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Facile Preparation of DNA-Tagged Carbohydrates

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Abstract—We report here that unprotected carbohydrates (maltose, lactose, cellobiose, and maltoheptaose) can be attached to the aminoalkylated oligonucleotides under mild reductive-amination conditions (aqueous borate buffer, pH 8.0, $NaBH_3CN$, 60 °C) without notable side reactions. Quadruplex-forming G-rich oligonucleotide, 5′-aminoalkyl d(TGGGGT), is glycosylated with maltoheptaose to afford a novel DNA-assisted tetrasaccharide cluster motif.

Carbohydrates occupy a central position in various biological cell-recognition events such as signal transduction, cell adhesion, and bacterial/viral infection.[1,2] However, individual carbohydrate–protein and carbohydrate–carbohydrate interactions are generally weak. To enhance the binding affinity, carbohydrate-mediated biological events typically utilize multivalent interactions,[3] wherein carbohydrates adopt oligomeric forms as assembled on natural scaffolds such as lipids, membrane proteins, and oligonucleotides. These multivalent interactions are characteristic of a variety of carbohydrate-mediated bindings and are well-known as 'glycocluster effect'.[4]

Various types of artificial glycocojugated molecules have been reported as glycocluster models, including polymers,[5] dendrimers,[6] calixarenes,[7] transition metal complexes,[8] and peptides.[9] However, it is still difficult to construct spatially controlled glycoclusters, which would serve as good models for better understanding of carbohydrate-mediated recognition events. A new facile method to construct number- and orientation-controlled glycoclusters is a challenge to expand the scope of glycotechnology.

Recently, considerable attention has been directed toward oligonucleotides as versatile building blocks of supramolecules. Seeman pioneered in construction of multi-dimensional nano-scale objects using programmed DNA assembly,[10] thus leading to a number of novel supramolecular structures such as two-dimensional DNA crystals and three-dimensional DNA nanocages. DNA-tagged carbohydrates are promising candidates toward spatially controlled glycocluster models.

DNA–carbohydrate conjugates have been prepared by attaching protected carbohydrate derivatives to the 5′-end of oligonucleotides or by phosphoramidite coupling of carbohydrate modified nucleotides.[11–13] Recently, Kobayashi and co-workers synthesized site-specifically galactosylated oligonucleotides using a galactose-modified deoxyuridine phosphoramidite and constructed periodic glycoclusters using half-sliding oligonucleotide strategy.[14] However, all of these coupling methods require protection of the OH groups or modification of the reducing end of carbohydrates prior to conjugation with oligonucleotides. These tedious protection/deprotection, and reducing-end-modification procedures prevent practical application to naturally occurring complex carbohydrates. Here we report on a highly convenient method to conjugate unprotected carbohydrates to 5′-end of oligonucleotides. We demonstrate the construction of a tetrasaccharide cluster using 5′-glycosylated quadruplex-forming oligonucleotides.

Direct coupling of unprotected carbohydrates to 5′-end of oligonucleotides was achieved by reductive amination reaction with 5′-aminohexyloligonucleotides in a borate buffer (Scheme 1).[15] To a solution (borate buffer, pH

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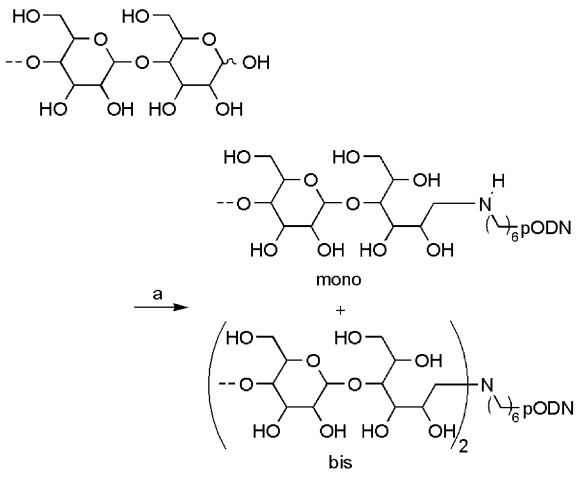

Scheme 1. (a) NH_2–$(CH_2)_6$–*p*ODN, sodium cyanoborohydride, borate buffer (pH 8.0), 60 °C, 12 h.

8.0) of carbohydrate was added 5′-aminohexyl-oligonucleotide and the reaction mixture was incubated at 60 °C for 12 h.[16] The course of the reaction was followed by C18 reverse-phase HPLC. Figure 1 shows a typical HPLC profile on a coupling of 5′-NH_2–$(CH_2)_6$–d(TTTTTT) with disaccharide lactose Galβ1-4Glc. As clearly seen in Figure 1, a clean reaction took place, yielding a single main product in addition to a minor foregoing product and an unreacted 5′-NH_2–$(CH_2)_6$–d(TTTTTT). The major and minor products were collected, concentrated by lyophilization, and were characterized by MALDI-TOF MS to be a monolactosylated ($[M+H]^+$ found 2269.9, calcd 2269.7) 1 to 1 adduct (78%) and bislactosylated ($[M+H]^+$ found 2595.9, calcd 2595.9) 1 to 2 adduct (4%). Control runs indicate that (1) the oligonucleotide backbone is intact even after 48 h incubation, (2) the yield of bis-adduct is less than 11% even after 36 h incubation, (3) no reaction takes place even after 48 h incubation in the absence of borate buffer which is known to facilitate reductive amination reaction, and (4) no reaction takes place either in the absence of sodium cyanoborohydride. All of these characteristic aspects are expected for reductive amination reaction.[17]

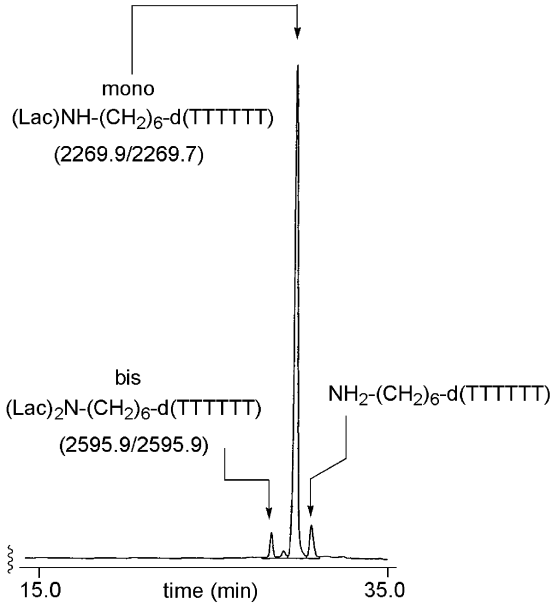

Figure 1. HPLC profile for a coupling of 5′-aminohexyloligonucleotide NH_2–$(CH_2)_6$–d(TTTTTT) with lactose. HPLC analysis was carried out on a WAKOsil 5C18 column (4.6×150 mm) eluted with 0.1 M TEAA buffer containing 0–20% acetnitrile linear gradient over 40 min at a flow rate of 1.0 mL/min, detected at 260 nm. Each product was identified by MALDI-TOF MS. Found and calculated MS data were shown in parentheses (found/calcd).

The high yield of the desired 1 to 1 product indicates that the oligonucleotide backbone and the aromatic amino groups abundant in nucleobases such as adenine, guanine, and cytosine are intact under the present reductive amination conditions using a borate buffer.[18] The mild coupling conditions described here resulted in a chemoselective 1 to 1 coupling between the reducing-end of carbohydrate and the non-aromatic 5′-amino group.

The present simple DNA-tagging method is also applicable to other oligosaccharides, maltose Glcα1-4Glc, cellobiose Glcβ1-4Glc, and maltoheptaose Glcα1-4(Glcα1-4Glc)$_3$.[19] The results were summarized in Table 1.

We then moved on the construction of number- and orientation-controlled glycoclusters, taking advantage of characteristic assembling properties of oligonucleotides. For this purpose, we prepared maltoheptaose-conjugated oligonucleotide, 5′-[Glcα1-4(Glcα1-4Glc)$_3$]-d(TGGGGT), according to the present method ($[M+H]^+$ found 3178.3, calcd 3180.4). G-rich sequence is well known to form a G-quadruplex structure in the presence of monovalent ions with full structural characterization by means of CD and NMR spectrometry. In order to gain insight into the solution structure of 5′-[Glcα1-4(Glcα1-4Glc)$_3$]-d(TGGGGT), CD spectrum was measured in a phosphate buffer (10 mM, pH 7.0) containing 100 mM KCl and 10 mM NaCl (Fig. 2). The CD spectrum shows a strong positive peak near 265 nm and a negative peak near 240 nm. The result indicates that 5′-maltoheptaose-conjugated d(TGGGGT) sequence adopts a parallel G-quadruplex structure under our experimental conditions[20] and the four sugar groups as spectators are presented in the same orientation to form a tetramaltoheptaose cluster motif (total 28 glucose residues).

In summary, the simple reductive-amination method works fine to introduce an unprotected carbohydrate moiety into the aminoalkyl terminus of oligonucleotides. The significance of DNA-tagged carbohydrates thus obtained may be multifold. The higher-order hybridization ability of oligonucleotides can be used to construct number- and orientation-controlled unique glycocluster motifs, as referred to here. The cell recognition ability of carbohydrates, on the other hand, may be applied to self-directing vectors in targeted oligonucleotide delivery. Still the third is the use of appended oligonucleotide as an amplifiable and sequenceable tag,

Table 1. Conjugation of 5′-NH_2–$(CH_2)_6$–d(TTTTTT) with unprotected carbohydrates[a]

Compd	Carbohydrate	Sequence	Mono		Bis	
			Yield (%)	$[M+H]^+$ m/z (found/calcd)	Yield (%)	$[M+H]^+$ m/z (found/calcd)
1	Lactose	Galβ1-4Glc	78	2269.9/2269.7	4	2595.9/2595.9
2	Maltose	Glcα1-4Glc	68	2271.3/2269.7	4	2596.7/2595.9
3	Cellobiose	Glcβ1-4Glc	73	2269.9/2269.7	3	2596.1/2595.9
4	Maltoheptaose	Glcα1-4(Glcα1-4Glc)$_3$	64	3080.3/3080.4	2	4217.9/4217.3

[a]Experimental details are dexcribed in ref 16.

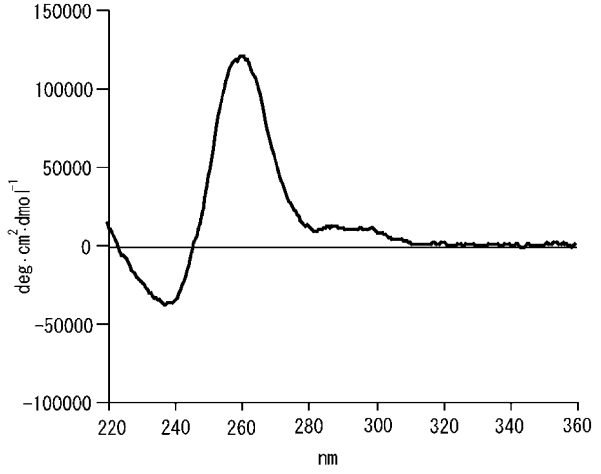

Figure 2. CD spectrum of 5′-[Glcα1-4(Glcα1-4Glc)$_3$]-d(TGGGGT). CD spectrum of oligonucleotides (120 μM base concentration) was measured in phosphate buffer (10 mM, pH 7.0) containing 100 mM KCl and 10 mM NaCl at 10 °C.

whose merit has been amply demonstrated in the identification of peptides as revealed by the phage- and mRNA-display techniques. Construction of DNA-barcoded carbohydrate libraries is now under way in our laboratory.

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16. All reactions were executed in a total volume of 200 μL with final concentrations of each species as indicated in parentheses. Carbohydrate (20 mM) was incubated with 5′-aminohexyloligodeoxynucleotide (50 μM) in a borate buffer (100 mM. PH 8.0) containing sodium cyanoborohydride (250 mM) for 12 h at 60 °C.
17. The stability of the linkage tethering carbohydrate to oligonucleotide was assessed by incubating the conjugate at 25 °C. Almost no significant hydrolysis products were observed after 24-h incubation.
18. Reductive pyridylamination of carbohydrates, which has been widely used for fluorescence labeling, requires more drastic reaction conditions (typically, concd CH_3CO_2H, 90 °C, 1 h, then sodium cyanoborohydride).
19. DNA-tagged products were not yielded in the case of *N,N′*-diacetyl chitobiose (GlcNAcβ1-4GlcNAc) under our experimental conditions.
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