REDOX POTENTIAL OF OLIGONUCLEOTIDE LINKED TO 5-DEAZAFLAVIN COENZYME MODEL. DETECTION OF HYBRID FORMATION BY CYCLIC VOLTAMMOMETRY

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Oligodeoxynucleotide linked to 5-deazaflavin was synthesized and its redox potential was investigated from the viewpoint of duplex formation with a complementary strand.

KEYWORDS 5-deazaflavin; oligodeoxynucleotide; redox potential; antisense molecule; DNA-probe

Naturally occurring 5-deazaflavin derivatives, such as Factor 420, are essential coenzymes involved in biological redox systems.[1] The redox reactions catalyzed by synthetic 5-deazaflavins have been well documented from a bioorganic chemical point of view.[2] In addition to its redox ability, 5-deazaflavins have inherent properties of aromatic planarity, characteristic ultraviolet-visible absorption and fluorescence. At the same time, there has been great interest in non-radioactively labeled oligonucleotides. The oligodeoxynucleotides linked covalently to biotin,[3] pyren[4] and fluorescein[5] have been reported as colorimetric or fluorescent probes. We have recently described the syntheses of oligodeoxynucleotides linked to 5-deazaflavin derivatives.[6] However, the synthetic methods were intricate and yields were considerably low. This intricacy was ascribed to the instability and insolubility of 5-deazaflavins possessing aminoalkyl linkage under the condition of linking to oligodeoxynucleotide. This paper describes a convenient synthesis of the oligodeoxynucleotide linked to 5-deazaflavin (dFL-ODN) (**1**) by means of a post-labeling of the 5-deazaflavin to oligodeoxynucleotide possessing aminoalkyl linker and the properties of **1**, especially its redox property.

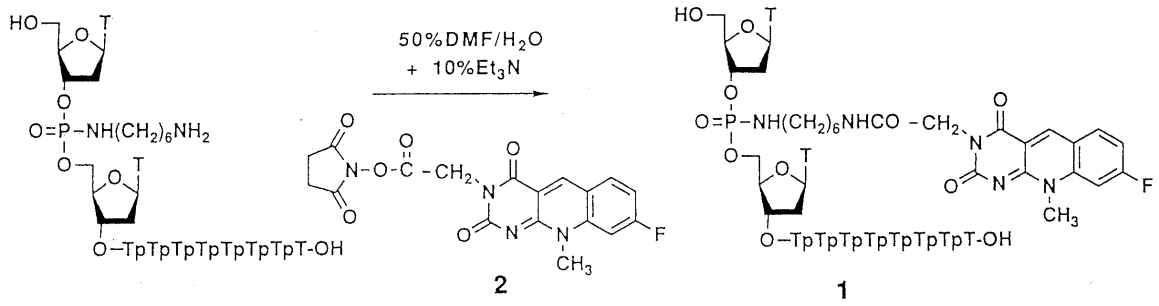

Chart 1. Synthesis of Thymidine Decamer Linked to 5-Deazaflavin

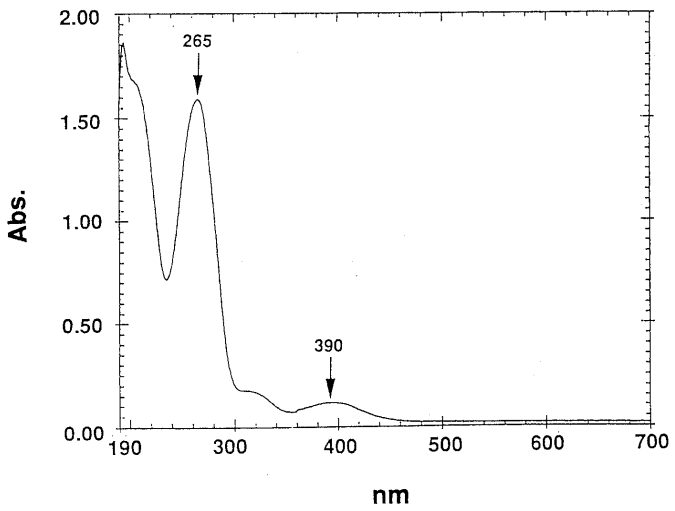

Fig. 1. UV-Visible Spectrum of **1**

Synthesis of **1** was performed as follows. The 5-deazaflavin-activated ester **2** was prepared by condensation of 8-fluoro-5-deazaflavin-3-acetic acid and N-hydroxysuccimide in the presence of dicyclohexylcarbodiimide.[7] Subsequently, **2** was coupled with the thymidine 10 mer bearing an aminoalkyl linker at the 5′-end of internucleotide linkage[8] in 50% dimethylformamide containing 10% triethylamine (Chart 1).[9] Purification using reverse phase HPLC gave dFL-ODN (**1**) in high yield (32 O. D. from 1.5 μmol scale). The UV-visible spectrum of **1** showed a characteristic visible absorption of 5-deazaflavin moiety at 390 nm in addition to an absorption at about 260 nm (Fig. 1). Furthermore, the structure of **1** was confirmed by 20% denaturing polyacrylamide gel electrophoresis[10] and by enzymatic digestion with snake venom phosphodiesterase and alkaline phosphatase.[11]

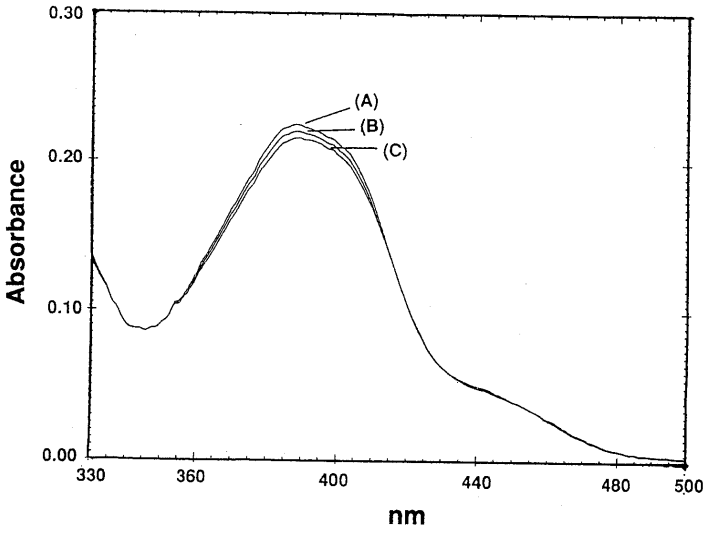

Fig. 2. Hypochromicity and Red Shift in Absorption Spectra of **1** upon Addition of Poly dA
The absorption spectra are given in the absence of poly dA (A) and in the presence of 0.25 (B), and 0.5 (C) equivalent of poly dA. Measurement was conducted at 10°C in 10 mM Tris-HCl buffer (pH7.0) under the concentration of 0.1M NaCl.

Because of its polycyclic planar structure, 5-deazaflavin derivative is expected to interact strongly with nucleic acid base. Figure 2 shows the visible absorption spectra of **1** in the presence of increasing amounts of poly dA at 10 °C. In this condition, **1** formed the duplex with poly dA. A small hypochromicity and a small red shift of absorption at 390 nm were observed by the addition of poly dA. The hypochromicity suggests the presence of stacking interacton between 5-deazaflavin moiety of **1** and nucleic acid base only in the case of where duplex is formed with poly dA, because it is known that a small hypochromicity and a small red shift of visible absorption spectrum are caused by the stacking interaction of flavin derivative with nucleic acid base.[12] Also, this interaction was corroborated by quenching of the fluorescence of **1** in the presence of poly dA at 10 °C.

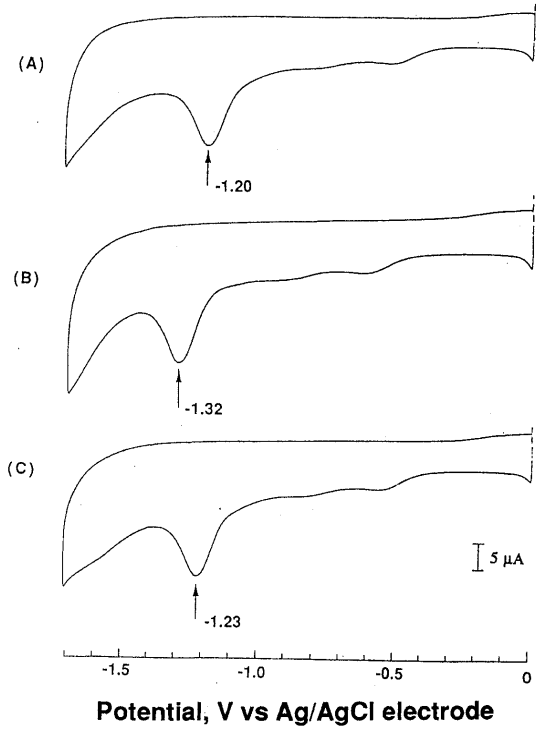

Fig. 3. Change of Redox Potential of **1**
(A) Cyclic voltammogram of **1**. (B) Cyclic voltammogram of **1** in the presence of equimolar of poly A. (C) Cyclic voltammogram of **1** in the presence of equimolar of poly U. Measurement was conducted at 10°C in 10 mM Tris-HCl buffer (pH7.0) under the concentration of 0.1M NaCl. The concentration of **1** was $8.96X10^{-4}M$.

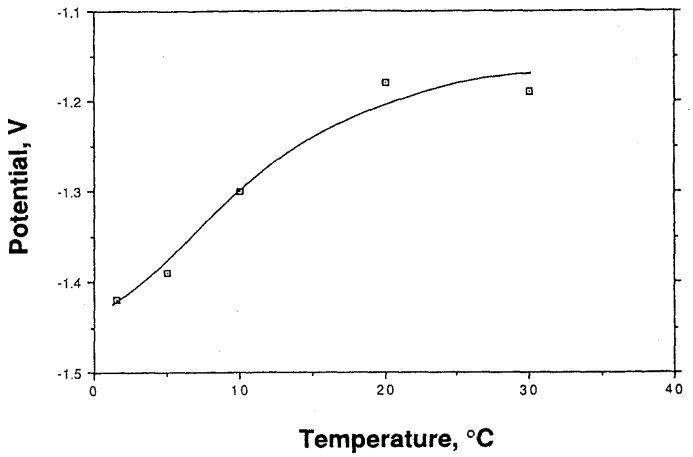

Fig. 4. Temperature Dependency of Redox Potential of **1** Measurement was conducted at 10°C in 10 mM Tris-HCl buffer (pH7.0) under the concentration of 0.1M NaCl, in the presence of equimolar of poly A.

Subsequently, the redox potential derived from 5-deazaflavin moiety of **1** was investigated. The redox potential of **1** shifted to a negative direction only in the presence of its complementary strand at 10 °C (Fig. 3). This shift showed a temperature dependency (Fig. 4). These results suggest that the shift of redox potential was ascribed to the hybridization of **1** with complementary strand. The interaction between 5-deazaflavin and nucleic acid base on the occasion of duplex formation will change the electronic structure of 5-deazaflavin moiety. The change of electronic structure of 5-deazaflavin would vary its redox potential, which is dependent on the energy level of the compound. However, at temperatures above 20 °C, the redox potential was the value of -1.20 V for a single strand of **1**, although the melting temperature value determined in a spectroscopic manner is 28 °C for duplex of **1** with poly A. Thus, the measurement of redox potential derived from 5-deazaflavin may be useful for detection of a slight structural change of the duplex structure around 5-deazaflavin.

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10) The compound **1** was observed to move at a slower rate relative to the native thymidine 10 mer in 20% denaturing polyacrylamide gel electrophoresis.
11) HPLC profile of the reaction solution of an enzymatic digestion showed the peaks of thymidine and thymidine dimer linking to the 5-deazaflavin via alkyllinker arm at the 5′-end of internucleotide linkage.
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