Separation of triplet repeat DNA by capillary electrophoresis and the conformational analysis by atomic force microscope

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ABSTRACT
We investigated the relationship between electrophoretic behaviors and higher order structures of triplet repeat DNA fragments by means of capillary electrophoresis and atomic force microscopy (AFM). It was suggested that the mobility difference between triplet repeat DNA and random sequence DNA should be correlated to differences in their dynamic conformation.

INTRODUCTION
An extension for repeats of trinucleotide, like CTG, is known to cause some inherited neuromuscular diseases, called triplet repeat diseases.¹ DNA fragments containing CTG repeats migrate faster than expected in non-denaturing polyacrylamide gels² and polymer solution.³-⁵ This unusual electric behavior of triplet repeat DNA is expected to be used for the detection of an extension of the triplet repeat. However, the separation mechanism has been remained to be unclear. In this work, we have investigated the higher order structure of triplet repeat DNA fragment in a buffer solution by means of AFM.

EXPERIMENTAL
Materials
We used two kinds of synthetic single-stranded DNA fragments labeled by FITC with PAGE-purification grade. One was 90mer of a triplet repeat DNA fragment, 30 repeats of CTG. Another was 80mer of the random sequence, which was used as a DNA marker. These DNA fragments were dissolved with TB buffer (50 mM Tris-borate, pH 8.0). Methylcellulose (4000 cP at 2% solution) was used as a separation polymer.

Capillary Electrophoresis
Beckman P/ACE 2100 capillary electrophoresis system was used for the separation of triplet repeat DNA under the following conditions: capillary, 27 cm total length and 20.2 cm effective length; running buffer, 1% methyl cellulose in 50 mM Tris-boric acid; temperature, 30 °C; electric field, 300 V/cm; sample injection, 5 kV for 10 s. Triplet repeat DNA and random DNA were dissolved with TB buffer into 0.09 uM and 0.16 uM, respectively. These DNA bands with FITC were detected with Laser-induced fluorescence (Ex. 488 nm, Em. 560 nm).

Preparation of samples for AFM
For deposition of DNA onto a mica substrate, the mica surface was functionalized with 3-aminopropyltriethoxy silane (APTES). The activation of mica in vapors of APTES for 1-2 h was suitable to obtain uniformly modified substrate surface.

AFM Imaging
The Nanoscope IIIa Multimode system (Digital Instruments) was operated in tapping mode for DNA imaging in liquid. Standard Si₃N₄ cantilever (100 μm long) was used under the following conditions: frequency, 9-10 kHz; scanning rate, 1 Hz.

RESULTS AND DISCUSSION
Figure 1 shows an electropherogram detected at a point of 20.2 cm effective length. The separation was achieved within 6 min (We have not yet identified each peak). A longer DNA fragment, (CTG)₃₀, migrates faster than a shorter random sequence fragment. In order to explain the anomalous mobility of triplet repeat DNA as
shown in Fig. 1., Mitchell et al. suggested compact structures of d(CNG)n oligonucleotides in solution. However, up to now, reported data has not allowed us to say definitely what these structures are.

Figure 2ab show topographic AFM images of double-stranded DNA (200 bp) and single-stranded triplet repeat DNA of (CTG)\textsubscript{30}, respectively. The single-stranded DNA does not seem like a strand, because of the higher order structure formed through the intra-strand hydration.

Figure 3 shows a histogram of the height of DNA from an AP-mica substrate measured by AFM. The average heights of each DNA are 1.54 nm ± 0.38 nm (200bp), 1.30 nm ± 0.31 nm (90bp) and 1.06 nm ± 0.24 nm (80bp), respectively. From these data, we cannot expect any definite compact structures for triplet repeat DNA. We speculate that the mobility difference between triplet repeat DNA and random sequence DNA should be correlated to differences not in their static structures but in their dynamic conformation. If so, the dynamical structure may be disturbed through the absorption process of DNA onto an AP-mica. Consequently, AFM measurements could not reveal the difference in the static structure. Further more investigations in the dynamical structure is required.

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