SINGLE-MOLECULE TUNNEL-CURRENT BASED IDENTIFICATION OF DNA/RNA TOWARDS SEQUENCING BY USING NANO-MCBJ

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ABSTRACT

We investigated a conductance-time e profiles DNA/RNA oligomers by mechanically controllable nano-break junction (Nano-MCBJ). Based on the difference in the conductance-time profiles, we sequentially identified the base-type in the oligonucleotide just passing through the sensing electrode, resulting in the determination of partial sequence. From the read signals, the assembled sequence contig was found to be comparable to the original oligomer sequence. This method could be applicable to single-molecule electrical identification of DNA/RNA oligomers.

KEYWORDS

Nanogap electrode, DNA, RNA, Tunnel-Current.

INTRODUCTION

Single-molecule genome sequencer by using solid-state nanopore device is one of the most promising candidates for a realization of $1000 personal genome-sequencing [1]. We have been proposed a tunneling-current based identification as a single-molecule DNA/RNA sequencing. This methodology is based on sequentially reading the tunneling-current across individual single-nucleotides in the sequence, resulting in a high-speed electrical discrimination of the nucleotides sequence without chemical probes and PCR amplifications Theoretical calculations have shown that the tunnel-current based identification can identify four base molecules by differences in electric current displayed when each one passes between metal gap electrodes [2-4]. In our recent study, each of the conductance values was determined for four deoxyribonucleoside monophosphates (dAMP, dCMP, dGMP, dTMP) by using nanofabricated, mechanically controllable break junction (nano-MCBJ) and four ribonucleoside monophosphates (rAMP, rCMP, rGMP, rUMP), and these values are found to be due to the individual molecular energy level. Each of the DNA and RNA conductance values were normalized by dGMP and rGMP, respectively, and these relative conductance values were used for the following base-typing in oligonucleotides [5-7]. Herein, we report on a tunneling-current based identification of DNA/RNA oligomers only by nanogap electrode. (**Fig.1**). We measured the electron-tunneling of DNA/RNA oligomer in the aqueous solution by using nano-MCBJ. When the nucleotide molecules passed between the nanoelectrodes separated by a sub-nanometer gap, the tunneling-current through the molecules was increased, relative to that in the absence of molecules. The current intensity was found to be closely related to the individual electronic conductance [7-12].. The sequential reading of the tunneling-current via nucleotide molecules just passing through the nanogap-electrode results in determination of the partial sequence. From analysis on the assembled the overlapped the partial read sequence, we found that the continuous sequence was comparable to the original nucleotide sequence, resulting in identification of the DNA/RNA oligomer.

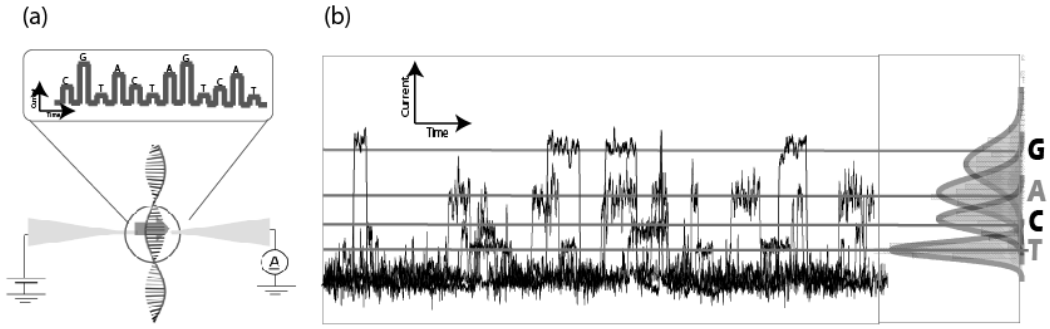

FIG. 1. Single-Molecule Tunnel-Current Based Electrical Detection by using Nano-MCBJ. Sequential reading of the tunneling-current across individual single-nucleotides in the sequence. (a)Schematic diagram of single-molecule electrical sequencing of DNA and RNA. (b) The four current–time profiles show dGGG, dAAA, dCCC, and dTTT profiles, respectively. The right figure represents data point histograms of the current profiles.

EXPERIMENT

The nano-MCBJ was prepared in the following procedure. First, a gold-wire was fabricated by a standard electron-beam lithography method. The gold-wire was mechanically broken by using a piezo-controller, resulting in forming a pair of gold nanoelectrodes. After the formation of a pair of electrodes, the nanogap-electrode was reconnected, and then the reconnected gold-nanowire was thermally broken under dc 0.1V apply [4]. We found that this gold-nanowire self-breaking phenomena reproducibly allow to form 0.5 nm, which is comparable to the size of one-gold atom. After the formation of nanogap, by using piezo-controller, the gap distance was tuned to be 0.8 nm, which would be comparable to the size of mono-nucleotide molecules, and used in the following electrical measurements. The gap distance formed was analyzed by the conductance measurements of the MCBJ devices, and

found to be 0.79 ± 0.05 nm. The fact that the gap distance of 0.83 ± 0.08 nm after the experiments was comparable to the formed gap distance indicated that the nanogap electrode was stable during the electrical measurements. Although electrode gaps far from the value of 0.8 nm were sometimes formed, such extraordinary gaps can be readily excluded because the gaps showed abnormal conductance profiles during the self-breaking process.

RESULT AND DISCUSSION

We investigated the current-time profiles for sample oligonucleotide in aqueous solution. When the nucleotide molecules passed between the nanoelectrodes separated by a sub-nanometer gap, the tunneling-current through the molecules was increased, relative to that in the absence of molecules. The current-increase in the signals is induced by the facilitation of tunneling-current through the nucleotide molecule, and is closely related to the individual electronic conductance.

First, homobase-oligonucleotides, *i.g.,* GG, GGG, GGGG, were investigated, and such the current-increased signals were observed. The current-signal intensity for these G oligomers was comparable to those of singlet-G nucleotides previously reported. Similarly, the current intensity of AAA, TTT, CCC oligomer were also comparable to those of A, T, C single-nucleotide, respectively. At the same time, the retention-time for the GGG, GGGG current signals was significantly long, relative to that for singlet-G nucleotides. These results indicate that the oligo-nucleotide required long time enough to flow through the gap-electrodes, relative to that for the single-nucleotides because of the difference in length of DNA strand. As it is well known that amino functional groups weakly bond to a gold surface, it is possible that the amino group of nucleotide base molecules could get temporally stuck while flowing through the gold electrode nanogap. In the previous study, we measured the single-molecule of benzenediamine in a liquid environment using the same technique employed in this study and the dwell-time length was experimentally found to about one-mililiseconds, which is comparable to the present duration signal-time. It was suggested that binding of these molecules occurred between the electrodes, so that the dwell-time length of residing at the nano-gap gold electrode would be longer.

Next, heterobase-oligonucleotides, *i.g.*, GTG, GCG, GAG and UGAGGUA., were observed. The characteristic current-signals with long retention-time were also obtained, but the current-fluctuation in the current-profile of the hetero-base nucleotide signals were completely different from homo-base nucleotide signals. The histogram of current-profiles reveals multiple-peaks. For example, the histogram of GTG current-signals revealed double-peaks, which indicates the G and T signals in the GTG sequence, respectively. Similarly, the double-peaks were also observed for TGT, ATA, CAC, GAG tri-nucleotides, and triple-peaks were observed for UGAGGU and TTGTATAGT, TGTGATAGT. This result shows that the number of the peak represent the number of base molecules in sample sequence. In addition, each of the peaks in the histogram of I-t profiles was assigned to their base-species, compared to the single nucleotide conductance values. For example, in the GTG I-t profile, the two peaks for relative conductance of 1.00 ± 0.25, and 0.35 ± 0.12 were comparable to the relative conductances of 1.00 ± 0.29 for single G, and 0.45 ± 0.12 for single T, respectively, so that they would be assigned to G and T in the GTG triplet-nucleotide, respectively. Similarly, the three peaks in the current histogram constructed from UGAGGUA I-t profiles were assigned to rGMP, rAMP and rUMP, since the relative conductance values of 1, 0.71 and 0.47 agree with those of these single base molecules for RNA. **Fig.3** show a typical I-t profiles of DNA oligomer, of which the sequence is comparable to let 7a TGAGGTAGTAGGTTGTATAGTT. The current histograms were constructed from the I–t profiles and reveals three-peaks. For these three peaks, the relative conductances of 1.00 ± 0.37, 0.73± 0.23, and 0.54 ± 0.33 were assigned to G, A, and T, respectively. These results indicates that the obtained signal detect the composition of base is found I-t profiles contain the base-species.

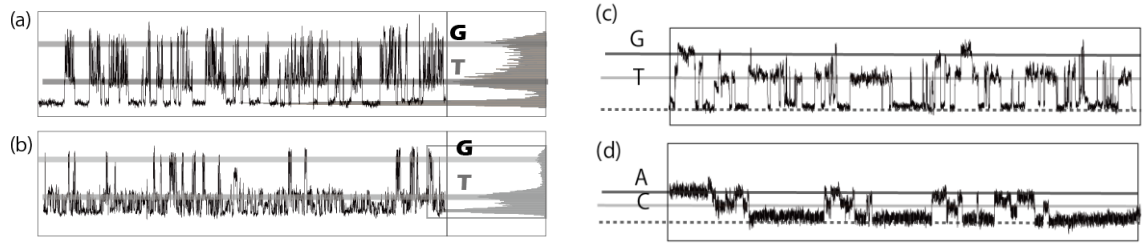

Fig.2 Conductance histograms and current–time (I–t) profiles of hetero-base tri-nucleotides (GTG (a), and TGT (B)). The left and right panels show the conductance histograms and I–t profiles The conductance histograms were constructed from full data points obtained from the corresponding right panels (I–t profiles). Each low- and high-conductance peak observed in each histogram corresponds to the base current and single-molecule conductance of the base molecules. Enlarged TGT and ACA current-profiles are shown in (c) and (d). Each of the conductance-steps can be assigned to the base-species.

Finally, based on the time-profiles, the fragmented sequence were determined, and compared to the original sequence. The signal assignment and reading procedure is shown in the following. First, we constructed current histograms using all the data obtained from the I–t profiles. Second, each of the peaks for the histograms was fitted to normal distribution, and the peak conductance values and standard deviation were determined. Third, for each of the peaks, the relative single-molecule conductance was calculated, and the base-species for the peak were assigned, compared to the single nucleotide conductance values. Fourth, each of the probability for each bases and the baseline were calculated from the equation of Gaussian function. Fifth, based on the highest-probabilities within the one

millisecond retention-time among the calculated base and baseline probabilities, the base-species or baseline were sequentially assigned, and then the fragment read signals were assembled into a continuous sequence. The read sequence represents the fragment sequence of sample nucleotide passing through the sensing electrode so that the read signals have two characteristics. First, the sequential base-type determined signals were found to be the partial sequence that reflects the "partial transit" of the nucleotides at the nanoelectrode. In the second, this duplication read is sometimes inserted in the read sequence. For accurate determination of the original sequence, the long and straight reading is preferable for the signal assembly. The fragmented and/or duplicated reading might be due to stochastic motions of the oligonucleotide around the gap-electrode, which originates from Brownian motion. In order to regulate the stochastic flow of the oligonucleotide through the electrode gap, a "gating nanopore", which is composed of solid-state nanopores and nanogap electrodes, could be one of the solutions because this nanopore is expected to control the dynamic flow through nanogap electrodes.

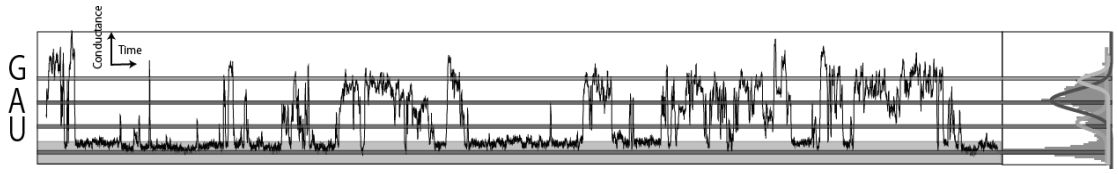

Fig.3 Conductance histograms and current–time (I–t) profiles of DNA oligomer (sequence: TGAGGTAGTAGGTTGTATAGTT). The left and right panels show Conductance–time profiles and the conductance histograms, respectively.

CONCLUSION

We developed a tunnel-current based single-molecule identification methodology for RNA/RNA oligomers. Based on the determined electrical conductivity, we sequentially identified the base-type in oligonucleotide just passing through the sensing electrode, resulting in the determination of partial sequence. The read signals were assembled into a continuous sequence, which was comparable to the original oligomer sequence. This single-molecule electrical sequencing using nano-MCBJ can be used to randomly identify sequences of single base DNA molecules. This method could be one of the promising whole-genome sequencing strategies for personalized medicine.

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