Drug Design for Genome Targeting

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Molecules that can target DNA or RNA with high efficiency and specificity have become of great interest because of potential applications in biotechnology as well as therapeutics through their capability of modulating gene expression at a specific site. A number of genetic as well as epigenetic disorders of genes have been identified as the cause of diseases, and they continue to increase in number. Thus, the molecules that can target specifically such disordered genes would play central role in future therapeutics. The objective of our research is to establish a concept of chemistry to develop new bio-functional molecules for targeting DNA and RNA. In this paper, I wish to present our recent results of drug design for DNA and RNA Targeting.

Figure 1 summarizes a general design concept, in which we have been attempting to target DNA or RNA to exhibit innovative bio-functions. Our methods rely on the use of functional oligonucleotides incorporating a reactive agent, and we expect to achieve followings.

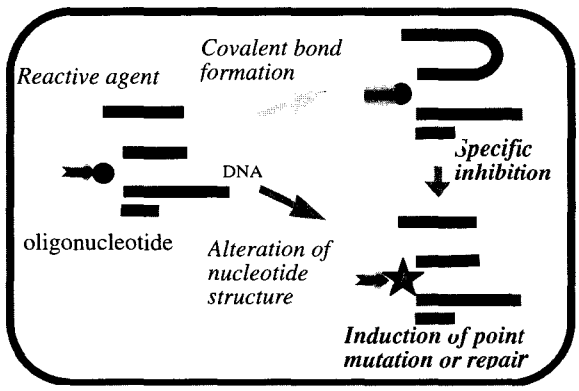


Figure 1. A General Concept for Functional Molecules for Targeting DNA or RNA.

(1) Specific covalent bond formation leading to specific inhibition of gene expression.
(2) Specific induction of point mutation.
(3) Specific reaction to alter nucleobase structure for application to chemical gene manipulation (or repair).

Problems to achieve the above mentioned functions are development of recognition molecules as well as selective agents that would work effectively in the living cells. In this presentation, I would like to focus on molecular design for such biological functions.

1. Functional molecules for specific covalent bond formation with inducible reactivity[1,2]

Specific covalent bond formation between the antisense and the target mRNA would improve antisense inhibition. However, there is no useful reagent available for in vivo use, except photo-reactive psolaren. In our study, the new strategy to form a covalent bond in a specific manner was designed based on MO calculations, and we have developed 2-amino-6-vinylpurine nucleoside (AVP). It has been shown that AVP derivatives exhibit efficient alkylation toward 4-amino group of cytosine with high selectivity. In the continuous effort to perform efficient chemical reactions in the cell, we further designed a new sequential reaction so as to induce the reactive AVP derivatives only within the duplex DNA by using its phenylsulfide or phenylsulfoxide derivative as a stable precursor.[1] The analogs of AVP have been applied to selective inhibition of gene expression in the antisense method and induction of point mutation in the antigene method.[2]

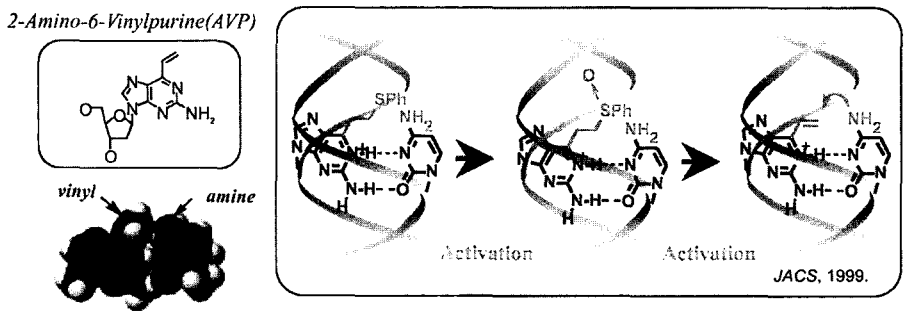


Figure 2. Chemical Structure of 2-Amino-6-Vinylpurine and Design of Hybridization-Assisted Activation

2. Cytidine-specific nitrosyl (NO) transfer reaction leading to deamination.[3]

It has been suggested that NO-induced mutagenesis arises from the initial nitrosation of the amino group of nucleobases, followed by deamination. If NO-induced deamination of a target nucleobase could be controlled, it would become an innovative biological tool to manipulate a gene at a single nucleobase level. We designed a NO-transfer reaction from *S*-nitroso thioguanine to an imino tautomer of cytosine (*Figure 3*). In this study, we have demonstrated the first example of sequence- and base-specific delivery of nitric oxide to cytidine and 5-methylcytidine, leading to the corresponding deaminated products, dC or d^{m}C, respectively. This innovative method would be useful to better understand the role of NO in DNA damage.

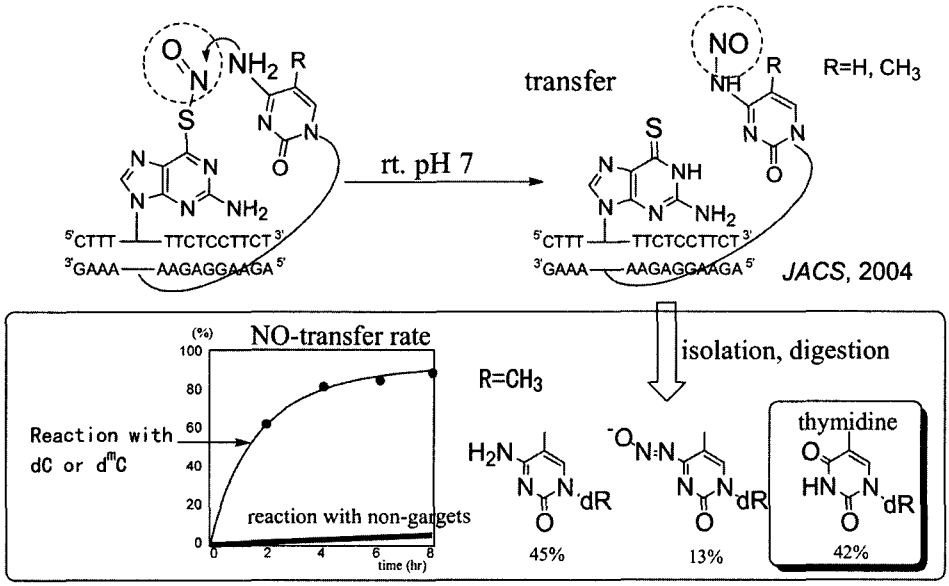


Figure 3. Cytidine-selective No-Transfer Reaction.

3. Non-natural nucleoside analogs for the formation of triplex DNA[4].

Triplex DNA is a powerful tool for gene targeting. The most stable triplexes are formed with homopurine/homopyrimidine sequences, and a pyrimidine base in the purine strand of the duplex interrupts triplex formation (Figure 4). Despite numerous studies, this limitation has remained an unsolved problem. Recently, we have developed new base analogs (W-shaped nucleoside analog; WNA) and demonstrated that WNA-βT with thymine and WNA-βC with cytosine stabilize non-natural antiparallel triplexes with a TA or a CG interrupting site, respectively (Table 1).

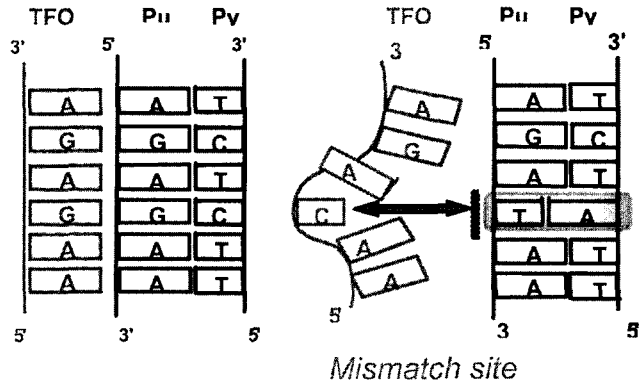
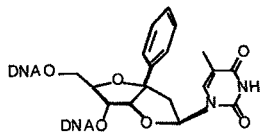
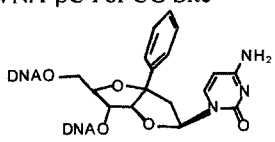


Figure 4. Schematic Structure of Antiparallel Triplexes and a Mismatch Site for Triplex Formation.

WNA-βT For TA-Site



WNA-βC For CG Site



Triplex Stability: *Ks*, 10^9 M^{-1}.

	TA	AT	C	GC	Cod
d	0.00	0.008	0.008	0.086	G:GC
dA	<0.00	0.074	<0.00	0.047	A:AT
WNA-βT	0.30	<0.00	0.015	0.082	βC:CG
WNA-β	<0.00	0.025	0.115	0.047	βT:TA

Table 1. The structure of WNA-bT and WNA-bC and Triplex Stability of Antiparallel Triplexes.

4. Delivery systems for the use of the functional oligonucleotides in the living cells.

We have investigated suitable delivery systems in order to use the functional oligonucleotides in the living systems, and recently found that polymer micelles of the functional oligonucleotide-PEG conjugates exhibit efficient antisense effect..

In conclusion, we have developed new recognition molecules as well as reactive agents that would target DNA or RNA. Furthermore, we have now an advanced technology of DDS systems to apply these new functional oligonucleotides into the living systems. Further study is now ongoing to establish an innovative biological technique with a combination of these new methods.

References

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4. Sasaki, S.; Taniguchi, Y.; Takahashi, R.; Senko, Y.; Kodama, K.; Nagatsugi, F.; Maeda M., *J. Am. Chem. Soc.*, **126**, 516-528 (2004).