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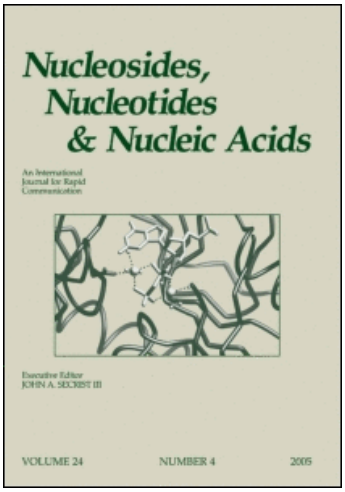

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DNA SEQUENCE RECOGNITION BY NFκB p50 HOMODIMER: STRICT AND OBSCURE RECOGNITION SITES IN THE BINDING SEQUENCE

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ABSTRACT

5-Formyl- and 5-(formylmethyl)-2′-deoxyuridines are introduced into a κB site instead of thymidine(s) in order to understand target sequence specificity of NFκB. It was found that one thymidine in the κB site is particularly important for the sequence specific recognition by NFκB.

The p50 subunit of transcription factor NFκB forms homodimer and binds to a decameric sequence motif named κB site (*a*: 5′-GGGACTTTCC-3′ and its complementary *b*: 3′-CCCTGAAAGG-5′) (1). The structure of NFκB p50 bound to DNA was disclosed by X-ray studies (2). We were interested in introducing thymidine derivatives having a formyl group into the κB site instead of thymidine(s)

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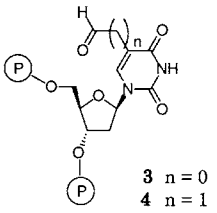
in order to understand target sequence specificity of NFκB (3). The formyl group in the κB site is also attractive due to possibility of forming DNA-protein crosslinks through Schiff bases with the primary ε-amino group of a lysine residue (4). We have demonstrated a reductive amination reaction of 5-formyl-2′ deoxyuridine and the ε-amino group of N^{α}-Boc-L-lysineamide to confirm the Schiff base formation (3a). Very recently, crosslinks of a single stranded oligonucleotide consisting of the repeated sequences of 5′-(TTX)$_n$T-3′ (**X** = **3**) with oligopeptides derived from RecA protein were detected (5).

We synthesized 5-(1,2-dihydroxyethyl)- and 5-(2,3-dihydroxypropyl)-2′-deoxyuridines (**1** and **2**) from 5-vinyl- and 5-allyl-2′-deoxyuridines, respectively, as precursors of 5-formyl- and 5-(formylmethyl)-2′-deoxyuridines (**3** and **4**). Nucleosides **1** and **2** were properly protected and phosphitylated to incorporate into the κB site by an automated DNA synthesizer (3,6). After purifying the oligonucleotide 26-mers by HPLC or gel electrophoresis, oxidative cleavage of the vicinal diol at the C5-side chain provided the formyl group on uracil base(s) in the following sequences in Scheme 1 (**X** = **3** or **4**):

Each 26-mer was annealed with its natural complementary strand. Molecular mechanics calculation based on the crystal structure of the NFκB p50 homodimer bound to DNA established by Müler *et al*. (2b) shows that the ε-amino group of Lys-244 could react with the formyl group of **3** and **4** in the protein-oligonucleotide complex (Fig. 1) (7).

Electrophoretic mobility shift assays demonstrated binding affinity of double strands of II–IV (**X** = **3** in the a strand) to the NFκB p50 homodimer (8) was almost equivalent to that of the natural duplex. However, the homodimer did not significantly recognize the duplex of I (**X** = **3** in the *b* strand) as the κB site (3a). According to dose experiments, duplexes II–IV (**X** = **4**) showed a 10-fold decrease in affinity to the protein compared with duplexes II–IV (**X** = **3**, data not shown). Although weak binding affinity was still observed in V–VIII (**X** = **4**) in spite of plural modifications in the strand a, duplex I with single modification in the strand *b* abolished binding to NFκB (Fig. 2).

These results strongly indicate that thymidine in the strand *b* is extremely important for recognition by NFκB. The recognition loop of NFκB would very strictly judge the structure of thymine base at this particular position (2). In the



A.
a. 5'-AGC TTC AGA G**GG GAC TTT CC**G AGA GG-3'
b. 3'-AG TCT C**CC CTG AAA GG**C TCT CCA GCT-5'

B.
I. 3'-AG TCT C**CC CXG AAA GG**C TCT CCA GCT-5'
II. 5'-AGC TTC AGA G**GG GAC XTT CC**G AGA GG-3'
III. 5'-AGC TTC AGA G**GG GAC TXT CC**G AGA GG-3'
IV. 5'-AGC TTC AGA G**GG GAC TTX CC**G AGA GG-3'
V. 5'-AGC TTC AGA G**GG GAC XXT CC**G AGA GG-3'
VI. 5'-AGC TTC AGA G**GG GAC XTX CC**G AGA GG-3'
VII. 5'-AGC TTC AGA G**GG GAC TXX CC**G AGA GG-3'
VIII. 5'-AGC TTC AGA G**GG GAC XXX CC**G AGA GG-3'

Scheme 1. **A**: Nucleotide sequence of the duplex 26-mer. The κB site is written in bold letters. **B**: The modified 26-mers synthesized are shown, in which T was replaced by **3** or **4** at **X** in the κB site.

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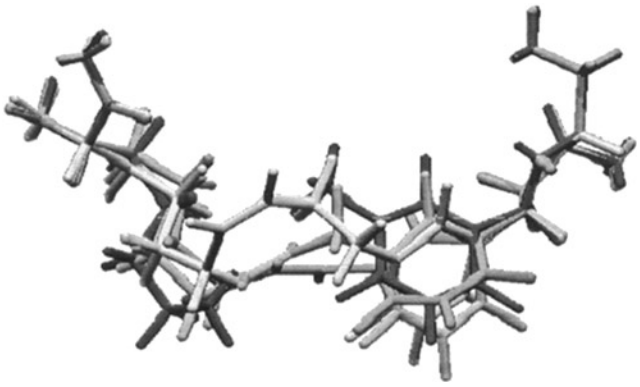

Figure 1. Computer modeling of Schiff base formation of **3** or **4** with Lys in the protein-oligonucleotide complex. The original positions of T and Lys-244 are also depicted (2b). The distance between the methyl group of T and the ε-amino group of Lys-244 is 4.197 Å.

case of 5-formyl-2′-deoxyuridine (**3**), the formyl group could induce the positive charge on the pyrimidine base (9). This charge effect probably account for the restrict discrimination by the protein.

In conclusion, we have synthesized oligonucleotides I–VIII containing **3** or **4** in the κB site and evaluated affinity to the NFκB p50 homodimer. As compared with **3** in duplexes II–IV, duplexes II–IV (**X** = **4**) showed a 10-fold decrease in

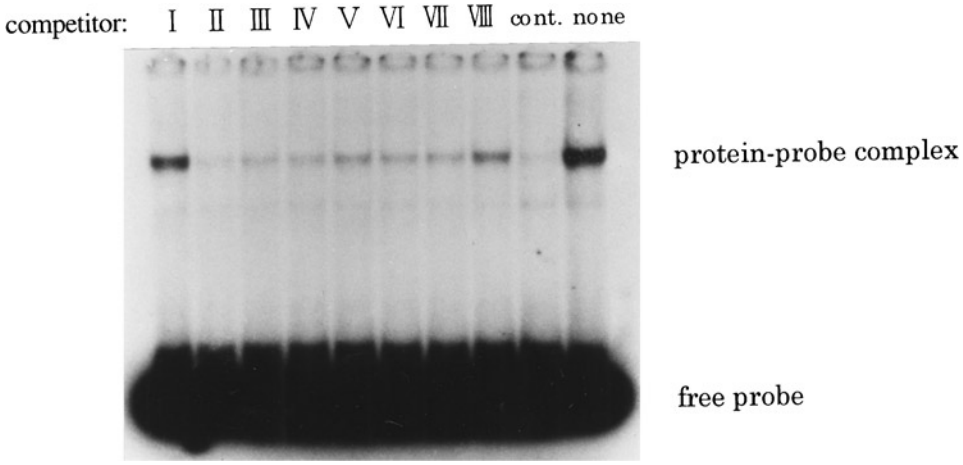

Figure 2. The 5′-^{32}P-labeled probe (wild type duplex, 1 ng) was incubated with the p50 homodimer (40 ng) in 20 μL of the binding buffer [15 mM Tris · HCl (pH 7.5), 75 mM NaCl, 1.5 mM EDTA, 1.5 mM DTT, 7.5% (w/v) glycerol, 0.3% (w/v) NP-40, 1 mg/mL BSA] at 25°C for 20 min, in the presence of 200 ng of cold competitor (I–VIII), which contains **4** at **X**, or cold wild type duplex (20 ng) as a control. DNA-protein complexes were analyzed on 4% non-denaturing polyacrylamide gels.

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affinity to NFκB. It was found that one thymidine in the κB site on the strand *b* is particularly important for sequence specific recognition by NFκB. We are currently investigating crosslinks through Schiff bases of oligonucleotides to NFκB with assist of design based on computer modeling.

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