Synthesis of DNA-Carbohydrate Conjugate *via* Diazocoupling: A New Class of Modified DNA with Enhanced Stability and Lectin-Recognition Ability

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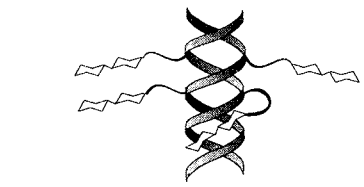
Salmon testes DNA was treated with a diazonium salt connected to *N*-β-lactoside via a spacer chain to yield a diazo coupling product with a degree of substitution of 0.14. The DNA-lactose conjugate kept a typical B-type conformation similar to native DNA, and showed higher melting temperature and stronger nuclease resistance than native DNA. The conjugate was found to acquire a strong binding affinity to galactose-specific lectin RCA_{120}.

Oligosaccharide chains of glycoproteins, glycolipids, and proteoglycans play important roles of recognition phenomena such as fertilization, antigen-antibody reaction, cancer metastasis, infection of viruses and bacteria, and so on.[1] Recently, an increasing attention has been paid to artificial glycoconjugate polymers substituted with pendant oligosaccharide chains as polyvalent recognition signals.[2] Various glycoconjugate polystyrenes,[3] polyphenylacetylene,[4] polypeptides[5] and others[2] have been synthesized and applied as glycoprotein models and biomedical materials.[2b] On the other hand, glycosylated DNAs have been discovered in T-even phages,[6] bacteriophage RL38JI,[7] bacteriophage SP[8,9] and *Trypanosoma brucei*,[9] although their biological roles have not yet been sufficiently elucidated.



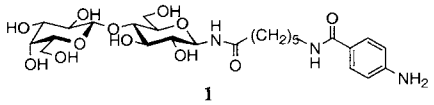


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Chart 1.

In this paper, we have synthesized a DNA-oligosaccharide conjugate mimic in order to develop a new type of functional DNA with lectin-recognition ability. As shown in Chart 1, a lactose derivative was introduced to DNA by diazo coupling[10] which was reported to react with 8-position of guanine base through little influence on the complementary base pairing of DNA. *p*-Aminobenzamide derivative carrying *N*-β-lactoside via a spacer chain (**1**)[11] was treated with $NaNO_2$ and HCl, and the resulting diazonium salt (17.4 mM) was allowed to react with fragmented salmon testes DNA (150-300 bp, 2.00 mg) in a borate buffer (0.2 M, pH 9.0) solution at 25 °C for 30 min. Purification of the product by ethanol precipitation and DEAE-ion exchange provided the DNA-Lac conjugate (1.85 mg) as a yellowish fiber. The degree of substitution of the lactose derivatives to nucleic bases in DNA was estimated to be 14 mol% of overall nucleobases (or 69 mol% of guanine bases) by enzymatic colorimetry using D-galactose oxidase and peroxidase.[12] In the UV spectrum of the conjugate appeared a band of nucleobases at 260 nm, together with a broad shoulder around 350 nm assignable to the azo function. The CD spectrum of the conjugate gave a typical pattern of B-type DNA duplex (Figure 1). Thus the native DNA conformation was little affected by this modification.

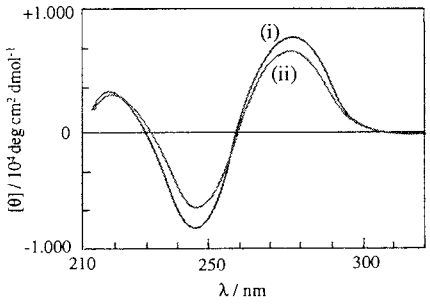

Figure 1. CD spectra of (i) native DNA and (ii) DNA-Lac conjugate. [DNA] = 73 μM-base in 0.25 mM NaCl (pH 7.0) at 25 °C.

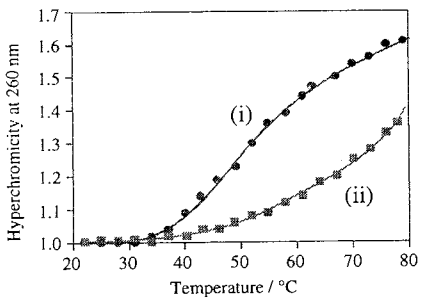

Figure 2. Melting behaviors of (i) native DNA and (ii) DNA-Lac conjugate. [DNA] = 67 μM-base in H_2O, pH 7.0.

Figure 2 shows that the melting curve of the conjugate (curve ii) was shifted to higher temperature by about 25 °C than that of native DNA (curve i). The introduction of the diazo compound to DNA stabilized the duplex of the conjugate. Figure 3 shows that nuclease digestion as detected with an increase of hyperchromicity was retarded by the modification of DNA. The retardation was much more effective to exonuclease III (*Escherichia coli* BE257/pSGR3) (B, curve ii) than to DNase I (endonuclease from bovine pancreas) (A, curve ii). The degradation with the exonuclease III from 5'-end of DNA chain may be resisted sterically by the modification.

Binding of the conjugate to *Ricinus communis* agglutinin (RCA_{120}, β-galactose-specific lectin) was investigated by fluorescence spectroscopy using tryptophan of RCA_{120} as a probe. With the addition of the conjugate, the fluorescence at 332

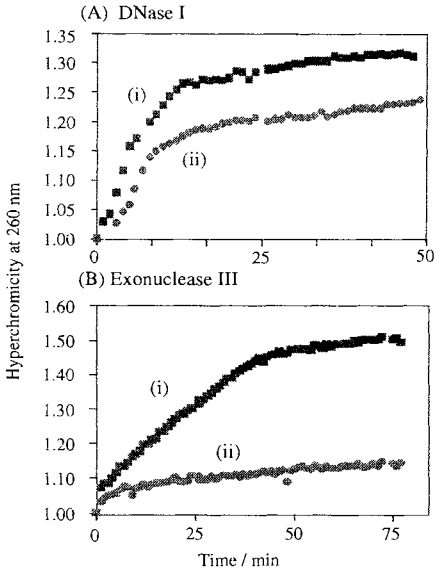

Figure 3. Nuclease digestion of (i) native DNA and (ii) DNA-Lac conjugate by (A) DNase I and (B) exonuclease III. (A) Each sample (64 μM-base) was incubated at 25 °C with DNase I from bovine pancreas (27 units / mL) in 3.0 mL of 83 mM AcONa buffer, pH 5.0, containing 25 mM NaCl and 4 mM $MgSO_4$. (B) Each sample (64 μM-base) was incubated at 37 °C with exonuclease III from *Escherichia coli* (50 units / mL) in 3.0 mL of 50 mM Tris-HCl buffer, pH 7.6, containing 1 mM $MgCl_2$ and 1 mM 2-mercaptoethanol.

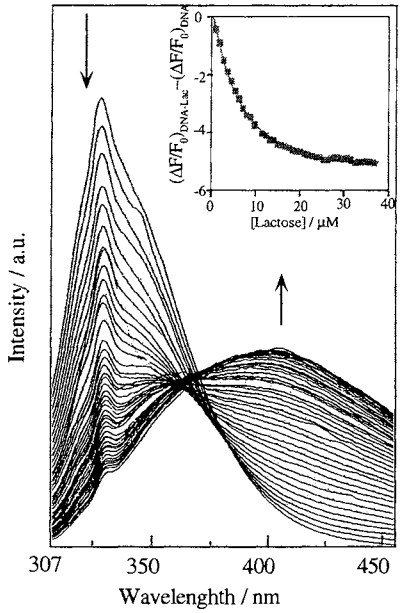

Figure 4. Changes in fluorescence spectra of RCA_{120} lectin (0.103 μM in PBS (pH 7.4)) with addition of 0.5 μL aliquots of DNA-Lac conjugate (0.87 μM-lactose) at 25 °C (excitation at 295 nm). **Inset**: Dependence of the normalized fluorescence intensity $[(\Delta F/F_0)_{DNA\text{-}Lac}-(\Delta F/F_0)_{DNA}]$ on concentration of lactose moiety of the conjugate, where ΔF is the change of fluorescence intensity at 332 nm of the lectin solution with addition of the conjugate or native DNA and F_0 is the fluorescence intensity of lectin alone, respectively.

nm was decreased significantly and also the fluorescence around 405 nm was emerged and increased (Figure 4). The latter fluorescence may be attributable partly to energy transfer from the excited tryptophan to the conjugate. As illustrated in the inset of Figure 4, a saturation curve against the lactose concentration was obtained when the fluorescence intensity at 332 nm was compensated with the small intensity change due to native DNA. The apparent association constant (K_a) between the conjugate and the lectin was calculated to be 2.5×10^5 M^{-1}. RCA_{120} lectin was bound to the lactose residues in the conjugate more strongly than to free lactose $K_a < 10^2$ M^{-1}. This is attributable to the glycoside cluster effect as reported for various glycoconjugate polymers.[2-5] On the contrary, concanavalin A (α-glucose- and α-mannose-specific lectin) was little bound to the conjugate (the data not shown). These results suggest that the conjugate may recognize various cells which bear different sugar receptors on the cell membranes.

In conclusion, a DNA-Lac conjugate could be synthesized simply and effectively via the diazo coupling method. The conjugate showed higher melting temperature and stronger nucleases resistance than native DNA. The B-type DNA duplex was stabilized by the conjugation. In addition, the conjugate was found to acquire a strong binding affinity to galactose-specific lectin RCA_{120}. The modification of DNA with various oligosaccharides via covalent bonds will be a useful protocol of molecular design for cell-targeted gene therapy such as antisences and antigenes[13] via receptor-mediated endocytosis.[14]

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References and Notes

1 M. Fukuda, O. Hindsgaul, "Molecular Glycobiology," IRL, Oxford (1994).
2 a) "Neoglycoconjugates: Preparation and Applications," ed by Y. C. Lee and R. T. Lee, Academic Press, San Diego, CA (1994). b) "Neoglycoconjugates Part B, Biomedical Applications," ed by Y. C. Lee and R. T. Lee, Methods in Enzymology 247, Academic Press, San Diego, CA (1994). c) T. Furuike, N. Nishi, S. Tokura, and S. Nishimura, *Macromolecules*, **28**, 7241(1995). d) D. Zanini and R. Roy, *J. Am. Chem. Soc.*, **119**, 2088(1997). e) D. D. Manning, X. Hu, P. Beck, and L. L. Kiessling, *J. Am. Chem. Soc.*, **119**, 3161(1997).
3 K. Kobayashi, A. Tsuchida, T. Usui, and T. Akaike, *Macromolecules*, **30**, 2016(1997).
4 K. Matsuura, S. Furuno, and K. Kobayashi, *Chem. Lett.*, **1998**, 847.
5 K. Kobayashi, E. Tawada, T. Akaike, and T. Usui, *Biochem. Biophys. Acta*, **1336**, 117(1997).
6 J. Lichtenstein and S. S. Cohen, *J. Biol. Chem.*, **235**, 1134(1960).
7 F. F. Hsu, P. F. Crain, J. A. McCloskey, D. L. Swinton, and S. Hattman, *Adv. Mass Spectrum*, **11B**, 1450(1989).
8 M. Ehrlich and K. C. Ehrlich, *J. Biol. Chem.*, **256**, 9966(1981).
9 J. H. Gommers-Ampt, F. V. Leeuwen, A. L. J. De Beer, J. F. G. Viliegenthart, M. Dizdaroglu, J. A. Kowalak, P. F. Crain, and P. Borst, *Cell*, **75**, 1129(1993).
10 a) E. N. Moudrianakis and M. Beer, *Biochemistry*, **53**, 564(1965). b) J. M. Rothenberg and M. Wilchek, *Nucleic Acids Res.*, **16**, 7197(1988).
11 Synthesis of **1** was as follows. ω-Aminohexanoic acid was acylated with *p*-nitrobenzoyl chloride and then condensed with β-lactosylamine in the presence of BOP and HOBt. The nitro function was hydrogenated with Pd/C and the product was chromatographed through Toyopearl HW40S to provide **1** in 59% yield from ω-aminohexanoic acid.
12 G. Avigard, D. Amaral, C. Asensio, and B. L. Horecker, *J. Biol. Chem.*, **237**, 2736(1962).
13 E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543(1990).
14 M. S. Wadhwa and K. G. Rice, *J. Drug Target.*, **3**, 111(1995).