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Single-Molecule Tunnel-Current based Detection Toward Amino-Acid Identification

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Abstract

We developed a single-molecule tunnel-current based detection method for single amino acids identification by using a nano-gap electrode. We measured the electron tunneling for ten kinds of the amino-acid molecules just through the nano-gap electrodes, and determined characteristics conductance values and retention-time for each of the amino acid species. In addition, we also determined the conductance of phosphorylated tyrosine site, and discriminated it from no-phosphorylated tyrosine. This tunnel-current based amino-acid identification method would be potentially applicable for peptide identification.

1. Introduction

A polypeptide is a single linear polymer chain typically consisting of twenty kinds of standard amino acids, folding into a particular biological functional form. The identification of amino acids and its sequence determination of polypeptides are a first step for fundamental understanding for conformations and functions of proteins. As a methodology to obtain primary sequence information of biopolymers, mass spectroscopy using soft ionization techniques and X-ray scattering techniques have been widely used. These methods analyzed accumulated detected signals, i.e., mass spectrum and x-ray diffraction pattern, from the crystallized or ionized target sample molecules. If the sample signals can be available at the single molecule level, ultra-low concentration sample can allow for the understanding of protein function not only at metabolic level but also up to signal transduction level. As a single-molecule analysis methodology, single-molecule tunnel-current based identification methods have been emerging because of the potential for its molecule resolution and high-throughput analysis [1]. We recently achieved single-molecule identification of DNA and RNA and read the sequence of oligonucleotides by a combination of electron transport profiles and signal assembly. Theoretical study also suggest that these tunneling-current methodology is based on the intensity of tunnel-current via single-molecules are related to the energy level.

In this study, we report on a single-molecule tunnel-current based identification of single amino acids by using a nano-gap electrode (Nano-MCBJ) (Fig.1: Schematics) [2]. We measured the electron tunneling for several kinds of the amino-acid molecules just through the nano-gap electrode, and determined each of the conductance values for the amino acid species. This analysis method would be used for amino-acid identification in peptides.

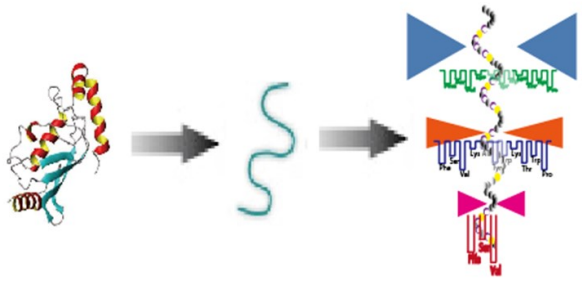

Fig.1 Single-Molecule Tunnel-Current Electrical Detection (a) Flow chart of Oligopeptide Profiling by Using Nano-Gap Electrode Devices. Higher-order structures of sample oligopeptide or protein were denaturing by chemical agents or local electrical field induced by nanostructures. When the stretched oligopeptide molecules are translocating through the nano-pa-electrode, tunnel-current signals were detected. Signal assignment for signals, and determination of partial sequence

2. Experimental Section

L-amino acid samples were purchased from Wako and Aldrich, TCI Corporation. The fabrication of the nano MCBJ is fabricated shown in the previous study (ref.X). These signals were obtained at a concentration of 10uM ~ 100nM amino-acid aqueous solution with phosphate buffer solution (pH 7.4). The current across the electrodes was recorded at 10 kHz using a custom-built logarithmic current amplifier and a PXI-4071 digital multimeter (National Instruments) under a DC bias voltage of 0.1 V.

3. Results and Discussion

First, we performed electrical measurements for standard amino acid molecules by using 0.7nm gap electrode, which was tuned by nano-fabricated mechanically controllable break junction. The 0.7 nm gap size is comparable to the, which is typical aromatic amino acid. Fig.2 shows a typical i-t profile for amino-acid in the aqueous solution. Of the twenty amino acid aqueous solutions, positive current-increase signals were detected. These signals would be due to the facilitation of tunnel-current via the amino acid molecules when they flow into the gap. Such signals for

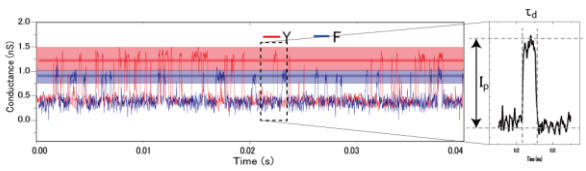

Fig.2 (a) Amino acid-Assignment for Conductance-Time Profiles for Tyrosine (Y) and Phenylalanine (F). Typical conductance-time profiles for Y for red-line and F for blue-line were obtained from each of the 10 um sample aqueous solution.

other amino acid solution were not detected or quite low-frequent. The signals of these amino-acid molecules were characterized by the extent of current increase (I_p), which are the maximum current in the signals, and the conductance histograms were then constructed from I_p data. Based on Gaussian Fitting for the Ip histogram, each of the conductance peaks has been found to be 953 pS for W, 874 for Y, 690 pS for F, 128pS for I and 168pS for E, and 183 pS for D, respectively. It suggests that the I_p peak values are the characteristic conductance values for each of the amino-acid molecules.

The conductance values of signal were based on the tunnel-current via molecules between the nanogap electrodes. The intensity of tunneling current (I) is defined as a following equation.

$$I=\exp(-(4\pi\sqrt{2m\phi})/h\,d)$$

, where d, m, φ, and h are the tunneling-distance, electron mass, work-function, and Planck's constant. Therefore, the existence of large molecules inside the gap would induce the smaller tunneling-distance, resulting in the large facilitation of tunneling-current. Since the W, Y, F, H and P were relatively large molecules among the standard amino acid molecules, In addition, the conductive molecules also would induce the facilitation of tunneling-current, relative to the non-conductive molecules because of the low-work function value φ. For example, the molecule size for P and H was comparable, but the aromatic imidazole ring for H induced a large tunneling current, relative to the saturated heterocyclic prolidine ring for P. Until now it has been reported that such the functional group influence the electron density and molecular energy levels, resulting in the extent of electron-tunneling.

The signals of these amino-acid molecules were also characterized by the retention-time (t_d) of the signal. The time-length histograms were then constructed from t_p data, and each of the characteristic retention-time peaks for amino-acids was determined by Gaussian fitting. From the statistical analysis, each of the t_d peaks were found to be 2.52 ms for W, 1.54 ms for Y, 1.53 ms for F, 1.15 ms for I and 1.57 ms for E, and 0.98 ms for D, respectively.

The signal retention-time (t_d) represents the translocation time through the gold electrodes. Therefore, the time-length would be related to the extent of the interaction between the amino acid and sensing electrode gap, electrophoretic force induced by local electrical field and/or the flow rate of molecular diffusion. Of the three factors, the interaction could be due to the variation of t_d. Until now, it is reported that the amino functional group weakly bound to the gold surface while flowing through the gold nanogap electrodes, resulting in one-millisecond transit for nucleotide and other amino molecules.

The magnitude of conductance peak values (G) and duration of this time peak values (T) classified amino acid molecules into several kinds of groups, which can allow for an identification of amino-acid molecules in the solutions

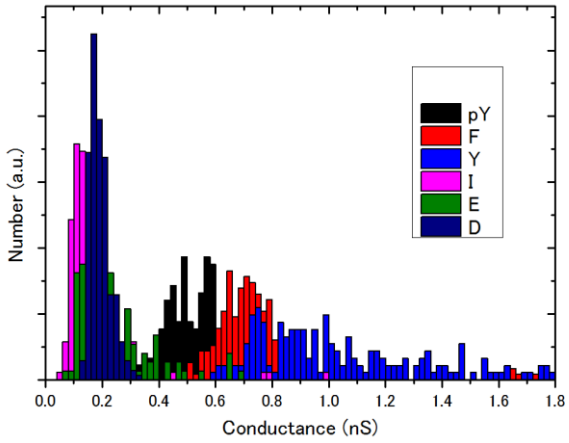

Fig.3 Amino acid-Assignment Signal histograms. This represent the characteristic conductance distributions by using 0.7nm Gap electrode for W, Y, F, H, P, and pY. From at least two hundreds the conductance peaks for each of signals, conductance histograms were constructed.

4. Conclusions

We achieved a single-molecule tunnel-current based identification of single amino acids by using a nano-gap electrode (Nano-MCBJ). We measured the electron tunneling for five kinds of the amino-acid molecules just through the nano-gap electrodes, and determined characteristic conductance values and retention-time for each of the amino acid species. This tunnel-current based identification method would be potentially applicable to be a single-molecule electrical protein-sequencing.

Acknowledgements

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