CONVENIENT SYNTHESIS OF OLIGONUCLEOTIDES LINKED TO 5-DEAZAFLAVIN COENZYME MODELS AT 3'-END. INCORPORATION OF 5-DEAZAFLAVIN TO CONTROLLED PORE GLASS(CPG) SUPPORT

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Using CPG support linked with 5-deazaflavin, the 5-deazaflavin modified oligodeoxynucleotides at 3'-end(ODN-dFI) were synthesized. The thermal stability of the duplex of ODN-dFI with its complement was higher than that of oligonucleotide linked to 5-deazaflavin at 5'-end internucleotide linkage.

KEYWORDS synthetic oligodeoxynucleotides; DNA probe; 5-deazaflavin; CPG modification; induced CD signal

The development of synthetic oligodeoxynucleotides(ODNs) linked to functional groups is important in explorations of efficient antisense ODNs and non-radioactive ODN probes.²⁻⁴⁾ On the other hand, flavocoenzymes are well-known redox coenzymes in biological systems.^{5,6)} Flavins have unique redox abilities as well as characteristic fluorescence and absorption. Additionally, flavin derivatives can cleave DNA under photoirradiation.⁷⁾ We have synthesized the ODN linked to flavin coenzyme models as multifunctional molecules⁸⁻¹⁰⁾ and have shown the ability of flavin-modified ODN to cleave the complementary strand under photoirradiation.¹⁰⁾ Furthermore, the redox potential and the intensities of fluorescence derived from flavin or 5-deazaflavin moieties were observed to vary with association with the target sequence.⁹⁾ In these experiments, flavin or 5-deazaflavin derivatives were linked to ODN via aminoalkyl linker at the 5'-end of internucleotide linkage, which was a phosphoramidate bonding (Fig.1). The presence of a phosphoramidate linkage in ODN produces a new chiral center at the phosphorus atom in addition to the inherent chirality of ODN. Thus, flavin and 5-deazaflavin-modified ODNs synthesized in previous studies consisted of a pair of diastereomers. We have now developed a new methodology to link a 5-deazaflavin to 3'-end of ODN by derivatization of controlled pore glass(CPG).

Fig.1. Structure of Oligonucleotides Linked to 5- Deazaflavin Derivatives

A 5-deazaflavin derivative attached to CPG support should be able to withstand drastic condition to remove protecting group in standard solid-phase DNA synthesis. Unfortunately, normal flavin derivatives are known to be unstable under basic conditions.¹¹⁾ The attack of the flavin ring by hydroxy ion under basic aqueous conditions causes decomposition of the flavins, which impairs their characteristic ability. As a flavin compound derivatized to CPG, we designed a 5-deazaflavin **1**, which possesses a diethanolamine substituent at C(8) position. Because the presence of an electron-donating group at C(8) position of 5-deazaflavin was expected to prevent the nucleophilic addition to 5-deazaflavin ring, **1** may be stable through the deprotecting step by conc. ammonia. Furthermore, a hydroxy group of diethanolamine moiety would facilitate the attachment of **1** to CPG support. The synthetic sequence is shown in Chart 1. Condensation of 6-methylaminouracil with 2,4-difluorobenzaldehyde gave 8-fluoro-5-deazaflavin **2**. Treatment of **2** with

diethanolamine at 115 °C gave **1** as yellow powder. Mono-dimethoxytritylation of **1** followed by the reaction with succinic anhydride yielded **3** as yellow gum. Standard incorporation of **3** to CPG support (100.1 $\mu\text{mol/g}$) gave **4** (5-deazaflavin CPG support). Incorporated amounts of **3** to CPG were determined by the releasing assay of dimethoxytrityl cation (15 $\mu\text{mol/g}$).

Chart 1. Incorporation of 5-Deazaflavin Derivative **1** to CPG Support

Synthesis of 3'-end 5-deazaflavin modified ODNs **5**, **6** using 5-deazaflavin CPG support was carried out according to the standard hydrogen phosphonate method.¹²⁾ Removing of protecting groups of amino substituents of bases was conducted at 60°C for 5 hours in conc. ammonia. Purification of the peak possessing both UV-visible absorptions at 260 nm and 440 nm by HPLC gave ODN linked to 5-deazaflavin at 3'-end **5**, **6** (ODN-dFl). UV-visible absorption spectra of purified ODN-dFl **5**, **6** are displayed in Fig.2. Spectra of both ODN-dFl **5** and **6** exhibited characteristic absorption of 5-deazaflavin possessing alkylamino substituent at C(8) position (arrow).

Fig. 2. UV-Visible Spectra of ODN-dFl **5** (A) and **6** (B)

The thermal stability of the duplex consisting of **5** and its complementary poly dA was investigated by spectroscopic means. ODN-dFl **5** could form a more stable duplex with poly dA than native thymidine 10 mer (29 °C for ODN-dFl **5**; 24 °C for native thymidine 10 mer). Furthermore, in order to assess the environment around 5-deazaflavin moiety, CD spectra of the duplex consisting of **5** and poly dA were measured. In addition to induced CD signal at 245 nm derived from formation of a duplex, induced CD signal at 440 nm was observed (Fig.3). Both induced CD signals exhibited a similar temperature dependency. The unequivocal manner of interaction between 5-deazaflavin and base array has not been made clear yet. However, induced CD signals obtained in the case of duplex formation showed a similar negative Cotton effect around both 245 nm and 440 nm. This finding suggests the presence of strong stacking interaction between 5-deazaflavin moiety and base array of duplex. Previously, the linker arm of flavin and 5-deazaflavin modified ODNs at 5'-end

Fig.3. Temperature Dependent CD Spectra of ODN-dFI **5** with Poly dA
All spectra were measured in 10 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl.

Fig.4. Temperature Melting Profiles of Oligodeoxynucleotides Linked to 5-Deazaflavins **5**, **7** with Poly dA in 10 mM Tris-HCl buffer (pH 7.0) containing 100 mM NaCl.
(a) Profile of **7** with poly dA; (b) profile of **5** with poly dA.

internucleotide linkage such as in **7** caused the disturbance of their duplex structure. These undesirable distortions might provoke the destabilization of their duplex. On the other hand, the duplex structure of ODN-dFI might not suffer from steric perturbation by the linker arm. In fact, the stability of the duplex consisting of ODN-dFI **5** and poly dA was higher than that of **7** and poly dA (Fig.4). Furthermore, dFI-CPG support could be applied to standard DNA synthesizer employing the hydrogen phosphonate method. This would facilitate the development of antisense ODNs linked to 5-deazaflavin as well as the application of ODN-dFI to structural probes.

REFERENCES AND NOTES

- 1) Present address: Center of Biotechnology, Baylor College of Medicine, The Woodlands, TX 77381.
- 2) E.Uhlman and A.Peyman, *Chem.Rev.*, **90**, 543 (1990).
- 3) K.Yamana and R.L.Letsinger, *Nucleic Acid Research*, **16**, 169 (1985).
- 4) A.Murakami, M.Nakaura, Y.Nakatsuji, S.Nagahara, Q.Tran-Cong, and K.Makino, *Nucleic Acids Research*, **19**, 4097 (1991).
- 5) T.C.Bruice, *Acc.Chem.Res.*, **13**, 256 (1980).
- 6) C.Walsh, *Acc.Chem.Res.*, **13**, 148 (1980).
- 7) M.Korycka-Dahl and T.Richardson, *Biochim.Biophys.Acta*, **610**, 229 (1980).
- 8) T.Akiyama, F.Yoneda, K. Tanaka, J.Kuwahara, and Y.Sugiura, *Nucleic acids Research, Symp.Ser.*, **25**, 69 (1991).
- 9) Y.Nakamura, T.Akiyama, Y.Yoneda, K.Tanaka, and F.Yoneda, *Chem.Pharm.Bull.*, in press.
- 10) T.Akiyama, F.Yoneda, J.Kuwahara, and Y.Sugiura, *J.Chem.Soc., Chem.Comm.*, submitted.
- 11) T.Harayama, Y.Tezuka, T.Tagu, and F.Yoneda, *J.Chem.Soc., Perkin Trans.I*, **1987**, 75.
- 12) B.C.Froehler and M.D.Matteucci, *Tetrahedon Lett.*, **24**, 469 (1986).

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