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Editorial

Nucleic Acids Chemistry and Engineering: Special Issue on Nucleic Acid Conjugates for Biotechnological Applications

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1. Introduction

Nucleic acids not only store genetic information in their primary sequence but also exhibit biological functions through the formation of their unique structures. Based on such functions and specific and designable complementary recognition through base pairing, nucleic acids are considered attractive molecules as functional units for biotechnological applications. Life in biological systems is maintained by the cooperative actions of various biomolecules, such as nucleic acids, proteins, lipids, and small organic molecules. With the development of chemical and biological technologies related to nucleic acids, the details of the mechanisms of such cooperative actions between nucleic acids and other biomolecules have been elucidated. This Special Issue focuses on the biotechnological applications of nucleic acid conjugates that transcend the natural cooperative systems.

2. Addition of Superior Functions to Natural Nucleic Acid through Covalent Conjugations

Nucleic acids show complementary recognition by base pairing, which cannot be achieved by other biomolecules. A specific region on messenger RNA (mRNA) can be targeted by rationally designing oligonucleotides, such as antisense and small interfering RNA (siRNA), that enable the suppression of the protein expression from the target mRNA. An artificial nucleic acid mimic typified by peptide nucleic acid (PNA) further enables targeting a specific region on genomic double-stranded DNA through the formation of a double-duplex invasion complex. When these oligonucleotides are applied for gene regulation, the delivery of the oligonucleotides into cells and localization in appropriate cellular compartments are issues that need to be addressed. The conjugation of a functional unit for providing cell permeability to oligonucleotides is a promising strategy for the intracellular delivery. D. Stetsenko et al. synthesized a novel type of lipid-oligonucleotide conjugates (LONs) based on their original chemistry, performing the conjugation on solid support. They demonstrated that siRNAs built of LONs downregulated gene expression through self-internalization into cells [1]. Since the constructed LONs spontaneously formed particles, the additional functionality of resistance to degradation for in vivo application is expected. Localization in a specific cellular compartment can be achieved by the conjugation of a short peptide tag, such as nuclear localization signal (NLS). Y. Aiba and O. Shoji et al. have constructed an NLS-PNA conjugate, which was superior to natural PNA in its invasion efficiency into the target DNA duplex [2]. The demonstration motivates the applicability for in cell studies because the NLS-PNA could form the double-duplex invasion complex even in the presence of physiological salt concentrations, whereas the invasion efficiency of PNA decreased as the salt concentration increased.

In addition to the complementary sequence, nucleic acids form complex tertiary structures that recognize other biomolecules such as proteins and small molecule metabolites. Aptamers, nucleic acids with potential to specifically recognize another molecule, have

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been applied as functional units in biosensors. Biosensors require a unit for recognizing the target molecule and another unit for outputting signal, which can be easily recorded and quantified such as fluorescence and luminescence. Since aptamers only recognize the target molecule, the construction of aptamer-based biosensors has been demonstrated by conjugations with other functional units. For example, the conjugation of an aptamer with a protein is one of the useful strategies because some proteins exhibit unique photochemical output signals. M. Mie et al. have demonstrated the construction of nucleic acid–protein hybrid molecules using a protein involved in viral replication initiation that acts as a conjugation unit. They constructed an aptamer–protein hybrid that detects the target molecule by a bioluminescence resonance energy transfer (BRET) through designing a fusion protein of the conjugation unit between NanoLuc luciferase and Venus, which output luminescence and fluorescence as a donor and acceptor of BRET, respectively [3]. The construct of the aptamer–protein hybrid would be applicable for sensing various aptamer targets because change in the BRET signal occurs upon steric rearrangement of the protein units upon the interaction of the aptamer and its target. When the target of the aptamer is a small molecule, it may be difficult to cause a large steric rearrangement of the protein units. In such cases, aptamer-based biosensors can also be constructed by optimally locating a small fluorophore, in which fluorescence is sensitive to the microenvironment, close to the aptamer unit. T. Morii et al. have constructed fluorometric sensors based on a ribonucleopeptide (RNP), which acts as a scaffold to locate RNA aptamer and fluorophore units in proximity. Although the prototype RNP-based sensors were just formed by a noncovalent interaction between RNA and peptide, the present study covalently linked the complex to improve chemical and thermal stability through optimization of the conjugation conditions [4]. The covalently linked RNP sensor would be applicable for the simultaneous detection of multi targets by preventing the interexchange of the functional units between RNP sensors.

The formation of covalent linkage between nucleic acid and protein can also be used for entrapping proteins that interact with a nucleic acid of interest. Although crosslinking chemical reagents for biomolecules have been developed, in order to capture a protein that binds to a specific site of the target nucleic acid, it is necessary to conjugate the reactive unit at the specific site of nucleic acid. F. Nagatsugi et al. have constructed oligonucleotides bearing an unnatural nucleotide consisting of 4-amino-6-oxo-2-vinyltriazine as the reactive unit and acyclic linker in place of deoxyribose. The vinyl group in the reactive unit enables covalent linkage with amino acid residues with nucleophilic species at a proximity that captures the proteins interacting with the specific region on the duplex [5]. The formation of covalent linkage is useful for the basic study of nucleic acid binding proteins. In addition, it is also expected to be possible to use the reactive oligonucleotide as an irreversible inhibitor of the proteins like a suicide inhibitor of catalytic proteins.

3. Molecular Assemblies Including Nucleic Acids for Functional Materials and Systems

The original and fundamental function of nucleic acids is to store the genetic information required to synthesize proteins. Since mRNA encodes the amino acid sequence of the protein to be expressed, it is attracting attention as biomedical material exemplified as a vaccine against SARS-CoV-2. When mRNA is used as a nucleic acid-based drug, it should be delivered into cells to express the protein of interest. Due to the considerably large size of mRNA compared to oligonucleotide therapeutics, chemical conjugation through the technology introduced above becomes difficult. In this case, the encapsulation of the mRNA into an assembled complex, which shows cellular internalization function, would provide an efficient strategy to deliver the mRNAs. K. Matsuura et al. have constructed an artificial capsule by using a short peptide unit derived from tomato bushy stunt virus. They have chemically conjugated the peptide with a short poly-T oligonucleotide and succeeded to encapsulate mRNA, which hybridizes to the poly-T strand through its poly-A tail. The capsule was further modified to internalize into cells and express proteins by the modification of the outside of the capsule with a cell penetrating peptide [6]. The

modification functions like a viral envelope and is expected to be applicable for targeting specific cells. In contrast to the use of nucleic acid as a drug, a strategy for internalizing a small drug molecule into an assembled complex via nucleic acid is also promising for the therapeutic application. M. Kuwahara et al. have constructed a bifunctional aptamer (bApt) consisting of two aptamer units for thrombin and a drug molecule, respectively. During the fibrin gel formation by the catalytic activity of thrombin, the thrombin aptamer unit enables the internalization of bApt into fibrin gel. Since another aptamer unit, which is interacting with the drug molecule, can be easily exchanged depending on the therapeutic target, the assembled fibrin gel can be a biocompatible material that stores and releases various drug molecules [7].

Assembly between nucleic acids and proteins is one of the major mechanisms that modulate gene expression in cells. For example, the assembly of Puf family proteins on the 3′ untranslated region of mRNA affects protein expression. Since the target RNA sequence of the Puf family proteins can be altered by rationally designing amino acids in their RNA binding domain, a strategy that assembles multiple Puf proteins on target mRNA would enable the efficient regulation of protein expression. M. Imanishi et al. have fused the engineered RNA binding domain of the Puf family protein with tristetraprolin (TTP), which is involved in mRNA decay and degradation. They demonstrated that a tandem assembly of four TTP-PUF fusion proteins effectively repressed protein expression from target mRNA [8]. Since the strong suppression was observed only when the four types of TTP-PUF fusion proteins are present as it is like a logic gate, the technology is expected to be applied as a gene regulation tool in the field of synthetic biology.

Molecular assembly is also applied for in vitro applications. The hybridization and self-organization of nucleic acids are designable based on the sequence complementarity. The reconstruction of assembly-based nucleic acid structures is applicable for controlling the subsequent output of functional units. As examples, Y. Ikawa et al. have demonstrated the regulation of catalytic activity of RNA through self-organization of split RNAs. They constructed an RNA triangle with six catalytic ribozyme units, in which the triangle complex was further supported by protein assembly on the corners. It is an attractive RNA-based nanoarchitecture and would be a unit for a system that exhibits more complex biological functions as the RNA triangle has succeeded to catalyze the trans-splicing of RNA with an arbitrary sequence [9]. S. Nishizawa et al. have synthesized a chemical probe for fluorometric detection of an orphan cytosine, which has no base paring partner due to an abasic nucleotide on the opposite strand. They also designed an oligonucleotide, which hybridizes to the target strand and locates abasic nucleotide opposite a specific cytosine, and demonstrated the detection of target nucleic acid by fluorescence signal through induction of the orphan cytosine and subsequent assembly of the chemical probe [10]. Since the assembly of the chemical probe is specific for the orphan cytosine sandwiched by matched base pairs, the technique is expected to be applicable for the detection of single nucleotide polymorphisms including viral mutations in relatively wide sequence variations.

4. Summary

In the published papers in this Special Issue, advanced research works involved in nucleic acid conjugates are reported in wide application fields, such as artificial gene regulation, biomolecular sensing, and therapeutics. In general, biotechnologies have been developed through discoveries and utilizations of functional mechanisms in life systems. The field of nucleic acid conjugates for technological applications will continue to develop since the full extent of natural nucleic acid functions has not been clarified.

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