indicated in black upper case, $\mathbf{A}_{n \times m}$,

$$\mathbf{A}_{n \times m} = \mathbf{E}_{n \times k} \mathbf{C}_{k \times m} \tag{2}$$

According to the singular value decomposition method [13], $\mathbf{A}$ can be decomposed into three multiplied matrices;

$$\mathbf{A} = \mathbf{USV} \tag{3}$$

where $\mathbf{U}$ and $\mathbf{V}$ are the $n \times n$ and $m \times m$ orthogonal matrices, respectively, and $\mathbf{S}$ is an $n \times m$ matrix, of which the off-diagonal entries are all 0's and the diagonal elements are in the following sequence,

$$s_1 \geq s_2 \ldots \geq s_m \geq 0 \tag{4}$$

the s_j^2 are the eigenvalues of matrix $\mathbf{A}$. If the samples are composed of k components, the first k eigenvalues are called the principal factors ($k < m$), which can be used to describe the main properties of matrix $\mathbf{A}$, whereas the rest of the eigenvalues are responsible for measurement error. The multiplied matrix of submatrices from the first k rows of $\mathbf{U}$, first k columns of $\mathbf{S}$ and $\mathbf{V}$, respectively, is called an abstract absorption matrix, and indicated as, $\underline{\mathbf{A}}_{n \times m}$,

$$\underline{\mathbf{A}}_{n \times m} = \underline{\mathbf{E}}_{n \times k} \underline{\mathbf{C}}_{k \times m} \tag{5}$$

where $\underline{\mathbf{E}}_{n \times k} = \mathbf{U}_{n \times k} \mathbf{S}_{k \times k}$, is called the abstract absorption coefficient matrix, $\underline{\mathbf{C}}_{k \times m}$ is called the abstract concentration matrix. The standard deviation (S.D.) between $\mathbf{A}$ and $\underline{\mathbf{A}}$ is,

$$\text{S.D.} = \Sigma\Sigma[(\mathbf{A}\text{-}\underline{\mathbf{A}})/(n \times m - k)]^{1/2} \tag{6}$$

The least square method is used to transfer the matrices $\underline{\mathbf{E}}$ and $\underline{\mathbf{C}}$ into $\mathbf{E}$ and $\mathbf{C}$. According to the least square method [16],

$$\underline{\mathbf{E}} = \mathbf{A}\underline{\mathbf{C}}^t \left(\underline{\mathbf{C}}\underline{\mathbf{C}}^t\right)^{-1} \tag{7}$$

$$\underline{\mathbf{C}} = \left(\underline{\mathbf{E}}^t \underline{\mathbf{E}}\right)^{-1} \underline{\mathbf{E}}^t \mathbf{A} \tag{8}$$

and the concentration for each component is a non-negative quantity,

$$\underline{\mathbf{C}} \geq 0 \tag{9}$$

According to Eq. (9), setting the values of negative elements in $\underline{\mathbf{C}}$ to 0, and apply to Eqs. (7) and (8) for several times until S.D. becomes stable, then $\underline{\mathbf{E}} = \mathbf{E}$ and $\underline{\mathbf{C}} = \mathbf{C}$.

The double logarithmic analysis is a linearization method for irreversible electrochemical processes, and has been described in a previous paper [20].

3. Results

3.1. Electrochemical reduction of DNA

The cyclic voltammogram of 0.35 mg ml^{-1} calf thymus DNA in pH 7.21 phosphate buffer including 0.2. M KNO_3 on bare GC electrode is shown in Fig. 1. An irreversible reduction peak of adenine or cytosine in the DNA molecule appears at approximately -0.55 V (Fig. 1 curve B) [8]. This peak increases and shifts to negative values when the GC electrode is modified with methyl green (Fig. 1, curve C). Methyl green (MG) has been used as a mediator to accelerate the electron

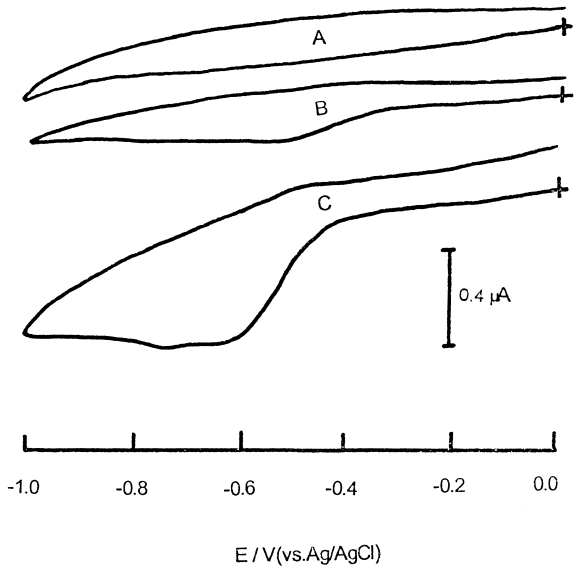

Fig. 1. Cyclic voltammogram of calf thymus DNA at GC electrode; 0.35 mg ml^{-1} calf thymus DNA in pH 7.21 phosphate buffer including 0.2 M KNO_3. Sweep rate: 100 mV s^{-1}. (A) background; (B) DNA at bare GC electrode; (C) DNA at GC electrode modified with methylene green.

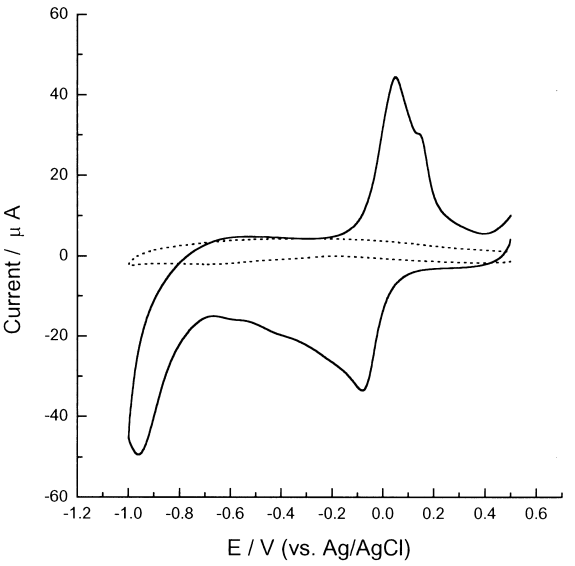

Fig. 2. Cyclic voltammogram of methylene green at GC; 1×10^{-3} M methylene green in 0.2 M KNO_3 including 0.01 M phosphate buffer solution (pH 7.21), sweep rate is 100 mV s^{-1}. Background in dotted line.

transfer of protein [21] when adsorbed at the GC electrode. The CV curve of MG under the same experimental conditions is given in Fig. 2, which shows a quasi reversible redox behavior at $E_{1/2} = -0.014$ V and an irreversible reduction peak at -0.962 V. Generally, a mediator accelerates a reduction process by shifting the peak potential to positive and increasing peak current, however, MG molecules accelerate the reduction of DNA by increasing the peak current only while shifting the peak potential to negative, which may be due to the strong interaction of MG with DNA molecules when the MG molecule inserted into the big groove in the double helix chain was similar to that of DNA oxidation with $[Ru^{II}(tpy)(phen)OH_2]$-$(ClO_4)_2$ case [22]. This result implies that MG can catalyze the irreversible reduction of DNA and the peak at -0.55 V corresponds to the reduction of DNA.

The reduction process was monitored by in situ UV spectroelectrochemistry with LOPTLC. The typical adsorption peak at 258 nm of double helix DNA in thin layer solution increases with applied potential shifting to -1.10 V from 0.00 V (Fig. 3) due to conformational changes of the DNA in-

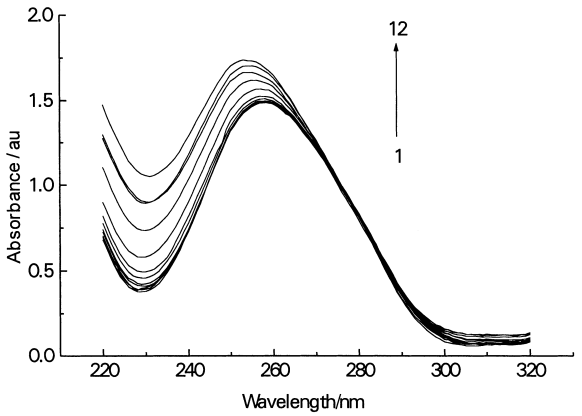

Fig. 3. UV-Vis spectra of DNA with different applied potentials. Applied potential: 1, open circuit; 2–12, from 0.0 to 1.1 V step −0.1 V.

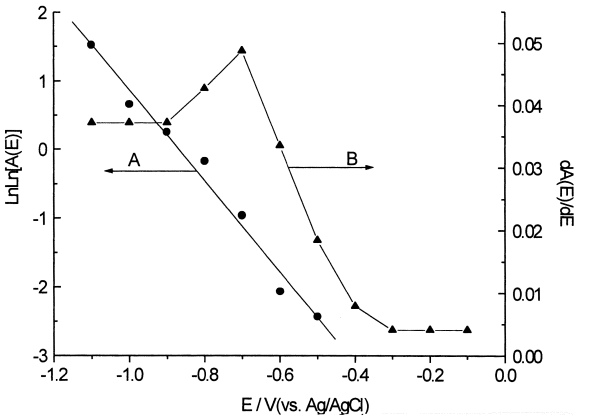

Fig. 4. Double logarithmic analysis. (A) Double logarithmic plot; (B) differential curve of absorbance–potential plot.

duced by electrochemical reduction, which shows a very similar tendency as that in denaturization process of DNA [6]. A plot of peak height at 258 nm against applied potential is called an absorbance–potential curve. A differential curve of the absorbance–potential plot with respect to potential is shown in Fig. 4B, from the peak position of which the apparent formal potential of the reduction process is obtained as −0.70 V. The double logarithmic curve shows an oblique line (Fig. 4A), indicating that the electroreduction of DNA corresponds to a simple electron transfer process. The product of electron number and electron transfer coefficient, $\alpha n = 0.17$, and standard heterogeneous electron transfer rate constant, $k^0 = 1.8 \times 10^{-5}$ cm s^{-1}, are obtained from the slope and intercept of the oblique line, respectively.

3.2. CD spectroelectrochemical results and SVDLS analysis

Calf thymus DNA (0.125 mg ml^{-1}) in 10 mM phosphate buffer (pH 7.21) including 0.2 M KNO_3 was used to perform the in situ CD spectroelectrochemistry. The CD spectrum of DNA changed with applied potential is shown in Fig. 5. The typical positive Cotton peak at 278 nm decreases with applied potential from 0.0 V shifting to −0.80 V, and increased gradually with applied potential from −0.80 V shifting to −1.20 V. This implies that there may be two conformation transition processes occurring in the potential range studied.

Three principal conformations [23] and their CD spectra are extracted from the experimental CD spectra by SVDLS. The first principal conformation shows a typical CD spectrum of native DNA (right-handed, Fig. 6 curve A), but the two smaller principal conformations have CD spectra with a very good mirror-image relationship. Curve B in Fig. 6 has two larger positive peaks at 263 and 253 nm and one small negative peak at 300 nm belonging to the right handed, and curve C in

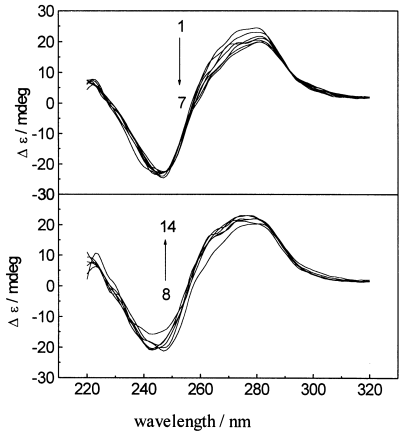

Fig. 5. CD spectra of DNA with different applied potentials. Applied potential (E/V): 1, open circuit; 2–14, from 0.0 to −1.3 V step −0.10 V.

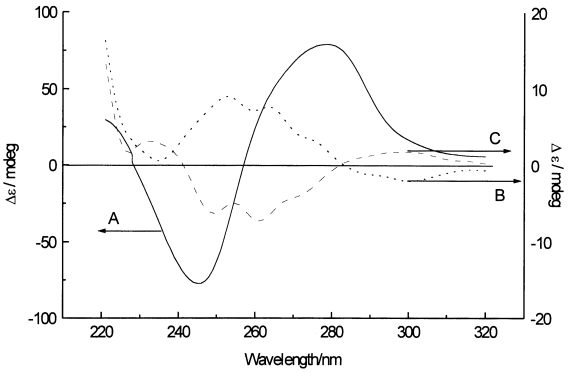

Fig. 6. Principal CD spectra of DNA. (A) $B_{10.4}$-DNA; (B) right-A; (C) left-A DNA.

Fig. 6 has two larger negative peaks at 260 and 250 nm and one positive peak at 300 nm belonging to the left-handed. Since the positive peak at 260 nm is characteristic of A-form DNA [24], the one with a positive peak at 260 nm may be considered as a right A-form DNA and the one with a negative peak at 260 nm as a left A-form DNA. Based on these three principal CD spectra and experimental CD spectra, the least square analysis was performed with a total standard deviation of 1.20. The resulting CD spectra are shown in Fig. 7. These three CD spectra are very similar to those of calf thymus DNA [25–28] with the A curve identified as $B_{10.4}$-DNA, B curve as C-DNA and C curve as $B_{10.2}$-DNA. Compared with the principal CD spectra, it can be seen that the so-called $B_{10.2}$-DNA may be the mixture of $B_{10.4}$-DNA with some left A-form DNA, while the C-DNA may be a mixture of $B_{10.4}$-DNA with right A-form DNA.

The fraction distribution of each component with applied potential is also obtained from the SVDLS analysis as shown in Fig. 8. The three conformations, i.e. $B_{10.4}$-, $B_{10.2}$- and C-form DNA coexist in the solution at 0.0 V with the fractions of 0.60, 0.34 and 0.06, respectively. When the applied potential shifts to −0.05 V, the fraction of $B_{10.2}$-DNA increases rapidly whereas the $B_{10.4}$-DNA fraction decreases rapidly and that of the C-form DNA decreases slowly. This implies that the conformational transition is mainly from $B_{10.4}$ to $B_{10.2}$. In the potential range from 0.0 to −0.5 V, the reduction of DNA does not occur, so this conformational transition may be driven by the electric field in the electric double layer due to the interactions between the non-unified electric field and multiple charges, permanent dipole interactions in DNA and water molecules. With the applied potential continually shifting from −0.50 to −1.1 V, the fraction of $B_{10.2}$ dramatically decreases from 0.56 to 0.0 while the fractions of $B_{10.4}$ increases rapidly from 0.42 to 0.865, and that of the C-form from 0.02 to 0.135, respectively. It means that there are two conformational transitions from $B_{10.2}$ to $B_{10.4}$ and from $B_{10.2}$ to C-form DNA. In the potential range from −0.5 to −1.1 V the reduction of DNA takes place, which may be responsible for the conformational transitions.

4. Discussion

Double stranded DNA is not really a rigid molecule, especially in solution. Therefore it is possible that several conformations coexist in one molecule. The conformational transition of DNA from B to Z in aqueous solution induced by cations has been studied [4–6]. It is suggested that B-form DNA is stable in aqueous solution due to the spine of hydration [5], and the salt ions-induced conformational transition may be due to the salt concentration-dependent part of

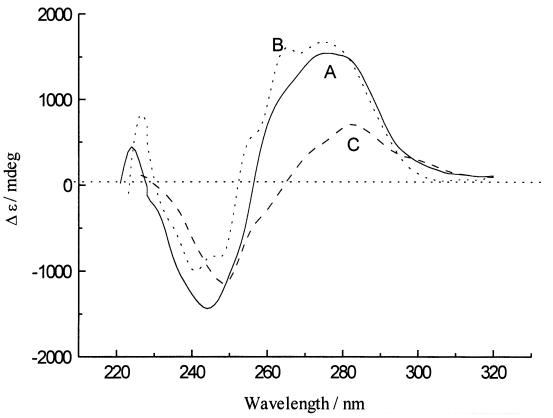

Fig. 7. CD spectra of DNA obtained by SVD-LS. (A) $B_{10.4}$-; (B) right-A; (C) left-A DNA.

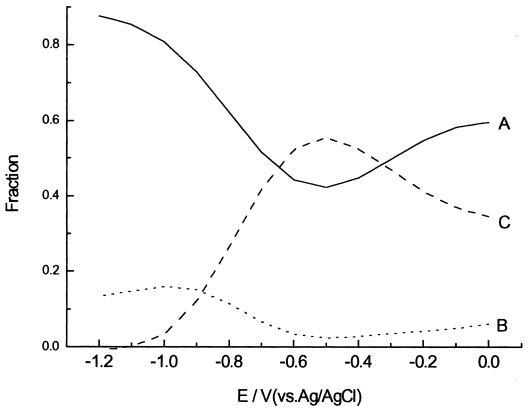

Fig. 8. Fraction distribution of each component of DNA with applied potential. (A) $B_{10.4}$-; (B) C-form; (C) $B_{10.2}$-DNA.

the free energy difference [29]. The double layer near the electrode surface [19] offers a very strong electric field (33.3 kV cm^{-1} for 10 mM electrolyte with a 0.10-V applied potential), which interacts with the macromolecules electrostatically through the charges, permanent dipole and induced dipole moments similar to those of ions. When a macromolecule is put into an electric field, it will reorientate with the largest dipole axis along the electric field, known as birefringence or reorientation effects. After the reorientation, the whole molecule has a smaller interaction energy with the external field, but some segments in the molecule may not be favorable in the electric field because their dipoles are in unfavorable directions in space. These dipoles will overcome the interactions between molecules and groups and rotate according to the electric field. For a unified electric field, there is only the interaction of torque. For a non-unified electric field, the gradient of the electric field from electrodes acts as another extra force on each dipole, which will drive the dipole rotating and moving away from the electric field. In a unified electric field, a rodlike DNA mainly reorientates along the electric field as described [5,6]. In a non-unified electric field, besides the reorientation effect, some segments in DNA molecule will be driven by extra force to change their position in some distance, which results in a partly conformational change of macromolecule to some extent. That may be the case of conformational transition from $B_{10.4}$ to $B_{10.2}$. When some components in the double chain of DNA take part in electrochemical reduction, the double chain would partly be damaged and result in conformation transitions from $B_{10.2}$ to C- and to $B_{10.4}$-form.

5. Conclusion

There are three conformations of native calf thymus DNA which coexist in aqueous solution with the predominant $B_{10.4}$ form; they are $B_{10.4}$, $B_{10.2}$ and the C-form. The non-unified electric field in the double electric layer of an electrode forces the DNA molecule to change its conformations from $B_{10.4}$ to $B_{10.2}$. Electrochemical reaction partly damages the rigid double chain of the DNA and changes the DNA conformation from $B_{10.2}$ to $B_{10.4}$ and to the C-form. In situ UV-Vis and CD spectroelectrochemistry with LOPTLC have been proven as useful tools to monitor the conformational change of macromolecules in an electrochemical process as well as the electric field. The SVDLS analysis is a powerful method to deal with dynamic CD spectra, which gives not only the number of components of conformations and the CD spectrum of each conformation, but also the conformation distribution with applied potentials. In situ thin layer CD spectroelectrochemistry with SVDLS opens a new way to study the structural changes of biomolecules the electrochemical processes.

Acknowledgements

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