【Grant-in-Aid for Scientific Research (S)】

Development of single-molecule peptide sequencing method

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Purpose and Background of the Research

●Outline of the Research

This project aims to develop a single-molecule peptide sequencing method. In conventional protein amino acid sequencing, peptide sequences are determined by Edman degradation or tandem mass spectrometry. However, single-molecule peptide sequencing has not been performed by these conventional methods. Therefore, we will develop a single-molecule peptide sequencing method by developing a synthetic antibody that specifically binds to the *N*-terminal amino acid of peptides (Figure 1). In this method, proteins from biological samples are hydrolyzed with proteases, and the resulting peptides are modified with Edman degradation reagent. After the peptides are covalently immobilized on a glass plate, the *N*-terminal amino acids of the peptides are identified by a synthetic antibody that binds specifically to each amino acid. By sequentially repeating the degradation and detection of the *N*-terminal amino acids, the amino acid sequences of the peptides are sequentially determined in single-molecule level. Moreover, using this technology, amino acid sequences containing post-translational modifications of diverse peptides can be determined in parallel. Therefore, we believe that the development of a single-molecule peptide sequencing method will bring innovations like next-generation DNA sequencers to the protein level.

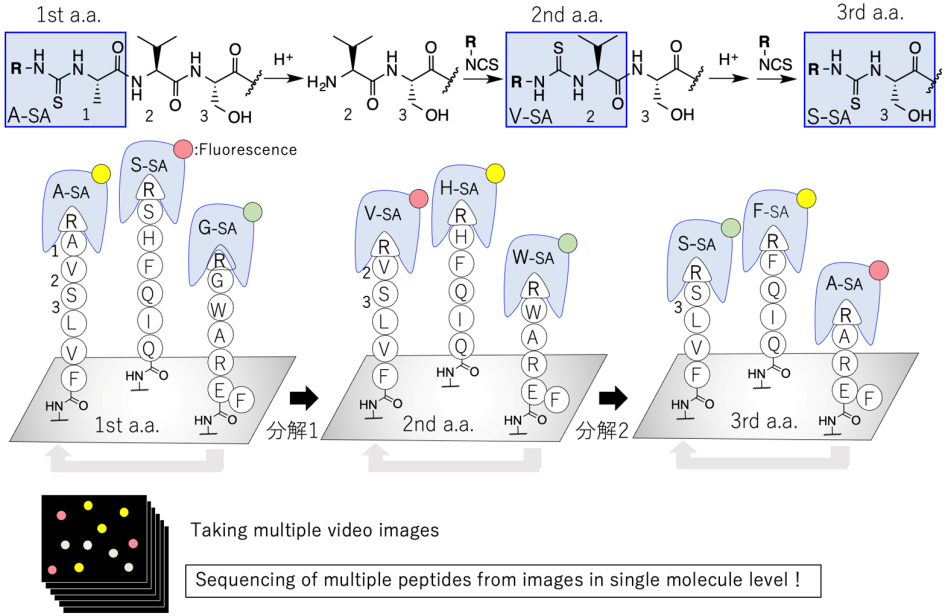

Figure 1. Development of single-molecule peptide sequencing method using synthetic antibodies

Expected Research Achievements

Key to this research is the development of synthetic antibodies (SAs) that recognize the *N*-terminus of peptides in an amino acid-specific manner. SAs have the advantages of being inexpensive to produce compared to antibodies, having a simple structure, and can be rapidly developed without immunizing animals. We have developed a rapid SA development method and have so far successfully developed SAs against various target proteins, such as SARS-CoV-2 neutralizing antibodies. In this study, we use an SA library consisting of 100 trillion SAs to select SAs against Edman-modified *N*-terminal amino acids of peptides (Fig. 2). Proteins produced by cells are basically composed of 20 types of amino acids (20 natural amino acids). Therefore, in this study, we first obtain SAs that can recognize each of the 20 *N*-terminal amino acids (Fig. 3). On the other hand, post-translational modifications are known to regulate the functions of many proteins in the cell, and the study of these post-translational modifications is important for understanding life. Therefore, in this study, in addition to 20 natural amino acids, we will develop SAs for typical post-translationally modified amino acids (Fig. 4). Using these SAs, we will determine amino acid sequences including post-translational modifications of diverse peptides at the single-molecule level in parallel.

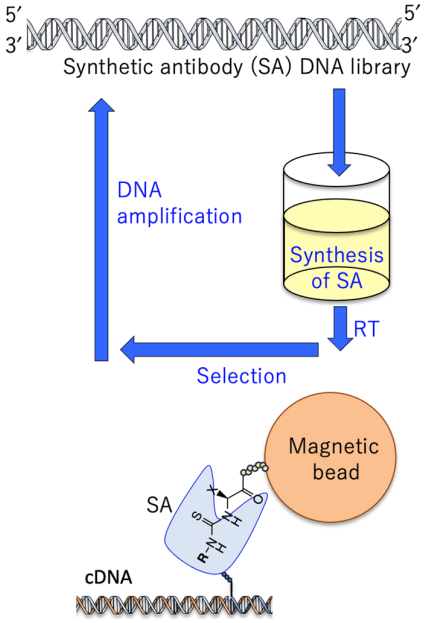

Figure 2. TRAP display

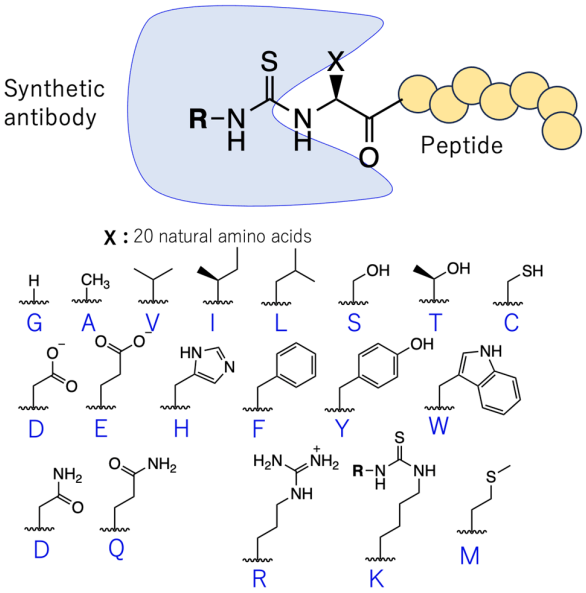

Figure 3. Selecting synthetic antibodies for 20 natural amino acids

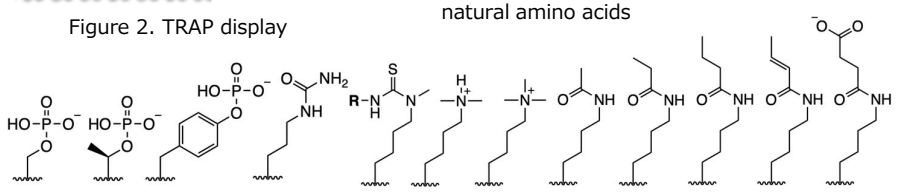

Figure 4. Selecting synthetic antibodies for post-translationally modified amino acids

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