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CHEMICAL CROSS-LINKING OF PEPTIDES DERIVED FROM RECA WITH SINGLE-STRANDED OLIGONUCLEOTIDES CONTAINING 5-FORMYL-2'-DEOXYURIDINE

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CHEMICAL CROSS-LINKING OF PEPTIDES DERIVED FROM RECA WITH SINGLE-STRANDED OLIGONUCLEOTIDES CONTAINING 5-FORMYL-2'-DEOXYURIDINE

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ABSTRACT

We report the first example of chemical cross-linking of 5-formyl-2'-deoxyuridine containing oligonucleotides with oligopeptides through a Schiff base formation. Twenty amino acid residue peptides investigated here were derived from the DNA binding site of RecA protein. We have demonstrated that the lysine residue placed at the 6th or 8th position from the N-terminus of the peptide directly contacts with DNA.

Escherichia coli RecA protein promotes the strand exchange between two homologous DNA molecules (1). RecA first polymerizes onto the ssDNA, producing a nucleoprotein filament. This complex then captures a dsDNA whose sequence is homologous to the resident ssDNA. A 20 amino acid residue peptide spanning the RecA loop L2 region (FECO peptide in Fig. 1a) has been shown not only bind to

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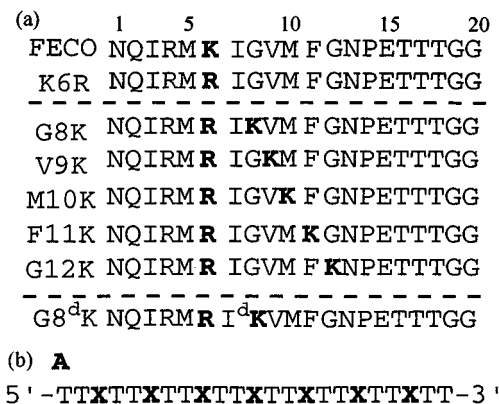


Figure 1. (a) The peptide sequences and (b) the DNA sequence used in this study. **X** denotes the 5-formyl-2'-deoxyuridine unit.

both ss- and dsDNA's but also catalyze the pairing reaction (2a). The peptide has one unique lysine. Thus, we conducted chemical cross-linking to the lysine residue to identify the contact points between ssDNA and the peptide.

It was reported that γ -irradiation to DNA causes oxidation of the thymidine 5-methyl group to the formyl group (3), and reactivity of the formyl group of 5-formyl-2'-deoxyuridine (**1**) to the lysine ϵ -amino group was recently proved by a reductive amination reaction through a Schiff base formation (4). Here we report the synthesis of a 23-mer oligonucleotide containing 7 units of **1**(**A**, Fig. 1b) and the cross-linking ability between the oligonucleotide and the RecA-derived peptide and its mutants.

We have synthesized a series of L2 analogues (Fig. 1a) whose central amino acid residues were sequentially replaced with *L*-Lys to find out possible Schiff base formation sites with the formyl group of 5-formyl-2'-deoxyuridine. All peptides were acetylated at the N-terminus, amidated at the C-terminus and designed to have a single Lys to avoid the complexity arising from multiple reaction points. Peptides were prepared by solid phase synthesis using standard Fmoc chemistry, then purified by HPLC and characterized by TOF mass spectrometry.

Incorporation of **1** into desired position(s) of oligonucleotides has been well studied (4,5). The oligonucleotide **A** (Fig. 1b) were synthesized using the modified Sugiyama procedure (4,5a) and labeled with DIG-11-ddUTP.

As the binding of the peptides to natural DNA was too weak to study with gel electrophoresis, it was assessed by CD spectroscopy. On binding to ssDNA, RecA peptides change the conformation from a random coil to a β -structure, which can be easily monitored by CD in the far-UV region where the spectroscopic signals primarily arise from the peptide bonds (2b). In our experiments, the CD from DNA was negligible. β -structure is characterized by a maximum at ~ 190 nm and a minimum at ~ 220 nm. As expected, the conformation of the control peptide K6R, in which Lys was replaced with Arg, changed from a random coil to a β -structure by the addition of ssDNA(Fig. 2).

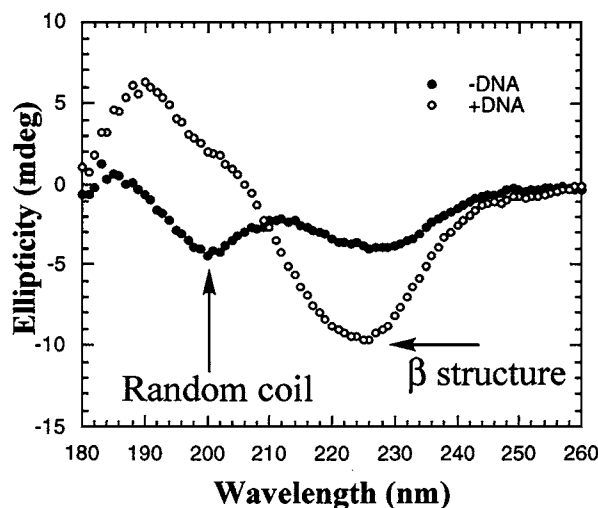


Figure 2. CD spectra of K6R (300 μ M) in the presence and absence of (dT)₂₁ (15 μ M). CD spectra were measured in 10 mM sodium phosphate buffer at pH 7.4.

Effects of ssDNA on the CD spectra of six peptides studied here are compared in Figure 3. The order of their propensity to form a β -structure was found to be FEKO > G8K > M10K, G12K > V9K > F11K, which reflects their affinity towards ssDNA.

Figure 4 shows the results of cross-linking experiment. DIG-labeled DNA (A*) was incubated with 160 μ M of peptide in PBS buffer containing 1 mM MgCl₂. The resultant Schiff bases were reduced by NaBH₃CN and analyzed on 10%

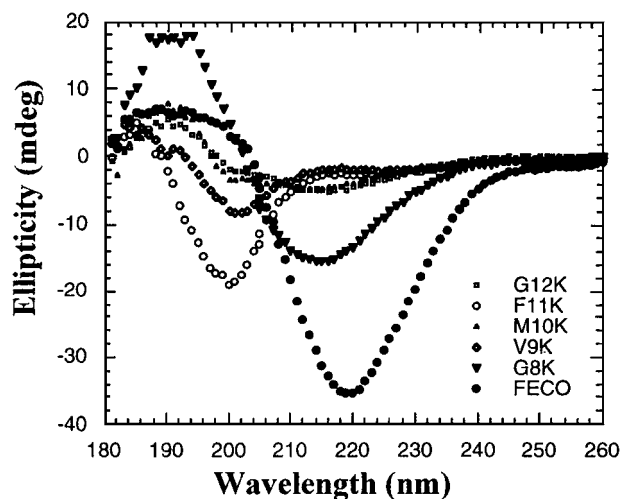


Figure 3. CD spectra of mutant peptides (300 μ M) in the presence of (dT)₂₁ (15 μ M). CD spectra were measured in 10 mM sodium phosphate buffer at pH 7.4.

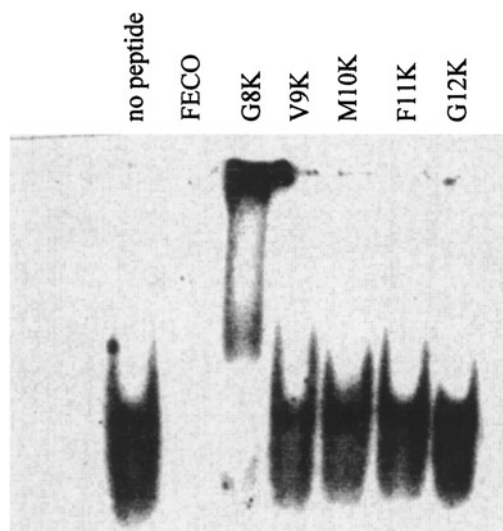


Figure 4. Gel mobility shift assay of chemical cross-linking. DIG-labeled DNA A* was incubated with 160 μM of each peptide for 30 min at room temperature in 137 mM NaCl, 8.1 mM Na_2HPO_4 , 2.68 mM KCl, 1.47 mM KH_2PO_4 , 1 mM MgCl_2 , (pH 7.4), 0.1% IGEPAL CA-630, 10% glycerol, followed by reduction with NaBH_3CN .

polyacrylamide gel electrophoresis. The reaction with FECO resulted in the complete absence of A* on the gel, indicating that the cross-linking reaction efficiently proceeded and caused the formation of insoluble aggregates in the reaction solution. G8K also cross-linked with A*, affording the high molecular weight molecules. Other four peptides did not give any detectable cross-linked product.

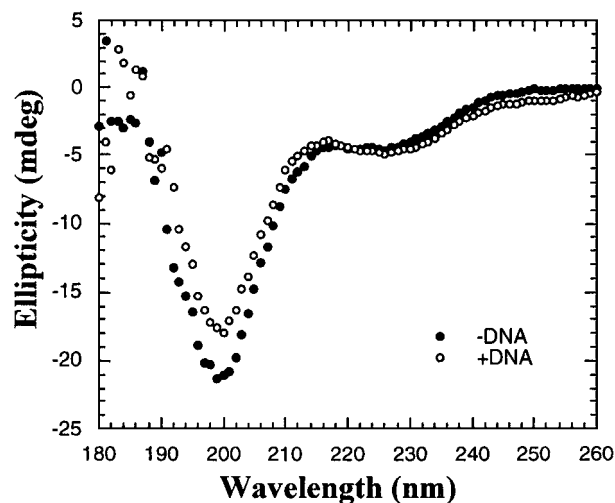


Figure 5. CD spectra of G8^dK(300 μM) in the presence and absence of $(\text{dT})_{21}$ (15 μM). CD spectra were measured in 10 mM sodium phosphate buffer at pH 7.4.



These results are consistent with those of CD, indicating that cross-linking through a Schiff base formation proceeded efficiently only when the peptides bound to ssDNA.

Interestingly Gly8 is totally conserved among 64 eubacterial RecAs. Since Gly has no side chain, we became interested in the effect of stereochemistry of amino acids at the 8th position on ssDNA-binding. Thus, we synthesized the peptide G8^dK in which *L*-Lys of G8K was replaced with *D*-Lys. CD spectrum (Fig. 5) has shown that the addition of ssDNA did not induce any conformational change in G8^dK, indicating the complete loss of DNA binding ability. The stereochemistry of Lys is rigorously limited to *L*-configuration.

In summary, the peptides which bound to ssDNA strongly and formed cross-links efficiently have the *L*-Lys at the 6th or 8th position from the N-terminus. These results indicate that the 20 amino acid residue peptides directly contact with ssDNA at least at the 6th and 8th positions. These findings suggest that further modification might be feasible for the 8th position. This is the first example of chemical cross-linking of DNA-oligopeptide complex through a Schiff base formation with the 5-formyl group on uracil.

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