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# B–Z transition of poly(dG-m$^5$dC) induced by binding of Lys-containing peptides

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Effects of oligopeptides containing Lys residues on the conformation of poly(dG-m$^5$dC) have been investigated by circular dichroism spectroscopy. Lys-Ala-Lys (KAK) and its longer analogs with Lys-Ala repeats are found to convert the B-form polynucleotide to the Z form very efficiently. The ability to induce the B–Z transition is characteristic of alternating Lys-Ala sequences and increases exponentially with increasing number of the repeats. The heptapeptide KAKAKAK has an ability comparable with that of spermine, one of the most effective inducers hitherto known. The present results provide the fist example of the B–Z transition of poly(dG-m$^5$dC) induced by peptide binding.

B–Z transition; DNA; Poly(dG-m$^5$dC); DNA/protein interaction; Peptide; Lysine

## 1. INTRODUCTION

One of the most drastic changes in DNA double-helical structure is the transition from the right-handed B form to the left-handed Z form. The B–Z transition of DNA was suggested by Pohl and Jovin to account for the inverted circular dichroism (CD) spectrum of double-stranded poly(dG-dC) in the presence of molar quantities of salt [1]. The structural details of Z-DNA were subsequently revealed by X-ray diffraction studies on oligo dG-dC crystals [2–4]. Z-DNA has a zig-zag arrangement of the sugar-phosphate backbone with a single deep groove in between, while the phosphate groups in usual B-DNA lie on a smooth helical line with two shallower grooves. Methylation of cytosine at position 5 greatly facilitates the formation of Z-DNA. Behe and Felsenfeld have demonstrated that double-stranded poly(dG-m$^5$dC) is readily converted from the B to the Z form by addition of a small amount of multivalent metal cations or polyamines as well as under high salt conditions [5]. The equilibrium between the B and Z conformations is poised by the subtle interplay of forces inherent in the DNA structure and arising from intermolecular interactions [6].

Whether peptides or proteins can induce the B–Z transition of DNA is an interesting problem in connection with conformational regulation of DNA by proteins and the biological role of Z-DNA. Klevan and Schumaker have reported that the complex of polyarginine and the Z form poly(dG-dC) prepared under high salt conditions remains stable against lowering the salt concentration [7]. This observation implies that particular peptides or proteins may favour Z-DNA. In this work, we have studied the effects of peptides on the conformation of poly(dG-m$^5$dC) and found that peptides composed of alternating Lys and Ala residues strongly induce the B–Z transition. This is the first report on the B–Z transition of poly(dG-m$^5$dC) caused by peptide binding.

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## 2. MATERIALS AND METHODS

Double-stranded poly(dG-m$^5$dC) sodium salt was purchased from Pharmacia and used without further purification. L-Lysine hydrochloride (K), L-lysyl-L-lysine dihydrochloride (KK) and spermine were obtained from Nacalai Tesque or Sigma. Lys-Ala-Lys (KAK), Lys-Ala-Ala-Lys (KAAK), Lys-Ala-Ala-Ala-Lys (KAAAK), Lys-Ala-Lys-Ala-Lys (KAKAK) and Lys-Ala-Lys-Ala-Lys-Ala-Lys (KAKAKAK) were synthesized on a solid phase peptide synthesizer (Pharmacia, Biolynx 4175) by using the 9-fluorenylmethoxycarbonyl method. The peptides synthesized were purified on a Jasco 880 HPLC using a reversed-phase column (Nacalai 5C18-AR) and/or a gel-filtration column (Asahi GS-320P). The final products were obtained as acetic acid salts and identified by elemental analysis and fast atom bombardment mass spectrometry.

Poly(dG-m$^5$dC) and the peptides were separately dissolved in 10 mM Tris-HCl buffer of pH 7.6 containing 10 mM NaCl. The concentration of poly(dG-m$^5$dC) (0.65–0.91 mM per nucleotide) was determined by using an extinction coefficient of 7000 $M^{-1} \cdot cm^{-1}$ at 255 nm [8]. Titration of the polynucleotide with peptide was made by stepwise addition of a few μl of peptide solution (0.7–25 mM) to the polynucleotide solution (initial volume, 200 μl) in a 1-mm quartz cell. The mixture solution was heated to 50°C for 5 min and then cooled to room temperature (21–23°C) for more than 10 min prior to recording CD spectra on a Jasco J-400X spectropolarimeter. The heat treatment was essential to achieve conformational equilibrium of the polynucleotide efficiently and after such treatment the CD spectra remained unchanged for days.

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## 3. RESULTS AND DISCUSSION

Fig. 1 shows the CD spectra of poly(dG-m$^5$dC) and its mixtures with KAK at peptide-to-nucleotide molar ratios ([P]/[N]) of 0.8, 1.0, 1.2 and 1.6. In the absence of KAK, poly(dG-m$^5$dC) is in the B form with a strong negative peak at 253 nm and a weak positive peak at 275 nm in the CD spectrum [5]. The B-form CD pattern persists at low concentrations of KAK ([P]/[N] ≲ 0.2, spectra not shown). With further addition of the peptide, the negative peak at 253 nm weakens and a new negative peak grows at 292 nm. Changes in the CD spectrum are completed at [P]/[N] = 1.6 with a strong negative peak at 292 nm and a weak positive peak around 260 nm. The final CD spectrum is almost identical to that of the Z-form poly(dG-m$^5$dC) in 3 M NaCl solution [5], demonstrating that the KAK binding converts the B form into the Z form. The appearance of an isoelliptic point at 272 nm suggests that the B–Z transition occurs directly from the B to the Z form without passing through any other stable conformations.

Since the amino groups of the Lys side chains of KAK are positively charged at neutral pH and expected to bind to the negatively charged phosphate groups of the polynucleotide, we examined the effect of amino acid Lys on the conformation of poly(dG-m$^5$dC). The CD spectrum, however, did not show any change even in the presence of 100-fold excess of Lys. Therefore, neutralization of the phosphate charge by amino group binding is not immediately responsible for the B–Z transition. Spatial arrangement of charged amino groups in the peptide is likely to be important in stabilizing the Z conformation.

Effects of spacing between Lys side chains were examined by recording the Cd spectra of poly(dG-m$^5$dC) mixed with other peptides containing two or more Lys residues. Since the molar ellipticity at 292 nm, $[\theta]_{292}$, changes from a small positive value in the B form to a large negative one in the Z form (see Fig. 1), we have employed $[\theta]_{292}$ as a measure of the B–Z transition. The $[\theta]_{292}$ values observed at several concentrations of peptide are plotted against [P]/[N] in Fig. 2. The curve of titration with KAK shows a steep fall of $[\theta]_{292}$ in a range of [P]/[N] = 0.6–1.4 with a midpont of transition at [P]/[N] = 1. Alternation of the number of Ala residues between two Lys ends drastically affects the titration curve. A dipeptide with no Ala insert, KK, does not change the conformation of poly(dG-m$^5$dC) even at [P]/[N] = 10. KAAK and KAAAK with longer Ala inserts never induce the Z form either, though a slight decrease in $[\theta]_{292}$ is seen at high peptide concentrations. The capability to induce the B–Z transition is, therefore, characteristic of the KAK sequence. The importance of the KAK arrangement is further evidenced by experiments with peptides having repeated KAK sequences. As Fig. 2 shows, KAKAK and KAKAKAK readily cause the B–Z transition with midpoints at

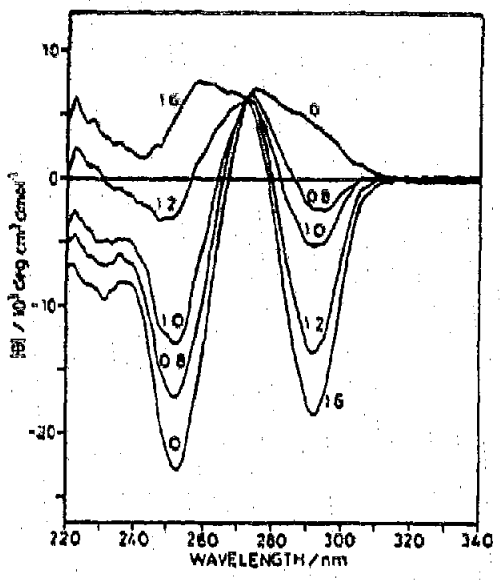


Fig. 1. CD spectra of poly(dG-m$^5$dC) and its mixtures with KAK in Tris-HCl buffer at pH 7.6 containing 10 mM NaCl. Aliquots of 16.9 mM KAK solution were added stepwise to 200 μl of 0.84 mM poly(dG-m$^5$dC) solution in a 1-mm cell. The number beside each spectrum indicates the corresponding peptide/nucleotide molar ratio. The intensity is converted into the molar ellipticity ($\theta$) per nucleotide.

[P]/[N] = 0.25 and 0.06, respectively. Addition of one AK unit makes the peptide about four times more effective in inducing the B–Z transition and the midpoints correspond to 2, 0.75 and 0.24 Lys side chains per nucleotide phosphate in the cases of KAK, KAKAK and KAKAKAK, respectively.

While the B–Z transition of poly(dG-m$^5$dC) has been reported to be induced by a small amount of metal cations [5] or positively charged polyamines [5,9–11], our results provide the first example of the transition induced by peptides. In order to compare the Z-inducing ability of the peptides with that of spermine, which is

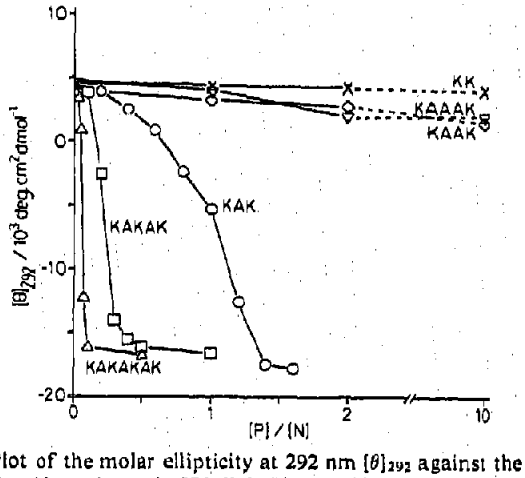


Fig. 2. Plot of the molar ellipticity at 292 nm $[\theta]_{292}$ against the peptide/nucleotide molar ratio [P]/[N]. The peptide added is KK, KAK, KAAK, KAAAK, KAKAK or KAKAKAK as indicated.

one of the most effective inducers of the B–Z transition [5], we titrated poly(dG-m$^5$dC) with spermine under the same experimental conditions. The midpoint [P]/[N] was found to be 0.03, which is of the order of that for KAKAKAK. This observation clearly shows that peptides with alternating Lys-Ala sequences belong to the class of the strongest inducers of the B–Z transition of poly(dG-m$^5$dC).

The dependence of the Z-inducing ability on the amino acid sequence of the peptide found here may be understood as follows: The spatial distribution of positively charged Lys amino groups in a stable conformer of KAK fits in with the zig-zag arrangement of the negatively charged phosphates in the Z-form poly(dG-m$^5$dC) but not with that in the B form. The selective binding to the Z form by electrostatic interaction reduces the energy of the complex and displaces the B–Z equilibrium in favour of the Z form. In contrast, KK, KAAK and KAAAK are unable to take such a Z-stabilizing conformation owing to shorter (KK) or longer (KAAK and KAAAK) separation between two Lys residues. The preferential binding of KAK to the Z form is exponentially enhanced by addition of AK units as found for KAKAK and KAKAKAK. This is probably because these peptides of the KAK family take a regular conformation and Lys side chains contribute to the binding cooperatively. Alanine, which has a small and neutral side chain, must be one of the good spacers for adjacent Lys residues to have a conformational freedom. Peptides with sequences other than KAK may also produce the Z-specific charge distribution. Studies are in progress to disclose more effective peptides that induce the B–Z transition of poly(dG-m$^5$dC).

In conclusion, we have demonstrated that oligopeptides composed of alternating Lys and Ala residues strongly induce the B–Z transition of poly(dG-m$^5$dC). This finding is partly analogous to the previous observation that the Z form of non-methylated poly(dG-dC) is stabilized by binding to polyarginine [7]. Such effects of peptides on the polynucleotide conformation may be related to a certain aspect of DNA/protein interaction. The B–Z transition of poly(dG-m$^5$dC) induced by oligopeptide binding is of particular interest in connection with the structural modulation and transcriptional regulation of DNA by proteins, because oligopeptides serve as models for interaction sites of proteins and the dG-m$^5$dC base sequences are frequently found in eukaryotic cells as an inhibition factor of gene transcription [12]. A run of 13 alternating Lys and Gly residues has been identified recently in the amino acid sequence of a mammalian DNA (cytosine-5)-methyltransferase [13], though the functional role of the long repeated Lys-Gly portion remains to be clarified.

## REFERENCES

[1] Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375–396.
[2] Wang, A.H.-J., Quigley, G.J., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G. and Rich, A. (1979) Nature 282, 680–686.
[3] Drew, H., Takano, T., Tanaka, S., Itakura, K. and Dickerson, R.E. (1980) Nature 286, 567–573.
[4] Crawford, J.L., Kolpak, F.J., Wang, A.H.-J., Quigley, G.J., van Boom, J.H., van der Marel, G. and Rich, A. (1980) Proc. Natl. Acad. Sci. USA 77, 4016–4020.
[5] Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sci. USA 78, 1619–1623.
[6] Jovin, T.M., Soumpasis, D.M. and McIntosh, L.P. (1987) Annu. Rev. Phys. Chem. 38, 521–560.
[7] Klevan, L. and Schumaker, V.N. (1982) Nucleic Acids Res. 10, 6809–6817.
[8] Gill, J.E., Mazrimas, J.A. and Bishop Jr, C.C. (1974) Biochim. Biophys. Acta 335, 330–348.
[9] Thomas, T.J., Bloomfield, V.A. and Canellakis, Z.N. (1985) Biopolymers 24, 725–729.
[10] Vertino, P.M., Bergeron, R.J., Cavanaugh Jr, P.F. and Porter, C.W. (1987) Biopolymers 26, 691–703.
[11] Thomas, T.J. and Messner, R.P. (1988) J. Mol. Biol. 201, 463–467.
[12] Doerfler, W., Toth, M., Kochanek, S., Achten, S., Freisem-Rabien, U., Behn-Krappa, A. and Orend, G. (1990) FEBS Lett. 268, 329–333.
[13] Bestor, T., Laudona, A., Mattaliano, R. and Ingram, V. (1988) J. Mol. Biol. 203, 971–983.