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論文 / 著書情報 Article / Book Information

題目(和文)	ミスアシル化tRNAによるPEG修飾および光応答性ペプチドアプタマー のin vitroセレクション			
Title(English)	Misacylated tRNA for PEGylation and in vitro selection of photo- responsive peptide aptamer			
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論 文 要 旨

THESIS SUMMARY

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要旨(英文800語程度)

Thesis Summary (approx.800 English Words)

The expansion of genetic code has attracted great attention because a great number of non-natural amino acids are incorporated into peptides and proteins. The incorporation of non-natural amino acids carrying special functional groups can provide powerful tools for generating peptides and proteins with novel physical, chemical, biological and pharmaceutical properties. Both chemical and biochemical approaches have been developed to incorporate the non-natural amino acids into peptides and proteins. One of the site-specific incorporation methods is utilizing misacylated tRNAs conjugating with non-natural amino acids which recognize a stop amber codon or a frameshift codon with high fidelity. Up to now, the peptides and proteins with non-natural amino acids have been applied to probe the structure and the functions, alter the properties and generate new materials and new drugs.

One of the most successful strategies is the incorporation of polyethylene glycol (PEG), which can overcome the drawbacks of peptides and proteins: the low stability, the bad biocompatibility and fast degradation in vivo. Many efforts have been done to incorporate PEGs. The site-specific PEGylation has been achieved; however, it is highly challenging to maintain the full activity of the proteins because the incorporated PEGs with the same lengths might mask or block the active sites. The incorporation of PEGs of different lengths could maintain the activity. Herein, I prepared two DNA sequences containing both the amber and the frameshift codon. Then PEGs with different lengths were genetically incorporated into one polypeptide backbone with tRNAs which were acylated with PEG-containing non-natural amino acids. All the in vitro translations were performed using RYTS kit according to the manufacture's protocol. Then the translated peptides were purified, analyzed by MALDI-TOF-MS and the amount of each peptide was estimated by comparing to 3 x FLAG peptide, which was used as the internal standard. First, I studied the incorporation of one PEG with various lengths into one polypeptide via the amber codon or the frameshift codon. The incorporation of longer PEG chain resulted in the less amount of the translation product. The PEG-length-dependence of the translation product from PEG8 to PEG24 may be explained by the steric hindrance between PEG and ribosome, and between PEG and EF-Tu. I also successfully incorporated two PEGs via the amber and the frameshift codon in one peptide. It was found that the translation efficiency depended on the length of the PEG, the codon and the incorporation sites. And I also found a mass corresponding to truncated peptides (lack of fMSKQIEVN or SKQIEVN). The amount of the truncated peptides increased as the molecular weight of PEG increased. It appears that the longer PEGs might block the peptidyl transferase reaction in the ribosome. This method could have the application in the precise synthesis of bioconjugate drugs.

Also, I incorporated one non-natural amino acid carrying an azobenzene residue which is a photo-responsive molecule into the peptides for the *in vitro* selection of the peptide aptamer. Aptamers are a special class of oligonucleotide acids or peptides which can bind to their targets with high specificity and high binding affinity. And the selected aptamers can be used as biomaterials, the biosensing probes, and the diagnostic and therapeutic tools. The classical selection methods include phage display, mRNA display, and ribosome display and so on. Among the *in vitro* methods, ribosome display is one very simple and effective method. Therefore, I employed ribosome display to select the peptide aptamer. In this study, I chose glutathione (GSH) as the target for the selection of the photo-responsive peptide aptamer. First, I prepared a random sequence library, which contained the amber codon. Through the amber codon, the non-natural amino acid carrying an azobenzene residue can be incorporated into the peptides. The *in vitro* transcription and translation was performed using PURESYSTEM. Then translated peptide solution incubated with the was glutathione-immobilized microbeads. The unbound complexes were washed away and the bound complexes were eluted by adding a glutathione solution or irradiating with UV light for the next round of selection. After eight rounds of selection, the selected sequences were cloning and analyzed. 215 types of sequences were found from 269 clones. 12 sequences showed multiple repetitions in the analyzed sequences. Two peptides derived from plural clones were chosen: B09 and B69. The dissociation constants (Kd) of B09 and B69 peptides bound to the GSH-immobilized microbeads were calculated as 5.21 and 1.19µm, respectively. And for peptide B09, it detached from the GSH-immobilized microbeads under UV irradiation. After the successful incorporation of one azobenzene molecule, two, three or more azobenzene molecules can be incorporated into peptides using the same method and the photo-responsiveness should be high. Besides the linear peptide aptamer, the cyclic photo-responsive peptide aptamer can also be developed, which would show high binding affinity.

In conclusions, I succeeded in incorporation of one and two PEGs of different lengths into one polypeptide backbone and in *in vitro* selection of a photo-responsive peptide aptamer with the incorporation of an azobenzene molecule.

備考: 論文要旨は、和文2000字と英文300語を1部ずつ提出するか、もしくは英文800語を1部提出してください。

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