Interaction of Synthetic J Protein of Bacteriophage φK with Its Circular Single-Stranded DNA

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The interaction of the chemically synthesized DNA-binding protein J (SynJ) of bacteriophage φK with its single stranded circular φK DNA (css-DNA) was examined by circular dichroism and fluorescence methods. A strong cooperative binding between the synJ and the css-DNA was observed in 20 mmol dm^{-3} Tris-HCl buffer (pH 7.3) with 200 mM NaCl. While an electrostatic simple binding was observed in the buffer without NaCl.

Small icosahedral bacteriophages φK, α3, G4, and φX174 have highly basic small proteins (J proteins, 24-37 amino acids long) binding their single-stranded (ss) DNA.[1-4] The function of the J proteins has been studied in vivo and vitro.[2,5-7] These J proteins have been shown to be essential for DNA packaging in phage morphogenesis. However, no detailed information on the mode of interaction between J protein and DNA has been obtained, except for the result that the φX174 J protein bound to both ss- and ds-DNAs.[8] Recently, Kodaira *et al.* showed that synthetic φK J protein tightly bound to the circular ss-DNA to form a compact complex in 50 mM (1M = 1 mol dm^{-3}) Tris-HCl buffer using gel retardation assay.[1] However, the gel retardation assay gave no detailed information of the structure of the J protein/DNA complex and a non-specific electrostatic interaction between J protein cations and DNA phosphate anions would involve in the tight binding observed under such low salt conditions. Thus the interaction mode of the J proteins with DNA is still unknown. As the amino acid sequences of the J proteins are highly conserved among the above four ss-DNA phages, the φK and α3 J proteins are thought to be smallest and intact DNA-binding proteins which have characteristic common feature of all four J proteins. Therefore, for better understanding of the J protein function, the smallest J protein (SynJ, KKARRSPSRRKGARLWYVGGSQF) was synthesized and the interaction mode with the circular ss-DNA of φK bacteriophage (css-DNA) was examined by circular dichroism (CD) and fluorescence methods.

The SynJ corresponding to φK and α3 J proteins without the N-terminal methionine was selected because it is demonstrated that φX174 J protein has no N-terminal methionine.[9] The SynJ was synthesized by an Applied Biosystem Peptide Synthesizer, Model 430A, using the Pam resin on Boc chemistry. After HF treatment of the peptide-resin, purification of the SynJ was done by reverse-phase HPLC using 0.1% TFA-CH_3CN solvent system. The final product was established by FAB-MS and the peptide content was determined by amino acid analysis. The css-DNA was supplied from Dr. Kodaira (Toyama University). Two kinds of 50 mM Tris-HCl buffers (pH 7.3) with 200 mM NaCl and without NaCl were used because the ionic strength in E. coli cells was found to be equivalent to about 200 mM NaCl.[10]

The effect of the SynJ on the structures of the css-DNA was examined by CD spectroscopy. The CD spectral change upon titration of the css-DNA with the SynJ in 50 mM Tris-HCl (pH 7.3) with 200 mM NaCl was observed as shown in Figure 1. Under these conditions, the SynJ took a random structure without characteristic CD absorption band in the range of 220-320 nm

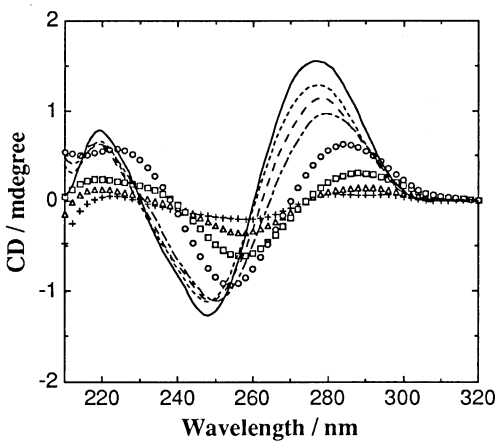

Figure 1. CD titration of css-DNA with SynJ in 50 mM Tris-HCl (pH 7.3) with 200 mM NaCl. R is the molar ratio of nucleotide/SynJ. The measurement was done using a JASCO J-720 spectropolarimeter. The nucleotide concentration range was 117-101 μM. 1/R: (——) 0, (-----) 0.039, (– – –) 0.065, (— — —) 0.079, (○) 0.092, (□) 0.105, (△) 0.118, (+) 0.144.

(data not shown). As the SynJ concentration increased, a minimum (248 nm) and two maxima (219 and 277 nm) became more positive and negative, respectively. In addition, these minimum and maxima were shifted to longer wavelengths. A small change was observed in the range of 1/R 0-0.065 (R is the molar ratio of nucleotide/SynJ) while relatively significant change in the range of 1/R 0.065-0.118. Above 1/R 0.144, these maxima and minimum almost disappeared, indicating that css-DNA might be saturated at this 1/R ratio 0.144. On the contrary to this, a different CD change was observed in the buffer without NaCl (Figure 2). As the SynJ concentration increased, these minimum and maximum (249 and 275 nm) observed for the css-DNA became more positive and negative in the range of 1/R 0-0.079, respectively. Then the SynJ/css-DNA complex showed a minimum at 258 nm above 1/R ratio 0.092. Interestingly, the inversion and disappearance of the minima and maxima in the CD spectra were observed under the both conditions with 200 mM NaCl and without NaCl. It is known that the transition of double helical DNA from B- and A-forms to Z-form induced by ions, solvents, and small molecules is accompanied by a quasi inversion of the CD bands.[11] Therefore, this phenomenon may arise from a partial formation of Z-form like paired bases and be characteristic of the condensation of the css-DNA.

As the fluorescence of the SynJ (Trp and Tyr) was quenched upon addition of the css-DNA, the binding capability of the SynJ to the css-DNA was proved by fluorescence titration with increasing amounts of SynJ in the buffers with 200 mM NaCl and without NaCl. The fluorescence intensity increases linearly upon addition of the SynJ up to 1/R 0.052 in the no NaCl buffer as shown in Figure 3B. The quenching ratio in this range was estimated below 20% by comparison to free SynJ (data not

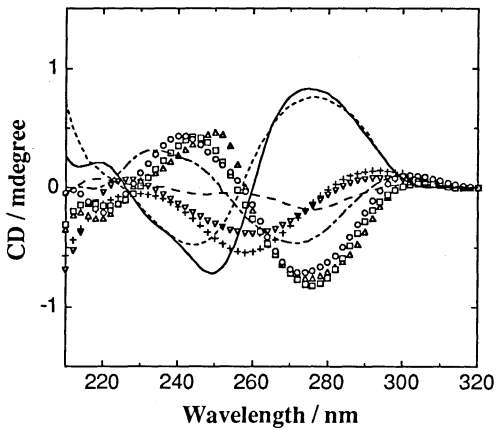

Figure 2. CD titration of css-DNA with SynJ in 50 mM Tris-HCl (pH 7.3). The mesurements were performed as shown in Fig. 1. 1/R: (——) 0, (-----) 0.013, (– – –) 0.026, (— — —) 0.039, (○) 0.052, (□) 0.065, (△) 0.079, (+) 0.092, (▽) 0.105.

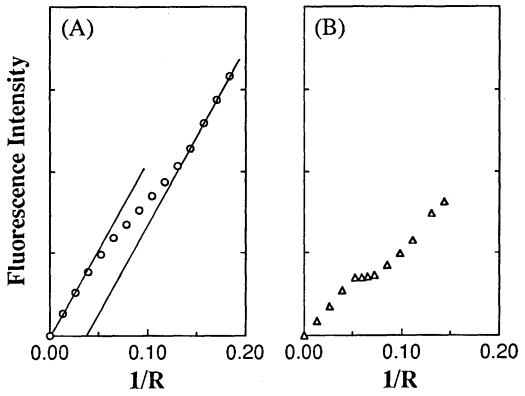

Figure 3. Fluorescence titrations of css-DNA with SynJ in 50 mM Tris-HCl (pH 7.3) containing 200 mM NaCl (A) and no NaCl (B), respectively. The nucleotide concentration range was 1.0-0.90 μM. The fluorescence intensities at 345 nm were monitored by exitation at 280 nm using a Hitachi F-3010 fluorescence spectrophotometer.

shown). In the range of 1/R 0.052-0.083, the fluorescence intensity was kept constant. This complete quenching suggested that a strong binding of the SynJ to the css-DNA or other secondary effect of the SynJ such as SynJ-SynJ interaction might occur. Above 1/R ratio 0.118, the given fluorescence intensity points were identical with that for increasing free SynJ, indicating that the css-DNA was saturated with the SynJ. On the contrary, a different titration curve was obtained in the buffer with 200 mM NaCl as shown in Figure 3A. In both 1/R ranges 0-0.039 and above 0.139, the given fluorescence intensity points seemed to be on the two parallel straight lines. These lines were identical with that for increasing free SynJ (data not shown). This result indicates that no binding occurs in these 1/R ranges and the css-DNA can be saturated with SynJ at 1/R 0.139. Around 1/R 0.039, the formation of the SynJ/css-DNA complex began because the points were deviated from initial sraight line showing increase of free SynJ. This titration curve is very similar to that for the gene 5 protein of bacteriophage M13 against poly(dA) in a buffer with 200 mM NaCl,[12] showing cooperative and strong binding between SynJ and css-DNA. It was deduced that one SynJ covered 12 nucleotides in the SynJ/css-DNA complex in accordance with the calculation method by Alma *et al.*[12,13] While in the no NaCl buffer, a cooperative binding of the SynJ to css-DNA may also occur. The mode of this interaction is complicated because a css-DNA condensation due to the reducing phosphate-phosphate repulsive interaction in DNA backbone should also occur in addition to the SynJ-SynJ interaction. Thus the number of nucleotides covered by one SynJ could not be determined under no NaCl conditions.

In conclusion, the binding of the SynJ to the φK css-DNA was found to be influenced by salt concentration. Under low salt conditions, the SynJ may electrostatically and cooperatively interact with css-DNA as seen in polycation-DNA interaction. While in the buffer with 200 mM NaCl, a non-electrostatic and strongly cooperative interaction occurred and gave rise to the structural change of the css-DNA. The cooperative and non-electrostatic binding may result from a stacking of the Trp and/or Tyr residues in the SynJ into the DNA bases and a hydrophobic SynJ-SynJ interaction. It was found that one φX174 phage has 60 J proteins in the phage particle.[9] From our results, however, about 500 SynJ would bind to css-DNA when saturated. It is still unknown whether the J protein/css-DNA complex become compact form in the presence of NaCl and how the J proteins participate in the DNA packaging. An electron microscopic study should be required. For the better understanding the J protein function, detailed binding studies using synthetic J protein analogs and polynucleotides must be usefull.

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13 The fluorescence intensity after complete saturation of the css-DNA with the SynJ can be written as:
$$F = [SynJ]_t F_{free} - Q_{max} F_{free} [Nucl]_t / n.$$
where n is the number of nucleotides covered by one SynJ; $[SynJ]_t$ and $[Nucl]_t$ are the total concentrations of the SynJ and mononucleotide in the css-DNA, respectively; F_{free} is the fluorescence intensity/mol free SynJ; Q_{max} is the fraction of the SynJ fluorescence quenched upon binding. Extrapolation of this line to the situation where F = 0 gives:
$$Q_{max}/n = [SynJ]_t/[Nucl]_t.$$
Therefore, the ratio Q_{max}/n can be obtained directly from the titration curve and the n value was determined using the Q_{max} value (0.41) obtained from other reverse titration experiment.