ELSEVIER

Biophysical Chemistry 112 (2004) 27–33

Biophysical Chemistry

www.elsevier.com/locate/bpc

Combing DNA on CTAB-coated surfaces

Hu-Zhi Zheng[a], Dai-Wen Pang[a,*], Zhe-Xue Lu[a], Zhi-Ling Zhang[a], Zhi-Xiong Xie[b]

[a]*College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P.R. China*
[b]*College of Life Sciences, Wuhan University, Wuhan 430072, P.R. China*

Received in revised form 18 June 2004; accepted 22 June 2004
Available online 20 July 2004

Abstract

A fluorescence microscope (FM) coupled with an intensified charge-coupled device (ICCD) camera was used to investigate the combing of DNA on cetyltrimethyl ammonium bromide (CTAB)-coated glass surfaces. DNA molecules can be combed uniform and straight on CTAB-coated surfaces. Different combing characteristics at different pH values were found. At lower pH (ca. 5.5), DNA molecules were stretched 30% longer than the unextended and DNA extremities bound with CTAB-coated surfaces via hydrophobic interaction. At high pH values (e.g., 6.4 and 6.5), DNA molecules were extended about 10% longer and DNA extremities bound with CTAB-coated surfaces via electrostatic attraction. At pH 6.0, DNA molecules could be extended 30% longer on 0.2-mM CTAB-coated surfaces. CTAB cationic surfactant has both a hydrophobic motif and a positively charged group. So, CTAB-coated surfaces can bind DNA extremities via hydrophobic effect or electrostatic attraction at different pH values. It was also found that combing of DNA on CTAB-coated surfaces is reversible. The number of DNA base pairs binding to CTAB-coated surfaces was calculated.

Keywords: CTAB; DNA; Fluorescence microscopy; Molecular combing; YOYO-1

1. Introduction

As genetic code carriers, DNA research is of great interest to biologists and chemists. Traditional research methods, for example, ultraviolet-visible spectroscopy [1], electrochemistry [2], nuclear magnetic resonance [3], Raman spectroscopy [1] and so on, are mainly based on studying a large number of DNA molecules in ensemble. Information about single DNA molecules is buried in thousands of DNA molecules using these methods.

Yoshikawa [4] used fluorescence microscope (FM) to investigate the conformation change of single DNA molecules in solution. Only long DNA molecules, such as T4 DNA of 166kb, can be studied because of the limits of optical resolution. T4 DNA molecules coil with a long axis length of about 3.5 μm in solution. Researchers usually focus on studying the transition of DNA conformations caused by some reagents [5].

Because DNA is string-like, it is easy to stretch so as to facilitate the observation. Many techniques have been developed to stretch DNA molecules, such as atomic force microscope (AFM) cantilever [6], optical [7] or magnetic tweezers [8] and traps [9,10], spin [11], flowing liquid [12] or flowing gas [13]. These techniques have been used for investigating the elastic characteristics of DNA molecules [8,9], dynamics of individual flexible polymers in a shear flow [12], interaction between DNA and proteins [14], photo-damaging of DNA molecules [15] and so on. But some of them involve complex instruments and unwieldy manipulations. One end or both ends of DNA molecules need modifying and anchoring on surfaces if they are stretched by AFM cantilever, tweezers, traps and spin. Only one DNA molecule can be stretched at a time using these techniques. Flowing liquid can be used for simultaneously stretching many DNA molecules, but one end of DNA molecules needs modifying and sophisticated control of flowing liquid is required. Flowing

* Corresponding author. Tel.: +86 27 87686759; fax: +86 27 87647617.
E-mail address: dwpang@whu.edu.cn (D.-W. Pang).

0301-4622/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2004.06.011

gas stretching is a simple technique without modification of DNA ends. But skilled operation is required, and the repeatability is poor.

Bensimon et al. [16] and Michalet et al. [17] developed a technique called "molecular combing", which can be used to stretch a large number of DNA molecules without modifying ends of DNA molecules. So, statistical analysis of imaging comes true. The most interesting merit is that "molecular combing" is suitable for stretching very long DNA molecules, such as genomic DNA. "Molecular combing" has been used for investigating DNA replication [18], fluorescence in situ hybridization [19,20], constructing genomic physical map [19,21] and nanomanipulation [22,23].

It is unnecessary to modify the ends of DNA molecules because "molecular combing" is based on the binding of DNA terminals to substrate surfaces [24]. It is believed that the double helix has a greater tendency to unwind at its ends than along its midsegments at proper pH values [24]. Consequently, the DNA is more hydrophobic at its extremities. And as reported by Stein et al. [3], the DNA's extremities are more negative than its midsegment. The specific binding of DNA extremities to surfaces is due to either the hydrophobic interaction with hydrophobic surfaces or electrostatic interaction with positively charged surfaces. On hydrophobic surfaces, DNA molecules extend about 30% longer than DNA contour length while about 10% longer on positively charged substrates [24,25].

As a cationic surfactant, cetyltrimethyl ammonium bromide (CTAB) has both a hydrophobic group and a positively charged group. It is also used as a germicidal chemical for it can bind to DNA. Yoshikawa's [5] work shows that CTAB could induce DNA molecules to transform from coils to compact globes in solution. In the present work, CTAB-coated glass was used as a substrate to comb DNA molecules. Different combing characteristics on CTAB-coated surfaces prepared with different concentrations of CTAB were found at different pH values. Our research will help choose new substrates to comb DNA molecules and will also be helpful in understanding the interaction of DNA with cationic surfactants.

2. Experimental setup

2.1. Materials

2-(*N*-Morpholino)ethanesulfonic acid (MES), cover glass (22×22 mm) and slides (25×75 mm) were purchased from Sigma. Stock solution of 1 mM YOYO-1 was obtained from Molecular Probes and diluted by ultra-pure water immediately before use. 4′,6-Diamidino-2-phenylindole (DAPI, Fluka) and 2-mercaptoethanol (2-ME, Fluka), λ-DNA (48 502 base pairs, Sino-American Biotech) and λ-DNA *Eco*RI markers (Sino-American Biotech) were used as received. λ-DNA *Eco*RI markers have six segments with the following lengths: 21,226; 7,420; 5,810; 5,650; 4,880; 3, 540 base pairs, respectively. The concentration of DNA was determined spectrophotometrically according to a molar extinction coefficient for DNA bases of 6600 M^{-1} cm^{-1} at 260 nm [5]. The ratio of the absorbance of DNA solution at 260 nm to that at 280 nm was 1.8. Absolute ethanol and CTAB were purchased from Shanghai Chemical, China. CTAB was recrystallized twice from ethanol. Ultra-pure water was prepared by a Labconco system (18.2 MΩ).

2.2. Instrumentation

The schematic diagram of DNA imaging system is shown in Fig. 1. An inverted fluorescence microscope (FM, Olympus IX-70) was equipped with an oil immersion objective (Zeiss, 100×, N.A.=1.25) and a U-MWB filter set (Olympus, 450-480/500/515 nm). A 100 W high-pressure mercury lamp (HBO) was used as illumination. An intensified charge-coupled device (ICCD) camera (512×512 pixels, I-PentaMAX Gene III, Roper Scientific) was utilized to acquire images. A 6% neutral density filter (ND filter) and a 25% ND filter were employed to decrease the excitation intensity, i.e., the excitation light was decreased to 1.5% of the original. The ICCD camera was mounted at the FM side port to obtain a wider field of view or at the single-lens reflex port (SLR) to improve the accuracy of measurement through a standard C-mount adapter. WINVIEW or WINSPEC software was used to acquire images and analyze data. AFM images were obtained using a Picoscan AFM (Molecular Imaging) with contact mode and a commercial MAClever type II probe (Molecular Imaging).

2.3. Procedure

Cover glass and slides were treated as follows. They were soaked in commercial liquid detergent for ca. 1 day,

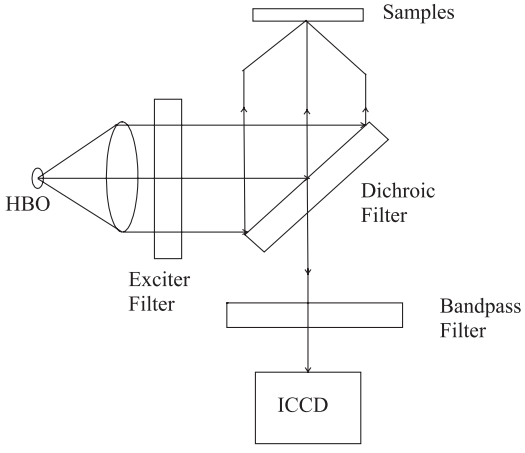

Fig. 1. Schematic diagram of the imaging system.

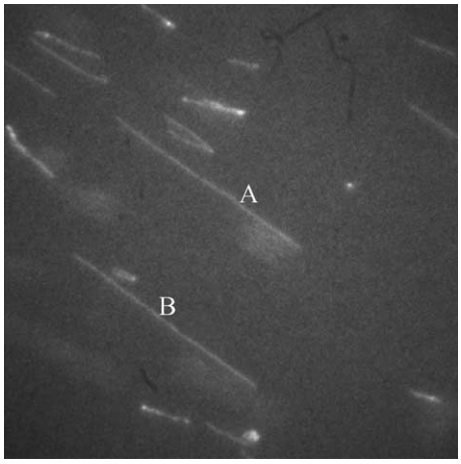

Fig. 2. Image of combed DNA molecules. Molecules A and B were selected for calculating the length. Image size: 43×43 μm^2. Experimental condition: pH value was 5.6, DNA molecules were combed on 0.2-mM CTAB-coated surfaces.

washed with water, then soaked in concentrated nitric acid for more than 5 h, again washed with water, subsequently soaked in 30% H_2O_2 for more than 5 h, rinsed with doubly distilled water and at last kept in absolute ethanol. Pretreated cover glass was rinsed with ultra-pure water and dried by flowing argon immediately before use. Pretreated slides were rinsed with ultra-pure water and soaked in different concentrations of CTAB solution for 24 h and dried by flowing argon prior to use.

DNA and YOYO-1 were diluted to 5 μM (in base pairs) and 1 μM with ultra-pure water immediately prior to use, respectively. DNA (5 μl) and YOYO-1 (5 μl) were added into 90 μl of 50 mM MES solution at different pH values, then 5 μl of 2-ME was added to reduce photo-bleaching and mixed gently. The mixture was incubated for 90 min at 4 °C in the dark before use. After incubation, the mixture was diluted with MES as needed. A total of 5 μl of the mixture was dropped onto cover glass, and the cover glass was placed bottom-up on a CTAB-coated slide carefully. After being kept for several hours, the slide was put on the FM stage and imaged.

Binding efficiency was calculated according to Bensimon's report [16]. However, the binding efficiency on the 0.5-mM CTAB-coated substrate surface was difficult to calculate because of the aggregation of DNA molecules.

DNA molecules combed straight and uniform were selected (Fig. 2) for the calculation of their length. Because long DNA molecules are easy to break when treated, the DNA molecules that were 30% shorter than the average length were not selected.

The force acting on DNA molecules was also calculated according to the equation $F=EA(l/l_0-1)$, where $E=1.1\times10^8$ N/m^2 is Young's modulus of DNA molecules, $A=3.8\times10^{-18}$ m^2 is their cross-sectional area, l_0 is the natural length of DNA (for λ-DNA, l_0=16.2μm) and l/l_0 is the relative extension [25].

3. Results and discussion

3.1. Imaging of single DNA molecules

DNA molecules were combed uniform and straight on a CTAB-coated glass surface (Fig. 3a) with a high binding efficiency of ca. 60%. Some combed DNA molecules whose both ends bound to the CTAB-coated glass surface look like a "U"(Fig. 3b) [25]. And some whose midsegments bound to the CTAB-coated glass surface look like a "V" (Fig. 3c). Combing characteristics of DNA molecules depend on both the concentration of CTAB used for the preparation of CTAB-coated glass surfaces and the pH value.

Lengths of DNA molecules straightened by combing and the force acting on DNA molecules are listed in Tables 1

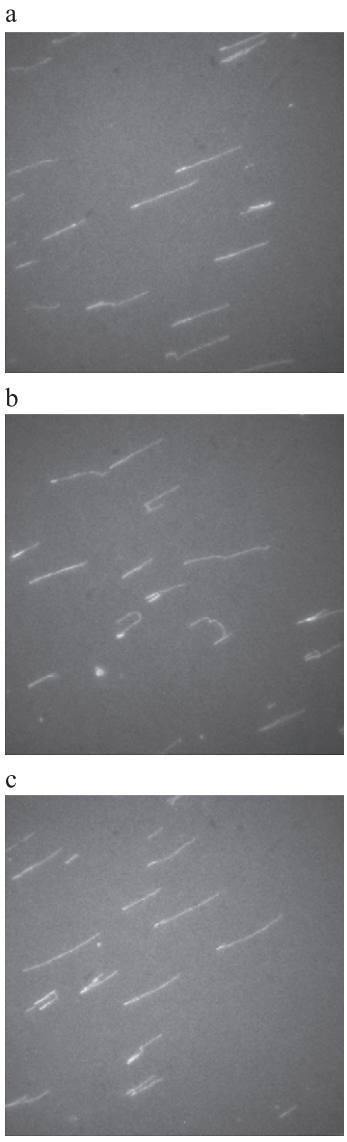

Fig. 3. Typical images of combed DNA (a), "U" pattern (b) and "V" pattern (c). Image size: 71×71 μm^2. Experimental condition: pH value was 5.6, DNA molecules were combed on 0.2-mM CTAB-coated surfaces.

Table 1
Combing characteristics of DNA on 0.1-mM CTAB-coated substrates

pH	5.4	6.5
Binding efficiency	ca. 60%	ca. 60%
Length of straightened λ-DNA (μm)	21.0±1.2[a]	18.0±0.4[c]
Length of straightened markers (μm)	8.5±1.5[b]	6.5±0.3[d]
Ratio of the lengths	2.5	2.8
Stretching force (pN)	ca. 130	ca. 50
Number of base pairs bound to surfaces (kb)	3	6

Confidence degree was 0.90. (a) 90, (b) 68, (c) 126 and (d) 79 DNA molecules were measured, respectively.

and 2. Because DNA bundles are not uniform in length and some of them are very long in some cases [26], existence of DNA bundles will affect accurate measurement of DNA lengths. So, single DNA molecules must be distinguished from DNA bundles. Fortunately, no DNA bundles were found on the CTAB-coated surfaces in our experiments.

The most convincing evidence for the presence of single DNA molecules can be obtained with AFM or transparent electron microscopy. But these two techniques cannot be used for the coarse CTAB-coated surfaces (Fig. 4).

Lyon et al. [15] reported three pieces of evidence indicating that the fluorescence signals arose from single DNA molecules and not from DNA bundles. First, the observed density of single DNA molecules on the surface was consistent with the value calculated from the total number of added DNA molecules and the total surface area. Second, cross-sectional line plots showed that all DNA molecules had similar intensities. Third, the measured physical lengths were consistent with the contour length of DNA.

The area of the 22×22 mm cover glass used was comparable to ca. 2.6×10^5 ICCD fields of view in the case of ICCD being mounted on the SLR port of the FM and a 100× oil immersion objective being used. A total of 5 μl of mixture of DNA and YOYO-1 containing about 6×10^5 DNA molecules was used each time, i.e., there were ca. 2–3 DNA molecules per field of view. This was just the case consistent with our observation. Cross-sectional analysis shows that all DNA molecules had similar fluorescence intensities (Fig. 5).

In Lyon et al.'s report, the measured physical lengths were only slightly larger than the calculated contour length. But the lengths of straightened DNA molecules were 10–30% larger than the calculated contour length on the CTAB-coated surface. So, an improved criterion to determine whether the DNA molecules observed were single or not was developed. λ-DNA and λ-DNA *Eco*RI markers were combed and imaged, respectively. Their straightening lengths were then calculated. λ-DNA *Eco*RI markers have six segments, but only the segment with a length of 21,226 base pairs could be straightened effectively. The ratio of their base pairs should be consistent with the ratio of their straightening lengths if the DNA molecules observed were single. As known, λ-DNA is 48,502 base pairs long. Our results show that the ratio of their straightening lengths of λ-DNA to markers was 2.5–2.8 (Tables 1 and 2), while the ratio of their base pairs is about 2.28. So, it is single DNA molecules that were observed.

3.2. Influence of CTAB concentration on combing

Three different concentrations of CTAB were used to coat glass slides. Different combing characteristics of DNA on different surfaces were found. On 0.1-mM CTAB-coated surfaces, DNA molecules could be combed uniform and straight with a high binding efficiency of ca. 60% at pH 5.4, 6.4 and 6.5 (Table 1). Straightening lengths were 21.0±1.2 μm at pH 5.4, which was ca. 30% longer than the contour length of λ-DNA and consistent with published results on hydrophobic surfaces [24,25]. Thus, it can be concluded that the binding of DNA extremities to CTAB-coated surfaces is attributed to hydrophobic interaction at pH 5.4. As reported [18], the addition of surfactants can decrease the stretching force, thus shortening the lengths of straightened DNA. But our results show that CTAB did not shorten the lengths of straightened DNA. The force acting on DNA molecules was calculated to be about 130 pN (Table 1).

At 6.5, straightened DNA molecules were 18.0±0.4 μm long on 0.1-mM CTAB-coated surfaces, which was about 10% longer than the contour length of λ-DNA and in agreement with lengths of straightened DNA on ionizable surfaces [24,25]. So, the binding of DNA ends to CTAB-coated surfaces is ascribed to electrostatic interaction [24,25]. The stretching force was about 50 pN (Table 1). At pH 6.4, similar results could also be obtained.

On 0.2-mM CTAB-coated surfaces, the optimal combing occurred at pH 5.6, 6.0, 6.4 and 6.5 (Table 2), with a binding efficiency of about 60% at pH 5.6, 6.4 and 6.5 and about 70% at pH 6.0. Straightening lengths were about 21.3±0.9 μm at pH 5.6, 20.9±0.5 μm at pH 6.0 and 18.2±0.8 μm at 6.5. Straightened DNA molecules were 30% longer than their contour length at pH 5.6 and 6.0 and 10% longer at 6.5. The corresponding stretching forces were about 130, 120 and 50 pN (Table 2), respectively. So, the binding of DNA ends to CTAB-coated surfaces is due to hydrophobic interaction at pH 5.6 and electrostatic interaction at 6.5. The higher binding efficiency could be obtained at pH 6.0, but

Table 2
Combing characteristics of DNA on 0.2-mM CTAB-coated substrates

pH	5.6	6.0	6.5
Binding efficiency	ca. 60%	ca. 70%	ca. 60%
Length of straightened λ-DNA (μm)	21.3±0.9[a]	20.9±0.5[c]	18.2±0.8[e]
Length of straightened markers (μm)	8.6±0.8[b]	8.5±0.9[d]	7.0±0.5[f]
Ratio of the lengths	2.5	2.5	2.6
Stretching force (pN)	ca. 130	ca. 120	ca. 50
Number of base pairs bound to surfaces (kb)	3	3	4

Confidence degree was 0.90. (a) 119, (b) 62, (c) 115, (d) 81, (e) 103 and (f) 75 DNA molecules were measured, respectively.

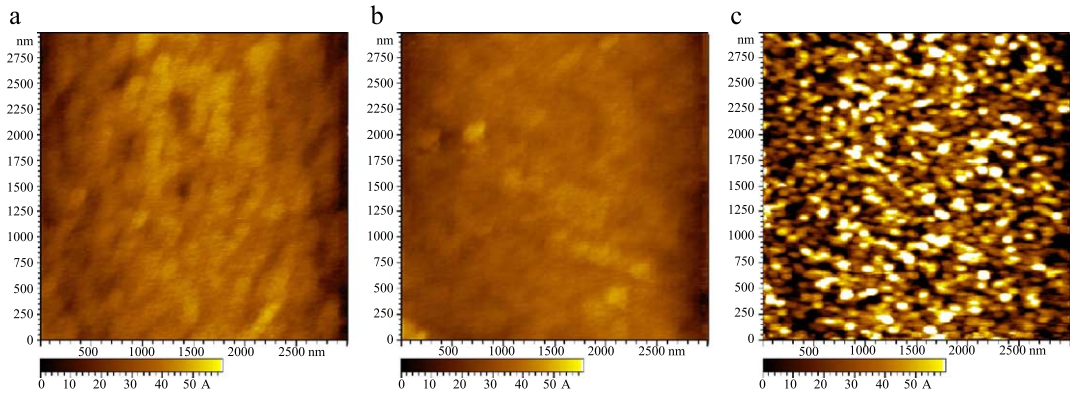

Fig. 4. AFM images of CTAB-coated glass substrates (a) on 0.1-mM CTAB-coated glass substrate, (b) on 0.2-mM CTAB-coated glass substrate and (c) on 0.5-mM CTAB-coated glass substrate.

the mechanism of binding of DNA extremities to the substrate surface is unknown.

At pH 6.0, DNA molecules can be combed straight on 0.2-mM CTAB-coated surfaces but cannot be combed on 0.1-mM CTAB-coated surfaces, which might be due to the difference in the CTAB surface coverage between two surfaces. The surface coverage of CTAB on 0.2-mM CTAB-coated surfaces should be higher than that on 0.1-mM CTAB-coated surfaces, and hence, more positive charges and more hydrophobic groups could be available.

On 0.5-mM CTAB-coated surfaces, DNA molecules cannot be combed straight. Almost no DNA molecules could be combed straight only at pH 6.5. The binding efficiency could not be calculated because of DNA aggregations (Fig. 6). And the lengths of straightened DNA could not be calculated because almost no DNA molecules could be combed straight.

As shown in Fig. 4c, the surface became rougher on 0.5-mM CTAB-coated glass surfaces, which might be characteristic of the formation of hemi-micelles [27]. The positively charged surface, high concentration of CTAB, which can shrink DNA molecules [5], and surface roughness caused DNA molecules only to be partly straightened at pH 6.5.

The above results are different from other published reports. On substrates used by other researchers, DNA molecules can be combed straight only over a narrow pH range. The most optimal combing straight occurred at pH 5.5±0.2 on hydrophobic surfaces [24]. On surfaces coated with ionizable groups, the optimal pH is linked to the surface charge and thus is linked to the pK_a of the surface groups and their density [24]. However, on the CTAB-

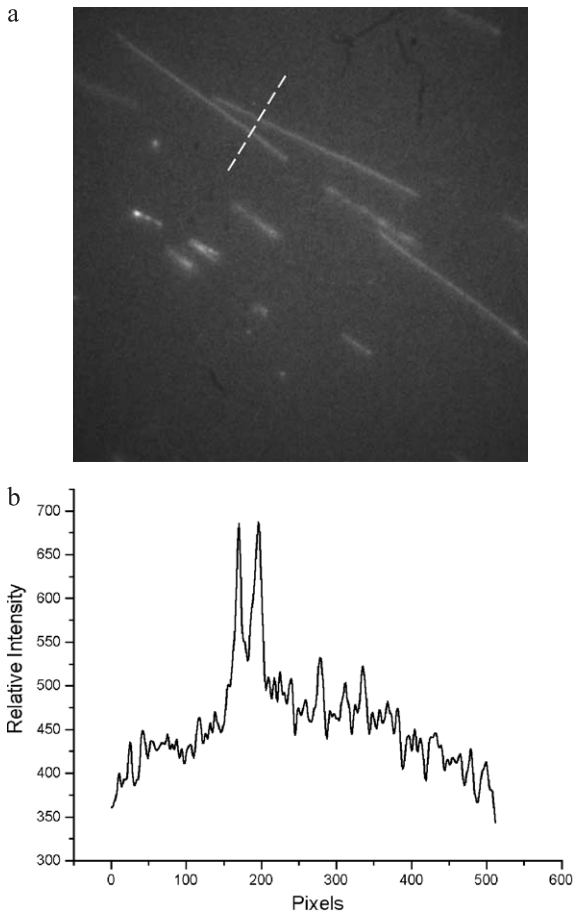

Fig. 5. Image of DNA (a) and related cross-sectional curve (b). Image size: 43×43 μm^2.

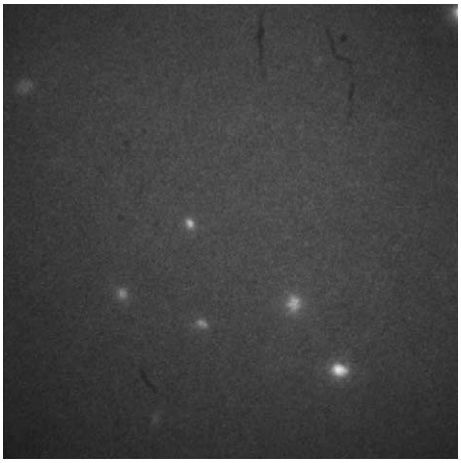

Fig. 6. Image of DNA on 0.5-mM CTAB-coated substrate. Image size: 43×43 μm^2.

coated surface used in this work, DNA can be combed straight and uniform at more pHs than that reported by others. At the same time, the combing is affected by the CTAB concentration that coats surfaces.

3.3. Influence of incubation time

The optimized incubation time was 5–7 h. Shorter incubation time caused low binding efficiency and poor combing, while longer incubation time caused contraction of combed DNA molecules (Fig. 7), which was different from the irreversible stretching of DNA on hydrophobic silanated surfaces. Combed DNA molecules on hydrophobic silanated surfaces kept straight and could be imaged after several months [16]. To distinguish it from the irreversible combing process on hydrophobic surfaces, the combing of DNA on CTAB-coated surfaces was named as "reversible combing". Maybe the contraction results from the combination of DNA and CTAB after long incubation [5].

3.4. Number of DNA base pairs anchored to CTAB-coated surfaces

The most important step of "molecular combing" is the specific binding of DNA terminals to substrate surfaces. However, it is still a problem to determine the number of DNA base pairs binding to substrate surfaces. Taylor et al. [28] stretched λ-DNA and then added fluorescent-labeled restriction enzyme *Eco*RI to investigate the interaction of DNA with the enzyme. Five *Eco*RI sites on DNA could be imaged for observation. But the first *Eco*RI site was closer to one end of the DNA than estimated. They believed that the end of DNA often formed a coil; hence, it was difficult to stretch. Two kinds of DNA were combed on CTAB-coated surfaces in our research, one with a length of 48,502 bp and the other with a length of 21,226 bp. As mentioned above, their base pair ratio was 2.29, while their combing length ratio was over a range of 2.5 to 2.8. Because some of DNA base pairs bind to CTAB-coated surfaces, the binding base pairs cannot be stretched.

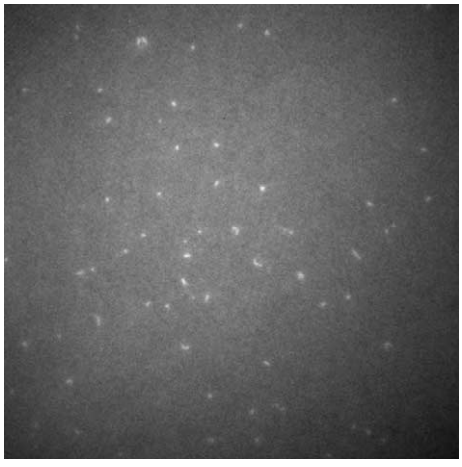

Fig. 7. Image of DNA stained with DAPI on a CTAB-coated surface kept for more than 15 h. Image size: 71×71 μm^2. Experimental condition: pH value was 5.6, DNA molecules were combed on 0.2-mM CTAB-coated surfaces.

The number of base pairs binding to CTAB-coated surfaces was calculated based on three hypotheses. First, under the same experimental conditions, i.e., the same pH value and the same substrate surface, DNA molecules with different lengths of base pairs were stretched at the same rate of extension. This hypothesis has been confirmed by some reports [24,25]. So, under the same experimental conditions, forces that act on all DNA molecules are the same according to the equation $F=EA(l/l_0-1)$ [25]. Molecular combing can be described below. DNA extremities first bind to substrate surfaces, and then DNA molecules are stretched by a moving air/water interface [16]. Some base pairs are required to bind to substrate surfaces to resist the stretching force; otherwise, DNA will be taken away from the substrate surface. Under given conditions, forces that act on all kinds of DNA molecules are the same, and the binding mechanism of DNA extremities to substrate surfaces is also the same. Hence, the second hypothesis can be proposed that all kinds of DNA molecules need the same number of base pairs to bind to the substrate surface. Third, the base pairs binding to the substrate surface have no contribution to the combing length of DNA. The length of DNA molecules is tens of folds smaller than their contour length because they form coils in solution. For example, T4 DNA (166 kb) with a contour length of about 55 μm is only about 3.5-μm long in the long axis because of coiling in solution [4,5]. When DNA molecules are combed, the base pairs binding to the substrate surface will keep coiled [28] while others will be stretched uniformly. The coil length of several kilos of base pairs binding to the substrate surface can be ignored compared to that of the uniformly combed part.

The number of base pairs binding to substrate surfaces can be calculated according to Eqs. (1) and (2):

$$(48,502 - x) \times y = \text{length of combed } \lambda - \text{DNA} \quad (1)$$

$$(21,226 - x) \times y = \quad \text{length of combed marker} \quad (2)$$

where x is the number of base pairs that bind to the CTAB-coated surfaces and y is the combing length per base pair. The number of base pairs binding to CTAB-coated surfaces is listed in Tables 1 and 2. It was reported [24] that combing caused a uniform extension of DNA on polystyrene or silane-coated surfaces and that the dependence of combing length on base pairs was linear and passed the coordinate origin. The difference between their reports and our experiment can be explained by the fact that they combed longer DNA (up to 200 kb) but shorter DNA was combed in our experiments. The binding part of DNA on the surfaces can be ignored relative to long DNA molecules. And the difference in substrates can also contribute to the different results.

4. Conclusion

DNA molecules can be combed straight and uniform on surfaces coated with different concentrations of CTAB, and the combing is reversible. Different combing characteristics at different pH values have been found because of different bindings of DNA extremities to CTAB-coated surfaces. At ca. pH 5.5, the binding is due to hydrophobic interaction, and at pH 6.4 and 6.5, the binding is owing to electrostatic interaction. At pH 6.0, ca. 70% of DNA molecules could be combed uniform and straight on 0.2-mM CTAB-coated glass surfaces. But the mechanism needs to be further explored. The number of DNA base pairs binding to CTAB-coated surfaces was also calculated.

Acknowledgements

The authors wish to thank Professor Li-Huang Zhu of the Institute of Genetics of the Chinese Academy of Sciences at Beijing, China, for his helpful discussion. This work was supported by the National Science Fund for Distinguished Young Scholars (Grant No. 20025311) and the National Natural Science Foundation of China (Grant Nos. 20299034; 20207005).

References

[1] C.G. Coates, L. Jacquet, J.J. McGarvey, S.E.J. Bell, A.H.R. AlObaidi, J.M. Kelly, Resonance raman probing of the interaction between dipyridophenazine complexes of Ru(II) and DNA, J. Am. Chem. Soc. 119 (1997) 7130–7136.

[2] D.-W. Pang, H. Abruna, Micromethod for the investigation of the interactions between DNA and redox-active molecules, Anal. Chem. 70 (1998) 3162–3169.

[3] V.M. Stein, J.P. Bond, M.W. Capp, C.F. Anderson, M.R. Anderson, Importance of coulombic end effects on cation-accumulation near oligonucleotide B-DNA using Na NMR, Biophys. J. 68 (1995) 1063–1071.

[4] Y. Kenichi, Controlling the higher-order structure of giant DNA molecules, Adv. Drug Deliv. Rev. 52 (2001) 235–244.

[5] S.M. Mel'nikov, V.G. Sergeyev, K. Yoshikawa, Discrete coil–globule transition of large DNA induced by cationic surfactant, J. Am. Chem. Soc. 117 (1995) 2401–2408.

[6] A. Engel, H.E. Gaub, D.J. Muller, Atomic force microscopy: a forceful way with single molecules, Curr. Biol. 9 (1999) R133–R136.

[7] C.G. Baumann, V.A. Bloomfield, S.B. Smith, Stretching of single collapsed DNA molecules, Biophys. J. 78 (2000) 1965–1978.

[8] S.B. Smith, L. Finzi, C. Bustamante, Direct mechanical measurement of the elasticity of single DNA molecules by using magnetic beads, Science 258 (1992) 1122–1126.

[9] T. Strick, J.F. Allemand, D. Bensimon, A. Bensimon, V. Croquette, The elasticity of a single supercoiled DNA molecule, Science 271 (1996) 1835–1837.

[10] Y. Arai, R. Yasuda, K.-I. Akashi, Y. Harada, H. Miyata, K. Kinosita Jr., H. Itoh, Trying a molecule knot with optical tweezers, Nature 399 (1999) 446–448.

[11] H. Yokata, J. Sunwoo, M. Sarikaya, G. van den Engh, R. Aebersold, Spin-stretching of DNA and protein molecules for detection by fluorescence and atomic force microscopy, Anal. Chem. 71 (1999) 4418–4422.

[12] P. LeDuc, C. Haber, G. Bao, D. Wirtz, Dynamics of individual flexible polymers in a shear flow, Nature 399 (1999) 564–566.

[13] J.W. Li, C.L. Bai, C. Wang, A convenient method of aligning large DNA molecules on bare mica surfaces for atomic force microscopy, Nucleic Acids Res. 26 (1998) 4785–4786.

[14] L.R. Brewer, M. Corzett, R. Balhorn, Protamine-induced condensation and decondensation of the same DNA molecules, Science 286 (1999) 120–123.

[15] W.A. Lyon, M.M. Fang, W.E. Haskins, S. Nie, A dual-beam optical microscope for observation and cleavage of single DNA molecules, Anal. Chem. 70 (1998) 1743–1748.

[16] A. Bensimon, A. Simon, A. Chiffaudel, V. Croquette, F. Heslot, D. Bensimon, Alignment and sensitive detection of DNA by a moving interface, Science 265 (1994) 2096–2098.

[17] X. Michalet, R. Ekong, F. Fougerousse, S. Rousseaux, C. Schurra, N. Hormigold, M. van Slegtenhorst, J. Wolfe, S. Povey, J.S. Beckmann, A. Bensimon, Dynamic molecular combing: stretching the whole human genome for high-resolution studies, Science 277 (1997) 1518–1523.

[18] Z. Gueroui, C. Place, E. Freyssingeas, B. Berge, Observation by fluorescence microscopy of transcription on single combed DNA, Proc. Natl. Acad. Sci. 99 (2002) 6005–6010.

[19] J. Herrick, A. Bensimon, Imaging of single DNA molecule: applications to high-resolution genomic studies, Chromosom. Res. 7 (1999) 409–423.

[20] K. Monier, X. Michalet, J. Lamartine, High-resolution mapping of the X-linked lymphoproliferative syndrome region by FISH on combed DNA, Cytogenet. Cell Genet. 81 (1998) 259–264.

[21] J.-F. Brugere, E. Cornillot, G. Metenier, A. Bensimon, C.P. Vivares, *Encephalitozoon cuniculi* (Microspora) genome: physical map and evidence for telomere-associated rDNA units on all chromosomes, Nucleic Acids Res. 28 (2000) 2026–2033.

[22] Z.-Q. Ouyang, J. Hu, S.-F. Chen, J.-L. Sun, M.-Q. Li, Molecular pattern by manipulating DNA molecules, J. Vac. Sci. Technol., B 15 (1997) 1385–1387.

[23] J. Hu, Y. Zhang, H. Gao, M. Li, U. Hartmann, Artificial DNA patterns by mechanical nanomanipulation, Nano Lett. 2 (2002) 55–57.

[24] J.-F. Allemand, D. Bensimon, L. Jullien, A. Bensimon, V. Croquette, pH-dependent specific binding and combing of DNA, Biophys. J. 73 (1997) 2064–2070.

[25] D. Bensimon, A.J. Simon, V. Croquette, A. Bensimon, Stretching DNA with a receding meniscus: experiments and models, Phys. Rev. Lett. 74 (1995) 4754–4757.

[26] N. Endlich, K.O. Greulich, Observation and manipulation of different structural variants of individual cation–DNA complexes in the light microscope, J. Biotechnol. 41 (1995) 149–153.

[27] B.D. Fleming, S. Biggs, E.J. Wanless, Slow organization of cationic surfactant adsorbed to silica from solutions far below the CMC, J. Phys. Chem., B 105 (2001) 9537–9540.

[28] J.R. Taylor, M.M. Fang, S. Nie, Probing specific sequences on single DNA molecules with bioconjugated fluorescent nanoparticles, Anal. Chem. 72 (2000) 1979–1986.