高速分子進化による歯周病原因プロテアーゼ‘阻害ペプチド’の創製

Generation of peptide inhibitors aimed for a periodontal disease-causing protease by evolutionary molecular engineering

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We have tried to develop peptide aptamers which can inhibit the causal protease, gingipain, for periodontal diseases using the *in vitro* selection method. For this aim, we could first constructed a DNA library which can generate circularized peptides by a disulphide bond, which will be performed after the i*n vitro* translation of peptides. The construct of a molecule used for the selection was made of three parts: a fluorescent moiety (GFP), endopeptidase Xa-recognition sequence (used for cutting out the peptide region) and the variable region of peptide sequence (which consists of ten amino acids sandwiched by two cysteines at both ends. Independently, MMV (multi-micro vessel)-based selection method was also improved for this purpose, which enables to detect positive clones, that is, gingipain-binding ones, by its GFP fluorescence in a parallel manner of more than 1000 clones. Preparation of the protease (gingipain Rgp and Kgp) was processed to its 80% purity beginning with a 10 liter scale culture. The succeeding experiments are continued toward the goal after the short contract term (3 months which were not sufficient to complete this work).

Through this study, the difference in the inhibitory effect and the stability between circular peptides and linear ones will be elucidated, possibly adding novel type of molecules for Evolutionary Molecular Engineering.

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