Construction of Glyco-Clusters by Self-Organization of Site-Specifically Glycosylated Oligonucleotides and Their Cooperative Amplification of Lectin-Recognition

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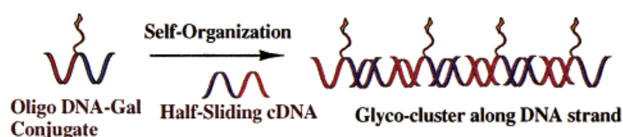
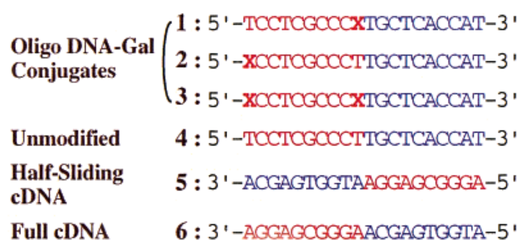
Self-organization has been a key concept in various scientific research fields. A number of supramolecular architectures have been constructed by self-organization of appropriate *tectons* (the components that undergo self-assembly) by hydrogen bonds, electrostatic interactions, and coordination bonds.[1] Recently, oligonucleotides have attracted much attention as a "molecular glue" for supramolecular architectonics based on the self-organization concept.[2] Oligonucleotides are promising construction materials to array functional components in controlled space and direction, since DNA forms a linear, rigid double-strand helix by complementary hydrogen-bonded base-pairing.[2]

Oligosaccharide chains, especially as glyco-clusters, on cell surfaces participate in various biological molecular recognitions and signal transductions through carbohydrate-binding proteins.[3] Various glycoconjugated polymers,[4−6] dendrimers,[7] calixarenes,[8] nanospheres,[9] and transition metal complexes[10] have been developed as glyco-cluster models and biomedical materials. It is well recognized that, if the intervals and directions of the carbohydrate ligands along the scaffolds can be controlled to fit strictly to the binding sites of the target carbohydrate-binding proteins, the binding ability of the glyco-clusters will be further enhanced. However, there has been no report for success in controlling the intervals and directions of the carbohydrate ligands. In this respect, it is of interest to apply DNA as a conformationally rigid scaffold of glyco-cluster models. Previously, we reported the preparation of covalent conjugates of multiple oligosaccharides attached to long DNA by a facile diazo-coupling method.[11] The conjugates acquired resistance to nucleases and strong recognizability to the corresponding lectin although the spaces between the oligosaccharides are random.





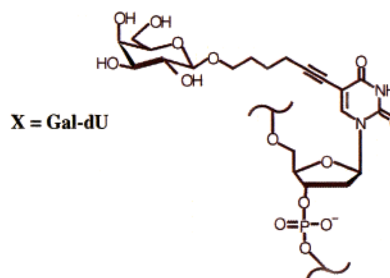


Figure 1. Schematic illustration of glyco-clusters constructed by sequentially addressed self-assembling of oligo DNA−galactose conjugates with the half-sliding complementary DNA. The red and blue sequences on **1**−**4** are respectively complementary to the red and blue sequences on **5** and **6**.

This paper proposes a new strategy to construct periodic glyco-clusters as illustrated in Figure 1. Site-specifically galactosylated oligonucleotide 20-mers **1**−**3** are synthesized and then hybridized with the half-sliding complementary oligonucleotide **5**. "Half-sliding complementary DNA" has been proposed as an *n*-mer DNA, in which the right half sequence (red) and the left half sequence (blue) are respectively complementary to the left half sequence (red) and the right half sequence (blue) of the target *n*-mer DNA. Their hybridization is expected to produce the gapped heteroduplexes. The resulting nanometer-sized macromolecular periodic DNA framework will construct a conformationally rigid scaffold to display carbohydrates in defined orientations. The oligonucleotide 20-mers are designed to display the galactose residues at 68 Å (**1** and **2**) and 34 Å (**3**) regular intervals to the same direction from their DNA duplex, since B-form DNA duplex has an average of 10 base pairs and 34 Å pitch per turn of helix.

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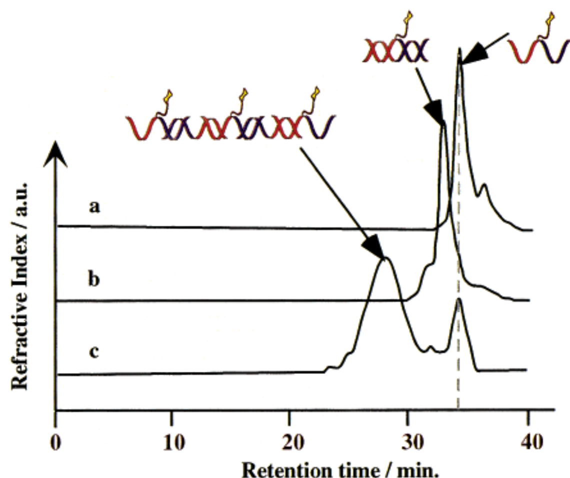
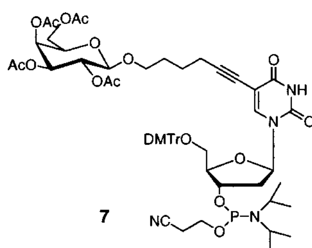


Figure 2. Size exclusion chromatography of galactosylated oligonucleotides. (a) **1** alone. (b) 1:1 mixture of **1** and **6**. (c) 1:1 mixture of **1** and **5**. *Conditions*: Shodex OH Pack SB-804 HQ to SB-803 HQ column, PBS (pH 7.4) as eluent, 25 °C, flow rate = 0.5 mL/min, injected [DNA] = 3.4 mM.

The synthesis of galactosylated oligonucleotides **1**−**3** was reported recently.[12] Galactose-modified deoxyuridine phosphoramidite **7** was synthesized via Heck reaction of 5-hexyn-1-yl



peracetyl-β-D-galactopyranoside with 2′,3′-bis-TBDMS-5-iododeoxyuridine, and then incorporated into the oligonucleotide strand at the desired positions on an automated solid-phase DNA synthesizer. The well-defined structures of the resulting site-specifically galactosylated oligonucleotides were confirmed by MALDI-TOF MS of the conjugates and HPLC analysis of their enzymatic digests. The base-pairing fidelity and the duplex stability of the oligonucleotides were maintained even after the introduction of the galactose moiety at the 5-position of the deoxyuridine unit.

The stability of the duplexes was evaluated by thermal denaturation. The T_m values of the duplexes between the glycosylated oligonucleotides and the half-sliding complementary DNA were 45 °C for **1**/**5**, 46 °C for **2**/**5**, 46 °C for **3**/**5**. These T_m values were comparable to 46 °C for **4**/**5**, but lower by about 20−25 °C than those of the duplexes of these oligonucleotides with the full complementary DNA (65 °C for **1**/**6**, 71 °C for **2**/**6**, 69 °C for **3**/**6**, and 68 °C for **4**/**6**). The typical CD pattern of B-type conformation was obtained for these duplexes of the glycosylated oligonucleotides with the half-sliding complementary oligonucleotide as well as with the full complementary oligonucleotide in phosphate buffer ([DNA] = 45 μM). Size exclusion chromatography (SEC) (Figure 2) and electrophoresis showed that the apparent molecular size of the duplex **1**/**5** was much higher than those of **1** itself and also of the duplex **1**/**6**.[13] The glycosylated oligonucleotides were self-organized with the half-sliding complementary DNA to form a unique type of glyco-cluster molecular assembly.

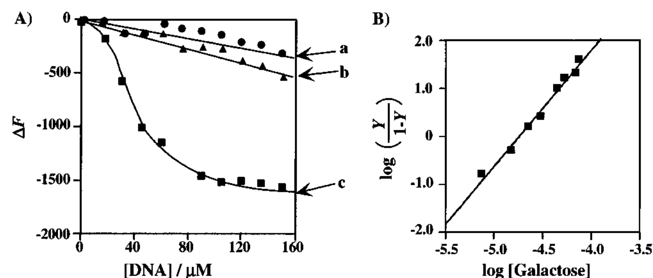


Figure 3. (A) Change in fluorescence intensity of FITC-RCA_{120} at 520 nm (E_x = 490 nm) depending on the concentration of oligo DNAs in PBS (pH 7.4) at 25 °C ([RCA_{120}] = 14.6 nM). (a) 1:1 mixture of **1** and **6**. (b) 1:1 mixture of **4** and **5**. (c) 1:1 mixture of **1** and **5**. The ΔF was corrected for the spontaneous quenching of FITC-RCA_{120} in PBS. (B) Hill plots of fluorescence intensity change of FITC-RCA_{120} with 1:1 mixture of **1** and **5**.

Binding affinity of the assembly to β-galactose-specific FITC-labeled RCA_{120} lectin has been evaluated quantitatively by fluorometry. As shown in Figure 3, the fluorescence intensity of FITC depended on the concentration of oligonucleotides. The glyco-cluster type duplex **1**/**5** was strongly bound to RCA_{120}, whereas the duplexes **1**/**6** and **4**/**5** were minimally bound to RCA_{120}. It is suggested that RCA_{120} recognized selectively the periodic galactose-cluster along the DNA duplex. The dependency of the change in fluorescence intensity on the concentration of **1** and **5** was sigmoidal, indicating that some galactose residues along DNA were cooperatively bound to RCA_{120}. The sigmoidal curve was treated with Hill equation (eq 1) to give the apparent affinity constant $K_a = 5.5 \times 10^4\ M^{-1}$ and the Hill coefficient $n = 2.4$.[14]

$$\log \frac{Y}{1-Y} = n \log [\mathrm{Gal}] + n \log K_a \quad \left(Y = \frac{\Delta F}{\Delta F_{max}}\right) \quad (1)$$

It is suggested that average 2.4 galactose residues are cooperatively bound to some of the four binding sites on RCA_{120} by organizing the conjugate **1** with **5**. Conjugates **2** and **3** were also found to bind to RCA_{120} cooperatively by organization with **5**. The Hill plots gave $K_a = 4.8 \times 10^4\ M^{-1}$ and $n = 2.9$ for **2**/**5**, and $K_a = 3.2 \times 10^4\ M^{-1}$ and $n = 3.7$ for **3**/**5**. Such a cooperative behavior was not observed in binding of allyl β-D-galactoside, glycopolymer,[6e] and glycoconjugated long DNA[11c] bearing β-galactoside to RCA_{120} lectin. Hence, the cooperative lectin recognition is characteristic of the self-organization of oligonucleotides in the present system.

In conclusion, we have demonstrated that the periodic specific presentation of galactosyl residues in space could be attained by hybridization of site-specifically galactosylated oligonucleotides with the corresponding half-sliding complementary DNA. The periodic galactosyl-clusters along the gapped heteroduplexes were cooperatively recognized by the specific RCA_{120} lectin. The interaction is similar to the cooperative binding of allosteric proteins, and the half-sliding complementary DNA functions as an "effector" for polyvalent binding of the galactose moiety to the lectin. The proposed strategy has high potential to address various critical problems in the field of glycoconjugate and DNA materials and also in supramolecular chemistry.

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(13) The apparent number average molecular weights compared with pullulan standards were as follows. **1** alone: M_n = 7830 (M_w/M_n = 1.04); the duplex **1**/**6**: M_n = 12 000 (M_w/M_n = 1.05); the duplex **1**/**5**: M_n = 78 500 (M_w/M_n = 1.89).

(14) The minimum inhibitory concentration of the galactose-cluster **1**/**5** for RCA_{120}-induced hemagglutination was 2.0×10^{-4} M, whereas the duplex **1**/**6** and D-galactose were not inhibited at the same concentration, and nonspecific concanavalin A-induced hemagglutination was not inhibited by **1**/**5**.