Extension of biochemical functions by the introduction of nonnatural amino acids and artificial nucleic acid analogs

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ABSTRACT
Extension of biochemical functions has been attempted by introducing nonnatural amino acids and artificial nucleic acid analogs. Nonnatural amino acids have been linked to tRNAs and the amino-acylated tRNAs were added to E.coli in vitro protein synthesizing system to produce nonnatural mutant proteins. The positions of the nonnatural amino acids have been assigned by the 4-base codons, like CGGG and AGGU. The extended codons have been introduced at a specific position or at random positions on a DNA. In the latter case, a DNA library that contains a single 4-base codon at random positions can be obtained. The combination of these new techniques opens a way to the introduction of artificial functions to biochemical systems.

INTRODUCTION
Biochemical systems can be extended by the introduction of synthetic analogs of biomolecules, such as nonnatural amino acids and artificial nucleic acid analogs. Position-specific incorporation of nonnatural amino acids into proteins (Nonnatural Mutagenesis) has been reported independently by Schultz and Chamberkin in 1989. They linked nonnatural amino acids to yeast tRNAs and the aminoacylated tRNAs were added to an E.coli in vitro protein synthesizing system. The position of the nonnatural amino acid has been directed by a UAG nonsense codon.

We have been extending their system, first by the introduction of a variety of nonnatural amino acids and, second, by the extension of the codon/anticodon pairs that can be assigned to the amino acids. By introducing several 4-base codons, we have successfully incorporated several different amino acids into a single protein. Furthermore, a DNA library in which randomly selected three consecutive bases are replaced by a CGGT 4-base codon has been developed. The "random insertion/deletion (RID) mutagenesis" will produce a protein library in which each protein molecule has a single nonnatural amino acid at random positions. Finally, an approach toward a novel type of peptide nucleic acids (PNAs) will be described.

NONNATURAL MUTAGENESIS AND EXTENSION OF CODONS
Key steps for the nonnatural mutagenesis are shown in Scheme 1.

We have been developing 4-base codons for assigning a nonnatural amino acid. We have found that several 4-base codons, such as CGGG, AGGU, and GGGG work independently from the existing 3-base codons and from each other. The orthogonality of the extended codons means that we can incorporate up to three different nonnatural amino acids into a single protein.
INCORPORATION OF FLUORESCENT AMINO ACIDS INTO PROTEINS

One of promising applications of the nonnatural mutagenesis is the incorporation of fluorescent amino acids into antibodies, receptors, and enzymes to detect very small amount of antigens, hormones, and inhibitors. For this purpose, however, a highly fluorescent amino acid must be incorporated into a very specific position where we can detect the binding of the ligand sensitively without suppression of the binding activity itself by the mutation. We have shown that a nonnatural amino acid that carries a methoxy-coumarine group (mchAla) satisfies the above requirement when it was incorporated at the 120th position of streptavidin. Actually, the 120mchAla-streptavidin could successfully detect biotin molecules down to nM concentrations.

RANDOM INSERTION/DELETION MUTAGENESIS

Introduction of nonnatural amino acids into proteins, however, often causes loss of the activity, probably due to the denaturation. To find the best position for a nonnatural amino acid, we have to synthesize a number of mutants and examine their activities. It is, therefore, desired to produce a protein library in which each protein contains a single nonnatural amino acid at random positions. To obtain the protein library and the corresponding DNA library, a novel method for the random insertion/deletion (RID) mutagenesis was developed. In the RID mutagenesis, an arbitrary base sequence including one of the 4-base codons, can be inserted in place of randomly selected three consecutive bases on a DNA. The concept of the RID mutagenesis is illustrated in Scheme 2.

![Scheme 2. Concept of the Random Insertion/Deletion (RID) Mutagenesis](image)

Fig.1. Melting curves of the 1:1 mixtures of OPNA(A12)-DNA(T12) and OPNA(A12)-DNA(T5CT5)

The very sharp melting curve and the precise base recognition as shown in Figure 1, suggest that the OPNA is a promising synthetic molecule for the regulation of biochemical systems.

CONCLUSION

Synthesis of molecules that work in biochemical systems is a promising approach toward the extension of biochemical functions in vitro and in vivo.

REFERENCES