Highlight Review

Central Dogma for a Molecular Design Based on DNA: DNB (Databasing/Designable Nanobio) → ENB (Engineering Nanobio) → FNB (Functional Nanobio)

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(Received March 31, 2005; CL-058005)

Abstract

Nanobiomaterials have become of broader interest over the past few years. In this review, we demonstrate functional biomolecules that we have constructed based on the biophysical data on nucleotides. We propose three concepts for the development of new nanobiomaterials; DNB (Databasing or Designable Nanobio) is the process of storing the biomolecular properties, ENB (Engineering Nanobio) is the process of conversion of the biomolecular properties to a molecular design, and FNB (Functional Nanobio) is the process of constructing functional molecules based on DNA technologies. Our approach of DNB → ENB → FNB can be the central dogma for a molecular design.

♦ Introduction

In biological systems, biomolecules accomplish highly specific and extremely efficient reactions even at low concentrations. They have significant properties, which cannot be attained by other molecules, such as accurate recognition of a target molecule, self-assembly into a well-defined structure, catalytic activity, signal transduction, response to a signal, and regulation of a molecular function, etc. Accordingly, biomolecules have the advantage of constructing new materials with functions for technology usages. Conjugation of a biomolecule with other chemical compounds including other biomolecules, dyes, redox materials, metal plate surfaces, and metal and polymer nanoparticles may expand the function. Because the sizes of most biomolecules and their complex compounds range from subnanometer to submicrometer, biomolecules are promising nanobiomaterials. Nanobio is categorizes as the interfacial research area of biotechnology and nanotechnology and specializes in the use of biotechnology in nanotechnology. Development of nanobiomaterials that utilize the properties of biomolecules in nanotechnology have become of broader interest over the past few years. The nanobiomaterial is fitted to a nano-scale material, a nano-sized device, and medicinal and therapeutic usages (e.g., nanoelectronics, nanorobotics, nanobiosensors, and nanochips).[1–3]

DNA and RNA recognize a complementary nucleotide sequence and can self-assemble into a compact structure, and may further have a catalytic activity.[4] The stem structure of nucleotides is a duplex form with Watson–Crick base pairs accompanied by hydrogen bonds in the plane of nucleotide bases and base stacking perpendicular to the bases. The shape of a DNA duplex can be approximated as a circular cylindrical sphere with a diameter of ca. 2 nm and a length reflecting the number of base pairs (0.34 nm per base pair in a B-form DNA). Mismatched nucleotides may change the shape of the duplex and provide a structural polymorphism. The nucleotide structure in consequence of the sequence is also subject to the solvent due to the nature of a highly hydrated and charged surface. Accordingly, nucleotides can also self-assemble into triplex, quadruplex, hairpin loop, and pseudoknot structures depending on the sequence and the solvent.[5] These structural motifs can be used as a structurally well-defined nano-sized scaffold.[6] It is notable that even a short oligonucleotide can adopt a desired structural motif in water. Nucleotides can be prepared biologically, biochemically, and chemically at a relatively low cost, and other types of molecules such as dyes, fluorescent probes, redox-active species, and amino acids can also be incorporated into a nucleotide strand. Moreover, nucleotides can be immobilized on a metal plate surface and attached to metal and polymer nanoparticles.

Although there is a limited diversity in the chemical structure of natural nucleotides and a rigid array of negative charges at the backbone, nucleotides with a desired function have been obtained by rational design and/or by selection from a large pool of random nucleotide sequences.[7,8] In this review, we demonstrate our approaches for constructing functional nucleotides rationally on the basis of biophysical data of nucleotides. The data are also useful for constructing new molecules combining a nucleotide with other compounds. We propose the concepts of databasing or designable nanobio (DNB), engineering nanobio (ENB), and functional nanobio (FNB) for constructing the functional nanobiomolecules designed for technology.

♦ Biomolecules Designed for Technology

The biophysical properties of biomolecules have been investigated. On the basis of the biophysical data, rational design of nanobiomolecules can be attained. Table 1 shows the functional biomolecules that we have demonstrated recently. The following

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Table 1. Functional biomolecules that we have constructed under the concept of DNB → ENB → FNB

	Functional molecule	DNB		ENB		FNB
(1)	Nucleotide nanowire	Characterization of the G-quadruplex structures	→	Search for small molecules that can induce the formation of the G-quadruplex in a parallel orientation	→	Formation of the nanowire structure as a structurally well-defined scaffold
(2)	DNA chip with an RNA cleavage activity	Determination of the minimum size of the DNAzyme	→	Immobilization of the DNAzyme on a metal plate surface	→	DNA chip that can recognizes the RNA sequence and the structure
(3)	pH sensor for a living cell	Energetic analyses of Hoogsteen base pairs	→	Design of a DNA duplex formed by Hoogsteen base pairs with a FRET activity	→	Measurement of intracellular pH to visualize cell apoptosis
(4)	Antigene molecule	Characterization of PNA and the NLS peptide	→	Sequence design of the NLS peptide and preparation of the PNA–NLS conjugate molecule	→	Inhibition of the target gene expression
(5)	Peptide inhibitor of protein reactions	Assignment of the target protein structure	→	Design of the oligopeptide sequence that can associate with the target protein	→	Inhibition of the protein assembly and reduction of the protein activity
(6)	Base pair mimic nucleoside	Quantification of the stacking interaction of nucleotides	→	Synthesis of the base pair-mimic nucleosides	→	Duplex stabilization and site-selective base unstacking and RNA cleavage

is a summary of the functional molecules.

(1) The self-assembly property of nucleotides is useful for constructing nano-sized structures and structurally well-defined scaffolds. We focused on a quadruplex structure formed by four-stranded DNA helices. A guanine-rich DNA sequence can adopt the G-quadruplex structure forming guanine quartets in an antiparallel or in a parallel orientation depending on the DNA sequence and the solvent. When the strand orientation is in parallel, multiple DNA strands may associate and form guanine quartet nanowires that can be used as a structurally well-defined scaffold. We found the DNA sequence of $5'$-$G_4T_4G_4$-$3'$ forming a nanowire structure by the addition of Ca^{2+} or PEG (polyethylene glycol) in solution while the DNA sequence adopted an intramolecular quadruplex in an antiparallel orientation in solution without Ca^{2+} or PEG.[9,10]

(2) The DNAzyme (deoxyribozyme) with a 15-mer catalytic loop that hydrolyzes a substrate RNA in the presence of divalent metal ions has been reported.[11] Based on the DNAzyme sequence, we constructed a short DNAzyme (11-mer at the catalytic loop) that became active only when Ca^{2+} was added.[12] We then immobilized the short DNAzyme on a gold plate surface to prepare a DNA chip for an SPR (surface plasmon resonance). It was demonstrated that the DNAzyme could recognize the secondary structure of the substrate RNA[13] and that the substrate RNA was cleaved when Ca^{2+} was applied on the DNA chip.[14] Consequently, our DNA chip can recognize the RNA sequence and the secondary structure by combining the DNAzyme activity and the DNA chip technology.

(3) Oligomer DNA was studied for monitoring pH in a living cell because of lower toxicity and a biodegradable property. To endow pH-sensitivity to a nucleotide, a DNA duplex formed by Hoogsteen base pairs was explored. Formation of a C/G Hoogsteen base pair requires protonation of the cytosine base of which the pK_a is shifted close to neutrality.[5] According to characterizations of the pH dependency and the salt concentration effect on the duplex formation,[15] a FRET (fluorescence resonance energy transfer)-active DNA that folds into a hairpin loop structure at acidic pH accompanied with an elimination of the FRET efficiency was engineered. We measured the intracellular pH of living *HeLa* cells by monitoring the FRET efficiency and observed that the intracellular pH was decreased after the induction of apoptosis.[16]

(4) Because PNA (peptide nucleic acid) can associate with a complementary DNA sequence tightly,[17] the molecule is supposedly effective as an antigene molecule. However, PNA shows less permeability into a living cell. To overcome this problem, we designed a new molecule of PNA conjugated with a modified NLS (nuclear localization signal) peptide that can be translocated into the nucleus. We prepared the PNA sequence complementary to the p53 gene and found that the amounts of mutated p53 protein and mRNA of the protein in an *NOS-1* cell were eliminated effectively by the PNA–NLS molecule (manuscript in preparation).

(5) Three-dimensional domain swapping is one of the strategies for protein oligomerization. RNase A (bovine pancreatic ribonuclease A consisting of 124-mer amino acids) can form domain-swapped dimers and oligomers. It is reported that the predominant dimer exchanges the C-terminal β-sheet with that of another.[18] On the basis of the structural data, we constructed a 14-mer oligopeptide containing the C-terminal amino acid sequence of RNase A. It was demonstrated that the peptide could associate with RNase A and was a potent inhibitor of the domain-swapped RNase A dimer formation.[19] In addition, the peptide was able to reduce the ribonucleolytic activity of RNase A (manuscript in preparation).

All of the functional biomolecules demonstrated in Table 1

Published on the web (Advance View) July 30, 2005; DOI 10.1246/cl.2005.1206

were engineered (ENB) and subsequently applied for the technology (FNB), based on the quantitative data on the self-assembled structures and the thermodynamics for molecular recognitions (DNB). Hereafter, we overview the recent progress in our research with the base pair-mimic nucleosides as a model case developing new molecules based on the concept of DNB → ENB → FNB.

♦ *Quantitative Evaluation of the Nucleotide Interactions for Nucleotide Engineering—DNB (Databasing or Designable Nanobio)*

The self-assembly and the folding of nucleotides can be evaluated with thermodynamic parameters. The nearest-neighbor model is widely used to account for the thermodynamic parameters of Watson–Crick base pairs.[4] The model was established based on the fact that the major interactions affecting the stability of the base pairs are the interstrand hydrogen bonds and the base stacking between adjacent base pairs. It was revealed that the nearest-neighbor parameters for the nucleotide duplex formations are context-dependent; $-\Delta G^\circ_{37}$ (Gibbs free energy at 37 °C) ranges from 3.4 to 0.2 kcal mol^{-1} per single Watson–Crick base pair formation in 1 M NaCl at pH 7.0.[20–23] The thermodynamic data enable accurate predictions of the secondary structure of nucleotides and their thermodynamic parameters.[24,25] Generally, an RNA duplex is more stable than both a DNA duplex and an RNA/DNA hybrid duplex with an identical sequence, by 0.7 kcal mol^{-1} in $-\Delta G^\circ_{37}$ on the average of the nearest-neighbor parameters.[21] This is inconsistent with the fact that both RNA duplexes and most RNA/DNA hybrid duplexes adopt an A-form conformation while typical DNA duplexes adopt a B-form.[4] Thus, it is concluded that the helical conformation may not be the major determinant for the duplex stability. We studied the role of the 2′-hydroxyl group on the duplex stability by thermodynamic analyses of the duplexes formed by chimeric RNA–DNA strand(s).[26] The data indicated that the stability of the nearest-neighbor interaction at the chimeric junction was influenced by the 2′-hydroxyl group on the 5′-nucleotide and that the 2′-hydroxyl group of a ribonucleotide provided substantial stabilization energy.

Interstrand hydrogen bonds play a central role in the formation of Watson–Crick base pairs that guarantees the base pairing partner. Base stacking between adjacent nucleotide bases is also important for the integrity of a duplex structure where the interaction does not require a particular partner, although the interaction energy between purine bases is greater than that between pyrimidine bases due to a larger overlapping area. Independent contribution of the hydrogen bond and the stacking of the base pairs is reported.[27] The stabilization energy for one hydrogen bond evaluated by the deletion of a hydrogen bond from a G/C base pair in an RNA duplex by replacement with an I/C base pair was 1.8–0.4 kcal mol^{-1} in $-\Delta G^\circ_{37}$, although that determined from DNA and RNA hairpin loops assumed in the absence of cooperativity in the hydrogen bonds was 0.5–0.2 kcal mol^{-1}.[28,29] The free energy of the stacking interaction estimated from the duplexes with a dangling end was 1.7–0.4 kcal mol^{-1} which was comparable to that of a hydrogen bond, and it was concluded that the hydrogen bond and the stacking energies competed in a base pair.[27]

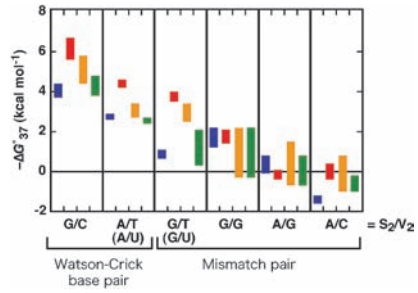

Figure 1. Comparison of the ΔG°_{37} values of a Watson–Crick base pair and a single mismatch at S_2/V_2 in the trinucleotide base pairs of 5′-$S_1S_2S_3$-3′/5′-$V_1V_2V_3$-3′ where S_1/V_3 and S_3/V_1 are G/C or C/G Watson–Crick base pairs. S and V nucleotides are DNAs (blue), RNAs (red), RNA and DNA (yellow), and DNA and RNA (green), respectively.

Mismatched nucleotides in a duplex may affect the nucleotide structure and the duplex stability. Because the conformation of a single mismatch is strongly affected by the base pairs on both sides as well as by the type of the mismatch nucleotides, a trinucleotide is convenient for evaluating the mismatch stability. Figure 1 compares the ΔG°_{37} values for the trinucleotide formations by Watson–Crick base pairs and mismatched pairs of DNA, RNA, and RNA/DNA duplexes. The duplex stability is greatly affected by the mismatch type. In all, G/U and G/T mismatches are relatively stable, in agreement with the similar base pair geometry to Watson–Crick base pairs and the formation of two hydrogen bonds.[4,5] It is an intriguing finding for the G/U and G/T mismatches that the relatively stable mismatches in an RNA/DNA duplex exhibit a stability similar to the G/U mismatches in an RNA duplex while unstable RNA/DNA mismatches show a stability similar to the G/T mismatches in a DNA duplex, implying a stability–structure relationship in RNA/DNA hybrid duplexes.[30] Our investigations with the mismatches in the hybrid duplexes also revealed that the methyl group on C5 of thymine enhanced the duplex stability by 0.5–0.1 kcal mol^{-1} affected by the type of adjacent base pairs and that the stabilization was likely to arise from hydrophobic interactions. Additionally, the 2′-hydroxyl group of ribouridine was found to stabilize the duplex by 0.6 kcal mol^{-1}, affected less by the type of adjacent base pairs, resulting from sugar puckering, hydration, and/or intrastrand hydrogen bonds with sugar or phosphate oxygen atoms.[31,32] The stabilization energy by the 2′-hydroxyl group is consistent with the greater energy in the nearest-neighbor parameters of RNA duplexes than in those of DNA duplexes (0.7 kcal mol^{-1} on the average). The data also indicated that the free energy changes due to the deletions of the C5 methyl group of thymine and the 2′-hydroxyl group of ribouridine, and by the nucleotide base exchange from U/G to G/T or from G/T to U/G in an RNA/DNA hybrid duplex did not affect other interactions and that the most dominant replacement related to duplex stability was the base exchange that altered the stacking interaction.[30] Replacement of a nucleotide by an abasic analog nucleotide is also reported to reduce the stability of a duplex dramatically (11–3 kcal mol^{-1})[33,34] because removal of a single nucleotide base causes a deficiency in the stacking interaction with base pairs on both sides and perturbs a duplex structure. These observations indicate the importance of the stacking interaction in the nucleotide structure formations.

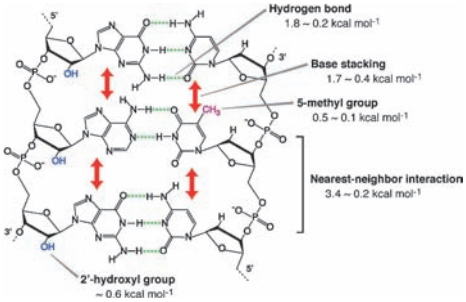

Figure 2. Interaction energy ($-\Delta G^{\circ}_{37}$) for a trinucleotide base pairs formation.

Figure 2 summarizes the $-\Delta G^{\circ}_{37}$ values for the base pairs formation and the contributions of each interaction,[5] although each may be affected by other interactions in a cooperative or a compensatory manner.

♦ *Nucleotide Engineering for Stabilization and Functionalization of a Duplex—ENB (Engineering Nanobio)*

Based on the quantitative data on the interaction energy for Watson–Crick base pair and mismatched nucleotides, nucleoside analogues that alter the nucleotide base structure have been explored to enhance the ability for nucleotide recognition and to provide functions beyond natural nucleotides. Artificial nucleotides changing the hydrogen bonding patterns and the number of hydrogen bonds of the base pair are reported.[35–37] On the other hand, nucleotides with an expanded base size are often examined to enhance stacking interaction where the planar aromatic molecules are used because the compounds have a high potential for stacking and may intercalate between base pairs in a duplex.[37–39] On the contrary, our investigation used the deoxyadenosine derivatives tethering a phenyl group [N^6-(N'-phenylcarbamoyl)-2′-deoxyadenosine, **X**] and a naphthyl group [N^6-(N'-naphthylcarbamoyl)-2′-deoxyadenosine, **Z**] at N6 of deoxyadenosine by an amide linker that were designed from a base pair structure (Figure 3).[40] The aromatic hydrocarbon group is expected to stack with a double helix when it orients in an appropriate position by adopting a geometry analogous to a Watson–Crick base pair. In addition, the presence of a linker that covalently connects adenosine with the aromatic hydrocarbon group may allow adoption of a conformation suitable for stacking with adjacent base pairs.

Our work demonstrated that the single **X** and **Z** at the 5′ dangling ends stabilized the self-complementary DNA duplexes of 5′-ATGCGCAT-3′ and 5′-TGCGCA-3′ by 1.7–0.9 kcal mol^{-1} per base pair-mimic nucleoside which was greater than that by a single dangling adenine (0.5 kcal mol^{-1}).[41,42] Importantly, the stabilization energy by **X** and **Z** was as good as or better than that of the formation of an A/T Watson–Crick base pair (1.0–0.9 kcal mol^{-1}). The strong stabilization suggests that **X** and **Z** stack efficiently on the adjacent terminal base pair of the duplex[43] by adopting the Watson–Crick base pair-mimic geometry as shown in Figure 4A. Addition of a second dangling residue to the 5′-dangling **X** or **Z** further increased the duplex stability by 0.7–0.6 kcal mol^{-1} for the additional **X** or **Z** and 1.1–0.9 kcal mol^{-1} for the additional adenine, although such stabilization energy was not given for the dangling end of adenines (only

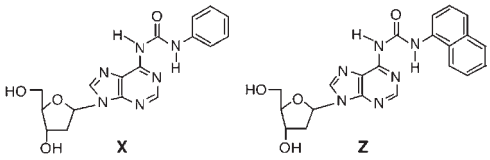

Figure 3. Structure of the base pair-mimic nucleosides of N^6-(N'-phenylcarbamoyl)-2′-deoxyadenosine (**X**) and N^6-(N'-naphthylcarbamoyl)-2′-deoxyadenosine (**Z**).

0.1 kcal mol^{-1}). On the other hand, the lower ability of the phenyl and naphthyl groups to stack on the 3′ end of the duplex was indicated (0.5–0.4 kcal mol^{-1}, close to 0.4 kcal mol^{-1} by the adenine dangling end). These observations could be accounted by the overlapping area with the adjacent base pair.[42] Figure 4B compares the stacking energies between A and the aromatic hydrocarbon group of **X** and **Z** and those between the phenyl and the naphthyl groups. When the A dangling end is more stable, **X** and **Z** can stabilize the duplex more and the additional energy due to the aromatic hydrocarbon group is greater. The data suggest a cooperative stacking interaction between the adenosine portion and the aromatic hydrocarbon portion in **X** and **Z**. In addition, the stabilization energies due to the phenyl and naphthyl portions correlate linearly, suggesting that these stabilization mechanisms are the same and that more stacking area provides more interaction energy.

The structure and thermal stability of the 11-mer RNA/DNA hybrid duplexes containing **X** in the middle of the DNA sequence were also investigated. The T_m (melting temperature) values among the natural duplexes containing A/A, A/G, A/C, and A/U pairs differed by 12.9 °C.[44] The large difference in the duplex stabilities is consistent with the previous reports (see Figure 1). In contrast, the T_m values of the duplexes containing **X** in place of the adenine forming **X**/A, **X**/G, **X**/C, and **X**/U were almost the same (only 0.8 °C differences in T_m). This observation suggests that the aromatic hydrocarbon group of **X** intercalates into the duplex, leading to the opposite nucleotide base flipping into an unstacked position (Figure 5A).[44] Similar observations were also obtained for the RNA/DNA hybrid duplex containing **Z** (manuscript in preparation) and the DNA duplexes.[45] Although the base unstacking involves a large energy penalty,[33,34] intercalation of the phenyl and the naphthyl groups could compensate for the energy. Therefore, it is concluded that the deoxyadenosine derivatives can adopt the base pair-mimic

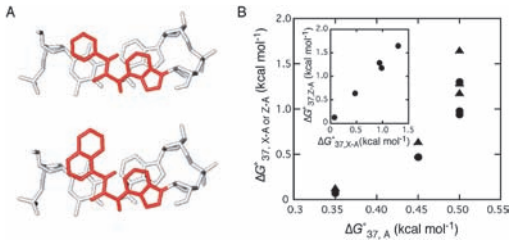

Figure 4. (A) Molecular modeling of **X** (upper) and **Z** (lower) on the 5′-terminus A/T base pair of a B-DNA duplex. (B) Plots of the stacking energy of A ($\Delta G^{\circ}_{37,A}$) against the energy of **X** (circles) or **Z** (triangles) subtracting that of A ($\Delta G^{\circ}_{37,X\text{-}A \text{ or } Z\text{-}A}$). Plots of the differences in the free energy between **X** and A ($\Delta G^{\circ}_{37,X\text{-}A}$) and that between **Z** and A ($\Delta G^{\circ}_{37,Z\text{-}A}$) are shown in the inset. All data were derived from Ref. 42.

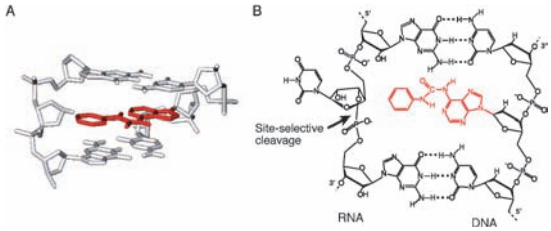

Figure 5. (A) A model of **X** (red) intercalating into a duplex. The opposite nucleotide of **X** is presented by an abasic nucleotide analog. (B) Illustration of the site-selective RNA cleavage by the DNA strand containing **X**.

geometry stably in the middle of the duplex as well as at the duplex terminus.

♦ Use of the Functional Nucleotide Designed for Technology—FNB (Functional Nanobio)

Site-selective RNA cleavage is important for biotechnology and therapy as well as biology. Natural ribozymes catalyze the RNA cleavage by utilizing metal ions and nucleotide bases as catalysts.[46,47] It is known that the ribonucleotide subject to be cleaved locates in an unpaired region and that the RNA hydrolysis proceeds via an in-line attack mechanism.[48] Thus, the RNA hydrolysis is favored if the arrangement of the 2′-hydroxyl group and the 5′-leaving oxygen atom adopts an in-line orientation during the transesterification reaction. It is indicated that the duplex backbone geometry prohibits the formation of the in-line attack arrangement while the unpaired nucleotides can be preferentially hydrolyzed in the presence of divalent metal ions.[48]

Because **X** caused the opposite nucleotide base flipping into an unstacked position, it was expected that the ribonucleotide opposite **X** was prone to be hydrolyzed. To confirm the idea, the 11-mer RNA/DNA duplex containing **X** in the middle of the DNA strand was examined for RNA hydrolysis. The RNA strand was found to be hydrolyzed on the 3′-side of the ribonucleotide opposite **X** in the presence of $MgCl_2$ (Figure 5B).[44] Importantly, the RNA cleavage was highly site-selective, and the RNA sequence to be cleaved was not restricted when the RNA strand hybridized with the DNA. The natural RNA/DNA duplexes containing a single mismatch showed no reactivity, and those with a single bulge revealed a lower amount of the cleaved product and less site-selective cleavage. These observations indicate that **X** stacks into the duplex stably and rigidly and that the ribonucleotide opposite **X** flips into an unstacked position regardless of the nucleotide species. The lack of pairing selectivity and the high T_m values have the advantage of site-selective base flipping in the target sequence and site-specific RNA cleavage. The functions of the adenosine derivatives suggest a use as a "universal" DNAzyme that cleaves exclusively a target RNA sequence. Additionally, the site-selective base flipping would also affect association of DNA-binding proteins and their reactions such as a polymerase reaction that may regulate and stimulate biological reactions.

♦ Summary and Future Directions

In this review, we argued the development of nanobiomolecules by the new approach of DNB, ENB, and FNB (Table 1). The approach can be attained notably for nucleotides because of their excellent properties such as recognition of a complementary sequence, self-assembly into a structurally well-defined structure, biological functions that can be applied to technologies (e.g., ribozyme, DNAzyme, aptamer, riboswitch, antisense, antigene, and RNAi), predictions of the secondary structure and the thermal stability according to the nearest-neighbor model, and availability of nucleotide analogues and conjugates with other compounds. Studies of the thermodynamic parameters of nucleotide interactions are useful for the rational design of nanobiomolecules, Moreover, it is also important to understand whether the interactions are cooperative or compensatory and whether adjacent base pairs and the environments affect the interaction.

The fact that the stacking interaction can provide a stabilization energy comparable to or greater than the interstrand hydrogen bond leads to the design of nucleotide analogues with a large planar aromatic moiety.[37–39] We designed the adenosine derivatives with a base pair-mimic structure (Figure 3). According to the expectation, the nucleosides stacked with the base pairs and adopted a geometry analogous to a Watson–Crick base pair at the duplex terminus and even in a duplex. The nucleosides could stabilize a duplex efficiently and flip the opposite nucleotide into an unstacked position. The property was subsequently used for a site-selective cleavage at the ribonucleotide opposite to **X**. Modification of the aromatic hydrocarbon group and the amide linker of **X** and **Z** may further expand the function. Furthermore, switching of the molecular function may be possible if the linker conformation can respond to a stimulation signal or molecule.

The RNA cleavage reaction is assisted by metal ions and water. Water is the major solvent component in aqueous solution and includes cationic and anionic molecules much more than the biomolecules. Thus, examination of the use of water and cosolutes might be effective for functioning a nanobiomolecule. For instance, natural ribozymes use divalent metal ions and water molecules for hydrolyzing a substrate RNA as acid–base catalysts, stabilizers for the transition state, and proton donors and acceptors.[46,47] If the molecules are assumed to apply to a cell, the intracellular environment should be considered because the environment in a living cell departs far from that in an aqueous solution. The total concentration of intracellular biomolecules including nucleic acids and proteins is estimated to be 300–400 $mg\,mL^{-1}$ which is much higher than that examined in typical in vitro experiments ($<1\,mg\,mL^{-1}$), and the biomolecules occupy an intracellular environment at 20–40% of the total volume. Moreover, the medium in a cell differed remarkably from a dilute solution, resulting from a number of water molecules associated with biomolecules, a small reaction volume, and a substantial surface area. Many properties of biomolecules that are not observed in vitro emerge as a result of such an intracellular environment, called molecular crowding.[49] Molecular crowding may also be significant for the molecules attaching on a metal plate surface and on a nanoparticle. Therefore, quantitative information on molecular crowding as well as the approaches using dilute aqueous solutions is important for the nanobiomolecule design, although the information is fairly limited.[50–52]

The central dogma for the gene expression is DNA → RNA → Protein, where DNA stores the genetic information, RNA converts the genetic information to proteins, and the pro-

tein exhibits functions. Likewise, the flow of nucleotide design described here is DNB → ENB → FNB originated from and based on DNA. The databasing or designable nanobio (DNB) is the process of storing the biophysical data on the biomolecules. The engineering nanobio (ENB) is the process of conversion of the biomolecular property to a molecular design. The functional nanobio (FNB) is the process of using the engineered molecule in technologies. Moreover, the FNB data regarding the molecular function can be regarded as the molecular property data in DNB and are feedbacked for further design of the molecules, similarly to the gene regulation by a product protein. Therefore, our new approach of DNB → ENB → FNB can be the new central dogma for a molecular design.

♦ Acknowlegements

The authors' work in this Highlight Review was supported in part by Grant-in-Aid for Scientific Research and "Academic Frontier" Project (2004–2009) from MEXT, Japan.

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