Single large DNA molecule analysis using fluorescence microscopy

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ABSTRACT
A large DNA analysis method which enable to obtain spatial information of positions of specific sequences along DNA molecule has been developed. Making use of the phenomenon that large DNA molecule is elongated stably under alternative current field in a concentrated linear polymer solution, direct observation of elongated individual λ DNA molecules with fluorescence probes was carried out using fluorescence microscopy. Then, the spatial positions of the fluorescence spot of the probe on the DNA molecule were determined by image analysis.

INTRODUCTION
The combination of gel electrophoresis and hybridization method is widely used to determine positions of specific sequences on large DNA molecules. In addition, there is another powerful technique to analyze chromosomal DNA, i.e. fluorescence in situ hybridization (FISH). On the whole, however, current wide use techniques for a large DNA molecule analysis are poor in the spatial resolution. In Figure 1, we propose an ideal method of detecting positions of specific sequences of DNA by single DNA molecule analysis using fluorescence microscopy. Recently, we found that large DNA molecules are elongated in concentrated neutral linear polyacrylamide (PA) solutions under alternating current electric field. This DNA elongation takes place by the entanglement effect between DNA and surrounding linear polymer chains. The size range of elongatable DNA is wide (from ca. 50 kb to ca. 1 Mb) and this DNA stretching phenomenon is expected to realize a new DNA analysis method as shown in lower part of Figure 1. Based on these results, we examined an effectiveness of single DNA molecule analysis using this new DNA stretching and straightening technique with fluorescent probes. Here, fluorescence labeled restriction endonuclease EcoRI were employed as fluorescent probes. EcoRI can recognize and just bind to the specific sequence of DNA, GAATTC. Therefore, fluorescence labeled EcoRI is an appropriate probe as a fluorescent probe for preliminary demonstration of single DNA molecule analysis.
RESULTS AND DISCUSSION

Figure 2 shows an example of a complex of λ DNA and fluorescence labeled EcoRI in the PA solution. A red fluorescence (rhodamine) spot of the probe was observed on the DNA which was visualized by YO-PRO-1 as a green fluorescence string. When there was no external field, the DNA molecule with fluorescence probe took a random coiled conformation (Figure 2, A). On the other hand, when the AC field was applied, the DNA took a stable loosely elongated and straight conformation (Figure 2, B). As a result, it became possible to obtain the spatial position of the fluorescence spot of the probe along the DNA molecule. In Figure 2, B, a schematic DNA molecule (thick bar) and positions of GAATTC sequence to which EcoRI is to bind (thin bands) are illustrated as a reference. One can see that the position of the fluorescence spot of the probe corresponds to a right end band of the five thin bands, i.e., the EcoRI is binding to the GAATTC sequence.

Recently, restriction endonuclease digestion of elongated individual DNA molecules has been imaged by fluorescence microscopy after fixation in agarose gel. Using this method, rapid construction of excellent ordered restriction endonuclease maps of yeast artificial chromosomes was achieved. These reports show that single DNA molecule analysis which enable to acquire spatial information of positions of specific sequences is very attractive method. Our method described here will contribute to such a new approach of DNA analysis. One of the benefits of our technique is that individual DNA molecules over the size range of 50 kb - 1 Mb can be elongated stably in the polymer solution without anchors. In addition, plural colors of fluorescent probes can be used to identify positions of different specific sequences on single DNA molecule in one experiment. This technique can be powerful for identifying positions of specific sequences, for instance, sequence of new-found markers, mismatch positions. Work on this topic is currently in progress in our laboratory.

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REFERENCES