pH Dependent Thermal Stabilization of DNA by Glcβ(1→3)GlcNAcβ1→STol

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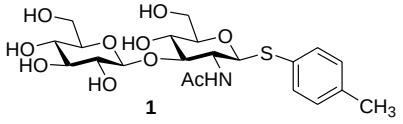
A disaccharide, Glcβ(1→3)GlcNAcβ1→STol (GGS, **1**), was synthesized and demonstrated to stabilize ct-DNA during the denaturing process. GGS at 50 μM shifted T_m of ct-DNA by 23 °C and the behavior was pH dependent. Poly(dA-dT)$_2$ was found to be the preferable type of DNA for GGS stabilization by circular dichroism spectroscopy study.

Keywords: DNA-carbohydrate interaction; Hydrogen-bond; pH dependent; Thermal stabilization.

Interactions of molecules with specific sequences of DNA or RNA to retrieve information stored in them are essential at various stages of a cell's life cycle. Molecules which bind with DNA/RNA and disrupt the normal functions of cancer cells and virus/bacteria can be used as antitumor or antiinfective agents. Many carbohydrate-containing antibiotics such as calicheamicin and erythromycin[1] have been proved to be useful therapeutic agents and their carbohydrate moieties were demonstrated to serve as the recognition domains to provide specific binding toward DNA[2] or RNA.[3] Recently, one of the elegant designs by using a monosaccharide to recognize a specific DNA sequence has been reported by Toshima and co-workers.[4] Neocarzinostatin, a carbohydrate-containing antibiotic, was simplified by replacing its aglycone moiety with a photoactive intercalator, and the resulting mono-amino sugar derivative was still found to direct the specific DNA sequence cleavage. Therefore, study of carbohydrate-DNA/RNA interactions may provide a new approach for the design of a new generation of antibiotics.

The carbohydrate domains of antibiotics usually are presented as deoxy and/or amino sugars to increase hydrophobic and electrostatic interactions with DNA/RNA.[5] Thus, common uncharged carbohydrates are generally considered to have obscurity in binding with highly negatively-charged nucleic acids. However, saccharides should be good candidates in association with DNA or RNA because a large number of hydrogen-bonds (H-bonds), potentially, can be generated from interactions of their hydroxyl groups with the phosphate anions of DNA/RNA backbones, and/or amino and carbonyl groups of nucleobases. Indeed, a single-stranded RNA has been demonstrated to bind with schizophyllan, a neutral β(1→3)-glucan with β(1→6) branch polysaccharide, through H-bond interactions.[6] Also, DNA ligands that bind tightly and selectively to neutral cellobiose were discovered[7] by SELEX[8] (systemic evolution of ligands by exponential enrichment) selection. These results demonstrate that neutral and common carbohydrates[9] can interact specifically with DNA or RNA through H-bond interactions and prompt us to study the capability of GGS **1**, Glcβ(1→3)GlcNAcβ1→STol,[10] to interact and stabilize a specific type of DNA in the denaturing process.



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The synthesis of GGS[11] is illustrated in Scheme I. Selectively protecting the primary hydroxyl group of **4** using TBDPSCl followed by per-benzoylation gave **5**. Removal of thiocresol from **5** with NBS was followed by treating with trichloroacetonitile to yield imidate **6**. Glycosylation of donor **6** with acceptor **7** gave disaccharide **8**. After manipulation of deprotection and protection steps, precursor **9** was obtained. The ester protecting groups of **9** were further hydrolyzed to afford GGS. Sulfation[12] and oxidation[13] of the primary hydroxyl group of **9** followed by basic hydrolysis yielded sulfated compound **2** and acid compound **3**, respectively.

Dedicated to Professor Sunney I. Chan on the occasion of his 67th birthday.

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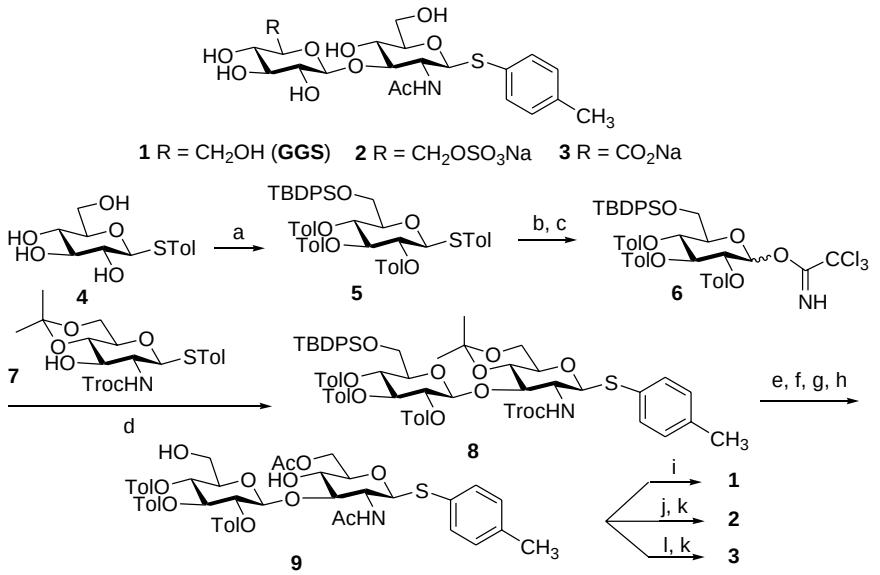
Scheme I Synthesis of GGS (**1**) and compounds **2** and **3**



Reagents and conditions: a) i. TBDPSCl, imidazole, DMF, rt, 3 h; ii. toluoyl chloride, pyr., DMAP (78% for two steps); b) NBS, acetone/H_2O (9/1), rt, 30 min, 73%; c) trichloroacetonitrile, CH_2Cl_2, Cs_2CO_3 (cat.), 0°C to rt, 1.5 h, 81%; d) TMSOTf (cat.), CH_2Cl_2, 0°C to rt, 1 h, 63%; e) TFA, CH_2Cl_2, H_2O, rt, 30 min, 74%; f) Ac_2O, pyr., DMAP (cat.), CH_2Cl_2, rt, 3 h, 96 %; g) i. Zn. HOAc, overnight; ii. Ac_2O, pyr. DMAP (cat), (96% in two steps); h) HF,/pyr, HOAc, THF, 85%; i) NaOMe (cat), MeOH, 83%; j) $(CH_3)_3N.SO_3$, DMF, 50°C, overnight, then Dowe50, MeOH, 1 h, 82%; k) 2M NaOH, THF, 0°C 6 h, 90%; l) PDC, DMF, 68%.

Interactions of these carbohydrates with double-stranded DNA were evaluated by measuring the changes of the DNA melting temperature (T_m).[14] Sonicated calf thymus DNA (ct-DNA) was used as the norm DNA in this study, and its T_m at 100 μM is 44 °C at pH 4.9 in 10 mM sodium phosphate buffer solution. After adding GGS (10 μM)[15] to the DNA solution, the T_m shifted to 51 °C. When the concentration of GGS was increased to 50 μM,[15] the T_m reached 67 °C (as shown in Fig. 1). The 23-degree shift of ct-DNA T_m suggested that GGS was capable of interacting with and stabilizing the double-stranded DNA during the denaturing process. Furthermore, the shift in T_m was found to be pH dependent under the same GGS concentration (50 μM).[15] When the pH value was changed from 4.9 to higher values (6.0, 7.1 and 8.2), the T_m shifts were reduced substantially. The pH dependent behaviors suggested that DNA was protonated at the nucleobases under acidic conditions, thus enhancing the H-bond interaction with GGS. Because the experiment was performed in phosphate buffer, the possibilities of the H-bond interactions being generated by the hydroxyl groups of disaccharide and DNA phosphate[6] moieties can be excluded.

When the hydroxyl group at position 6′ of GGS was modified to anionic sulfate (**2**) or carboxylate (**3**), the T_m shifted by only 4-5 °C under similar conditions. These results suggested that the hydroxyl group at position 6′ may play an important role in the disaccharide-DNA interactions.

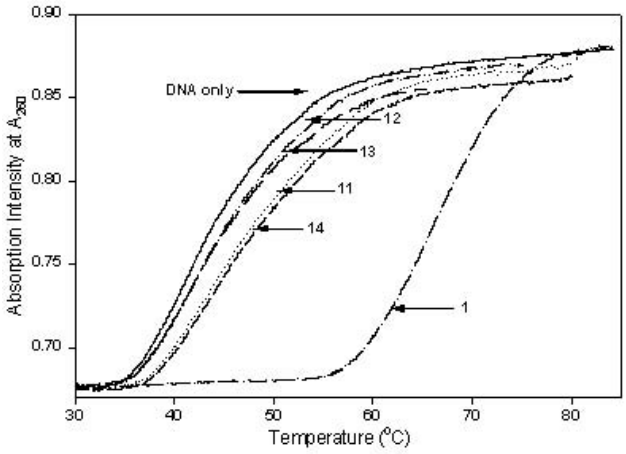


Fig. 1. DNA melting temperatures in the presence of various disaccharides. The absorption intensity is the value at the wavelength at 260 nm. DNA solution contained 100 μM of ct-DNA and 10 mM sodium phosphate (pH 4.86) with 100 uM of compounds **11-14**, respectively, or 50 μM of GGS.

A variety of sugars, as listed in Fig. 2, were also examined to demonstrate the unique properties of GGS in reaction with DNA. Disaccharide **10** was used to compare the influence of different disarccharide conformations (β1→3 vs β1→4 *O*-linkage). Disaccharides **11-13** were applied to study the orientation effects of the hydroxyl groups at the axial and equatorial positions. Sugars **14-20** were examined to see the binding affinity differences between mono- and di-saccharides, and to ascertain the effects of the functional groups such as methylthiophenyl and amino groups. The results showed that most of the saccharides did not have any influence in shifting the T_m of ct-DNA, except for **17**, **18** and **20** which possess free amino groups and slightly increased the T_m by only 2-3 °C in the DNA denaturing process. These results also demonstrated that the interactions of GGS with DNA were not through intercalator (methylthiophenyl) with nucleobases because both compounds **10** and **15** contained the same intercalator but could not shift T_m.

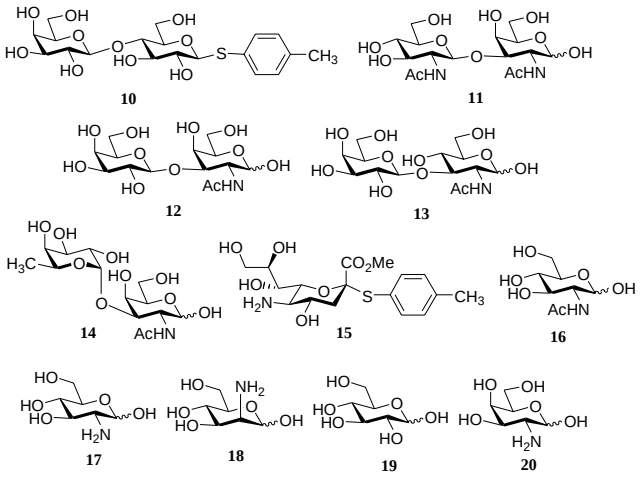


Fig. 2. List of mono- and di-saccharides used for DNA binding studies.

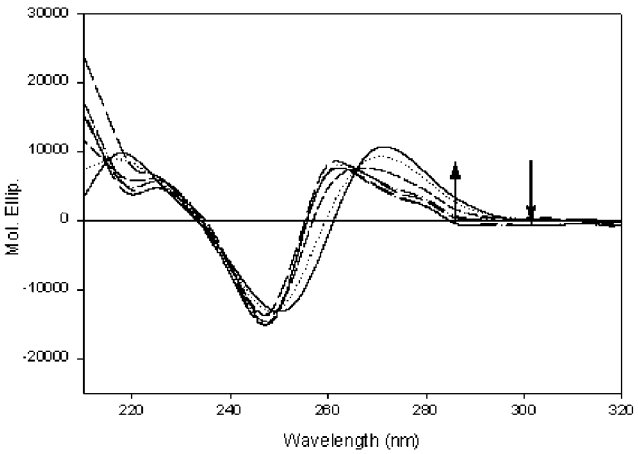


Fig. 3. CD absorption spectra of Poly(dA-dT)$_2$ titrated with compound **1**. [Poly(dA-dT)$_2$] = 100 μM, [compound **1**] = 0, 10, 20, 30, 40 and 50 μM, respectively, at 10 mM sodium phosphate buffer (pH 4.86), at 25 °C.

The preferable type of DNA for GGS was determined by measuring the shift of the absorption wavelength[16] of DNA in circular dichroism (CD) spectroscopy. Disaccharide GGS itself did not show any change in CD spectra even with increasing its concentration up to 200 μM, suggesting that the aggregation effect of carbohydrate can be ignored. When poly(dA-dT)$_2$ was used to react with GGS (10 - 80 μM) as shown in Fig. 3, the absorption wavelength of DNA CD spectra was shifted from 272 to 262 nm, while those of disaccharides **2-6** remained unchanged. Furthermore, the use of poly(dG)-poly(dC), poly(dA)-poly(dT) or poly(dG-dC)-poly(dG-dC) to react with GGS only slightly increased the DNA absorption intensities without shifting wavelength under similar conditions. The results indicated that disaccharide GGS preferred to bind with poly(dA-dT)$_2$.

In brief, we have demonstrated that GGS, **1**, can stabilize poly(dA-dT)$_2$ double helix DNA in a thermal denature process. This novel phenomenon is pH dependent and may be attributed by the H-bond interactions between the hydroxyl groups of disaccharide and the protonated amino bases of DNA. The disaccharide interacted and changed the local environment of double-stranded DNA resulting in T_m shift. More detailed information at the molecular level is needed to investigate this pH dependent thermal stabilization interaction. Study of interactions between well-defined oligonucleic acid and GGS is in progress.

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10. ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.06 (s, 3H), 2.37 (s, 3H), 3.32-3.58 (m, 6H), 3.74-3.84 (m, 3H), 3.93-3.94 (m, 3H), 4.52 (d, J = 8.0 Hz, 1H), 4.91 (d, J = 8.4 Hz, 1H), 7.29 (d, J = 7.7 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 19.70, 21.78, 52.88, 60.11, 60.33, 67.97, 68.96, 72.42, 75.02, 75.48, 79.20, 83.54, 86.05, 102.64, 127.46, 129.49, 129.49, 131.96, 131.96, 138.66, 173.85. HRMS (FAB) calcd. for $C_{21}H_{31}NO_{10}SNa$ ($M+H^+$): 512.1566. Found: 512.1576.
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