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Specific Recognition of Unusual DNA Structures by Small Molecules: An Equilibrium Binding Study

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Abstract: The binding interaction of ethidium to a series of synthetic deoxyoligonucleotides containing a B-Z junction between left-handed Z-DNA and right-handed B-DNA, was studied. The series of deoxyoligonucleotides was designed so as to vary a dinucleotide step immediately adjacent to a B-Z junction region. Ethidium binds to the right-handed DNA forms and hybrid B-Z forms which contain a B-Z junction, in a highly cooperative manner. In a series of deoxyoligonucleotides, the binding affinity of ethidium with DNA forms which were initially hybrid B-Z forms shows over an order of magnitude higher than that with any other DNA forms, which were entirely in B-form DNA. The cooperativity of binding isotherms were described by an allosteric binding model and by a neighbor exclusion model. The binding data were statistically compared for two models. The conformation of allosterically converted DNA forms under binding with ethidium is found to be different from that of the initial B-form DNA as examined by CD spectra. The ratio of the binding constant was interestingly correlated to the free energy of base unstacking and the conformational conversion of the dinucleotide. The more the base stacking of the dinucleotide is unstable, or the harder the conversion of B to A conformation, the higher the ratio of the binding constant of ethidium with the allosterically converted DNA forms and with the initial B-Z hybrid forms. DNA sequence around a B-Z junction region affects the binding affinity of ethidium. The results in this study demonstrate that ethidium could preferentially interact with unusual DNA structures.
Key words: allosteric DNA, binding interactions, B-Z junction, CD characteristics, sequence effect.

DNA has been considered to be conformationally flexible, a dynamic structure in which different conformations are in equilibrium with each other (Rich *et al.*, 1984; Jovin *et al.*, 1987). DNA conformations might play an important role in regulating gene expression. The potential existence of Z-DNA is one of the most extreme cases of a sequence-dependent conformational change. Z-DNA has been found *in vivo* (Nordheim *et al.*, 1981; Wittig *et al.*, 1989) and recently, potential Z-DNA forming sequences have been mapped in human genes (Schroth *et al.*, 1992). There are other possible examples of unusual DNA structures (Wells, 1988): cruciforms, triple-helices, bent DNA, bulges, base-pair mismatches, and junction regions between different DNA conformations. These structures may be recognized by proteins or antibiotics. In the recognition process, unwinding or unstacking of the DNA double strand helix may occur. In such cases, the disruption of stacked base pairs lowers the energy barrier at the binding sites in the event of binding interaction.

Since an unusual structure, the B-Z junction, is a local region of structurally distorted DNA, it has been thought to provide a target for drug binding (Walker *et al.*, 1985). Recently, favorable binding of ethidium to the B-Z junction has been reported (Suh *et al.*, 1991; Chaires *et al.*, 1992; Suh, 1993). Reviews of the biological chemical properties of B-Z junctions between right-handed B-DNA and left-handed Z-DNA have also been published (Jovin *et al.*, 1987; Klysik *et al.*, 1981). Recently, several properties of a newly synthesized oligomer containing a B-Z junction, BZ-1, have been demonstrated (Sheardy, 1988; Doktycz *et al.*, 1990; Winkle and Sheardy, 1990; Guo *et al.*, 1991; Lu *et al.*, 1992; Sheardy *et al.*, 1993, 1994). However, the exact nature of a B-Z junction has not yet been fully described. Several important characteristics of a B-Z junction can be described as follows. First, the length of a B-Z junction region was found to be about 3 base pairs (bps) long, by examining short synthetic oligomers (Sheardy and Winkle, 1989; Dai *et al.*, 1989).

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Second, formation of the junction was energetically unfavorable, presumably because of the base unstacking of the double helix, since it was found that the junction contained at least one distorted base pair (Sheardy and Winkle, 1989). This feature may be related to the transient single-strandedness of the junction. Third, junctions are sequence dependent but their length may not be dependent on the base sequence (Dai *et al.*, 1989). In this study, a series of deoxyoligonucleotides were designed and synthesized by changing dinucleotide steps immediately adjacent to the B-Z junction region. They are referred to as BZ-2, BZ-5, and BZ-6, based on the information about the location of the junction region at BZ-1 (Suh *et al.*, 1992). The purpose of this study is to test the sequence effect on the binding interaction of ethidium and to monitor the conformational transitions by the binding interaction of ethidium. In this report, it is demonstarted that the flanking sequences affect significantly the binding of ethidium to deoxyoligonucleotides containing a B-Z junction. Binding isotherms of these oligonucleotides show a positive cooperativity. Ethidium binds tighter to the allosterically converted DNA forms in high salt, which initially favors the hybrid B-Z forms over other DNA forms. These results demonstrated that ethidium could preferentially interact with a specific conformation in unusual DNA structures accompanied by allosteric changes of DNA conformation.

Materials and Methods

Materials

Deoxyoligonucleotides, which are designated BZ-1, BZ-2, BZ-5, and BZ-6, are synthesized on an Applied Biosystems 380B DNA Synthesizer using the phosphoramidite method (Caruthers, 1991) and purified by trityl-selective reverse-phase HPLC, as previously described (Sheardy, 1988). Each strand of linear DNA oligomers is mixed at equal concentration, heated up to 90℃ and then slowly cooled down to 20℃, in order to anneal non- and self-complementary sequences. The other two oligomers, which are designated CG-8 and AT-8, and used as reference sequences, are purchased from Clonetech (Clonetech Laboratories, Inc.; Palo Alto, USA) and from American Synthesis (American Synthesis Inc.; Pleasanton, USA), respectively. Ethidium was purchased from Sigma (Sigma Chemical Co.; St. Louis, USA). Ethidium concentrations were determined by absorbance, at 480 nm assuming $\varepsilon=5{,}600\ M^{-1}cm^{-1}$. Experiments were performed in BPE buffer (low-salt) containing 6 mM Na_2HPO_4, 2 mM NaH_2PO_4, and 1 mM EDTA, pH 7.0. The BPE buffer plus 4.5 M NaCl (high-salt) was prepared by adding stock NaCl solution to the BPE buffer up to 4.5 M NaCl.

Spectral measurement

Absorbance measurements were performed with a Cary-219 spectrophotometer (Varian Co.; Palo Alto, USA) equipped and controlled by a Gateway personal computer. Circular dichroism was measured with a JASCO J-500A spectropolarimeter, interfaced to and controlled by an IBM PS/2 computer.

Fluorescence titrations

Ethidium binding was monitored by steady-state fluorescence measurements on a Perkin-Elmer 650-40 spectrofluorometer, with $\lambda=525$ nm for excitation, and $\lambda=605$ nm for emission. Titrations were conducted at 20℃ with 400 ml samples at a concentration of 38 μM (bps). The bound ethidium concentration (C_b) was calculated from the relation

$$C_b=C_t\ (I-I_o)/(V-1)I_o$$

where C_t is the total ethidium concentration, I is the observed fluorescence intensity, I_o is the fluorescence intensity of the identical concentration of ethidium in the absence of DNA, and V is the experimental coefficient determined in BPE buffer and in BPE plus 4.5 M NaCl. Free ethidium concentrations (C_f) were obtained from the conservation relationship, $C_f=C_t-C_b$. The binding ratio r is defined as $r=C_b/[DNA]$.

Results

Non-junction forming hexadecadeoxynucleotides

Two non-junction forming deoxyoligonucleotides were designed and synthesized as reference sequences. Fig. 1 shows the deoxyoligonucleotide which is designated CG-8 and contains alternating $(5'\text{-methyl-dCdG})_8$, while the other deoxyoligonucleotide AT-8 contains $(dAdT)_8$, which cannot be converted to Z-DNA from B-DNA. The conformational changes of CG-8 and AT-8 in low and high salt, respectively, were monitored by circular dichroism. It was found that both CG-8 and AT-8 in low salt were in the B-form DNA, even though the detailed characteristics of the CD spectra is different. The positive peak appeared at 280 nm for CG-8, but appeared at around 270 nm for AT-8. The negative peaks are at 250 nm with different magnitude at the same concentration of DNA. In high salt, their behaviours are interestingly different. The 16-mer CG-8 shows an inverted CD spectrum referring to the Z conformation as expected, shown in Fig. 2(a). The CD spectrum of AT-8 in high salt seems to remain in a B-form like DNA, which revealed a diminishment in the positive peak in 260~300 nm. The negative peak

BZ-1 : 5'-CGCGCGCGA*C*TGACTG
3'-GCGCGCGC**T***G*ACTGAC

BZ-2 : 5'-CGCGCGCGA*TC*GACTG
3'-GCGCGCGC**T***AG*CTGAC

BZ-5 : 5'-CGCGCGCGA*TT*GACTG
3'-GCGCGCGC**T***AA*CTGAC

BZ-6 : 5'-CGCGCGCGA*CC*GACTG
3'-GCGCGCGC**T***GG*CTGAC

CG-8 :5'-CGCGCGCGCGCGCGCG
3'-GCGCGCGCGCGCGCGC

AT-8 : 5'-CATGCATGCATGCATG
3'-GTACGTACGTACGTAC

Fig. 1. Hexadecadeoxynucleotides used in these studies. Italic letters represent the consecutive dinucleotides, at or nearby, at the B-Z junction region in BPE buffer at 4.5 M NaCl. Initial C's in bold style are 5'-methyl-deoxycytosines.

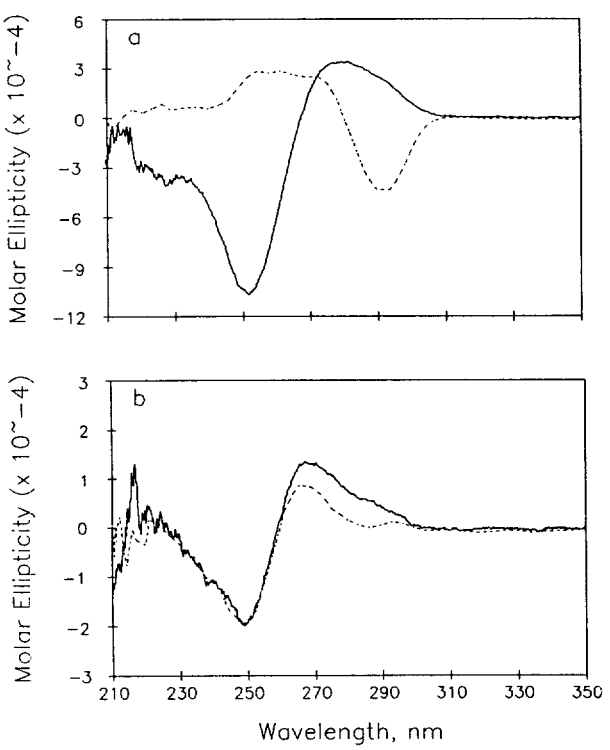

Fig. 2. Circular dichroism spectra of hexadecanucleotides in BPE buffer and in BPE buffer at 4.5 M NaCl. (a) CG-8, (b) AT-8. The solid lines show CD spectra of B form DNA in low-salt and the dashed lines show CD spectra of different forms in high-salt.

in 210~260 nm falls on the same region of the CD spectrum in low salt, shown in Fig. 2(b). By the comparison of the CD spectra in Fig. 2(b), the DNA conformations of AT-8 at the two NaCl concentrations seemed to be different. However, the conformation of AT-8 in high salt seems still to be a non-Z form DNA, which will be discussed later.

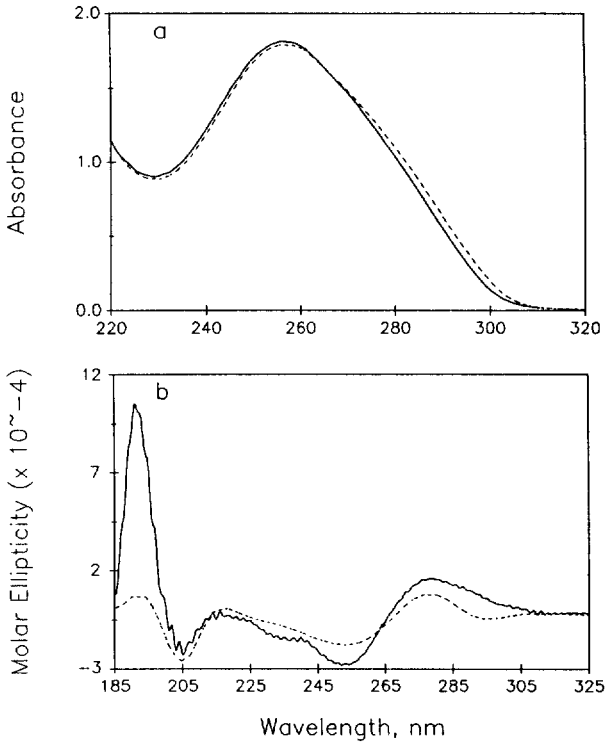

Fig. 3. UV absorption spectra and CD spectra of BZ-1, as an example, in BPE buffer and in BPE buffer at 4.5 M NaCl. The solid lines represent spectra of B form DNA in low salt and the dashed lines represent spectra of the hybrid form DNA containing a B-Z junction in high salt. (a) UV absorption spectra. There is a little red-shift and the biggest difference of absorbance between two spectra is appeared at 295 nm. (b) CD spectra. The minimum of a trough, so called, is appeared at ~295 nm and there is a positive peak at ~195 nm in high salt which can be contrasted to that peak of B form DNA in low salt.

The spectral characteristics of DNA containing a B-Z junction

The techniques for the measuring of the B to Z conformational transition have been reviewed elsewhere (Rich, 1984). The characteristics of the u.v. absorption spectra of deoxyoligonucleotide containing a B-Z junction has not yet been demonstrated, even though it shows unusual optical properties. In solution of low NaCl concentration, BZ-1, as an example, remains in the B conformation, but in high NaCl concentration, BZ-1 undergoes a conformational transition from a right-handed B form to a hybrid DNA form containing a B-Z junction between a left-handed Z-DNA and a right-handed B-DNA. This conformational transition is accompanied by a change in the u.v. absorbance spectrum as shown in Fig. 3(a). Unlike the u.v. spectra of

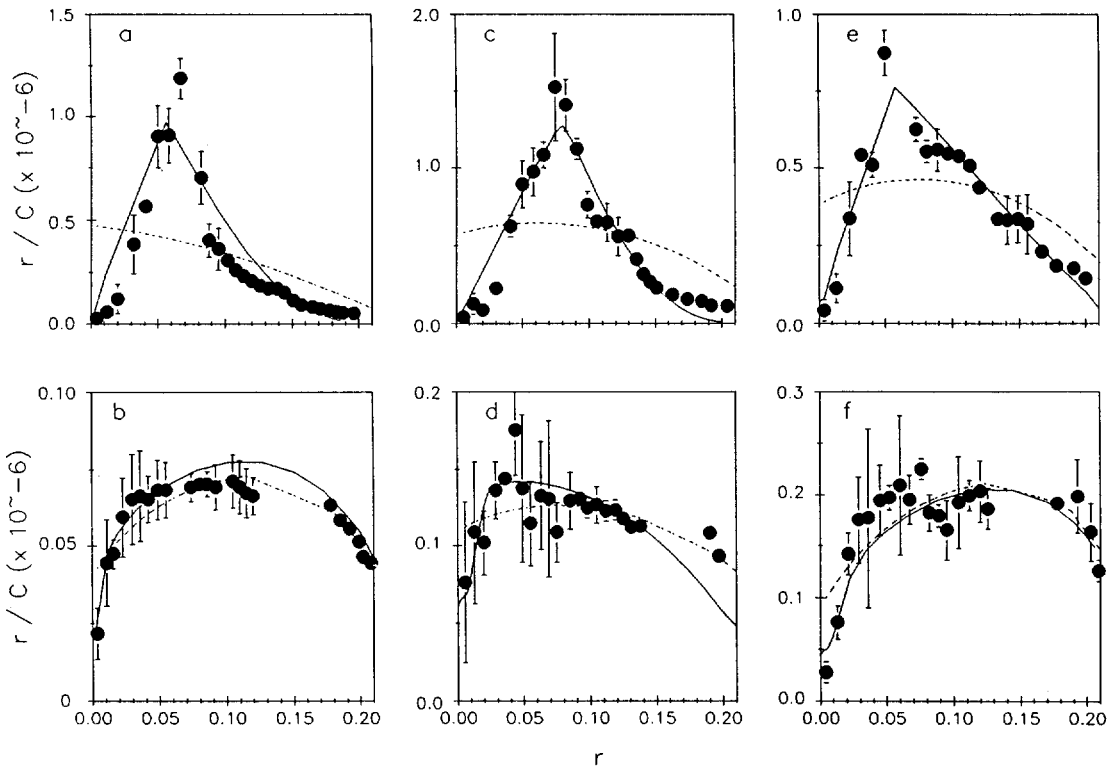

Fig. 4. Binding of ethidium to BZ-2 BZ-5 and BZ-6 at two NaCl concentrations. (a) BZ-2, (c) BZ-5, (e) BZ-6, in BPE buffer at 4.5 M NaCl, respectively. (b) BZ-2, (d) BZ-5, (f) BZ-6, in BPE buffer, respectively. Data are represented as symbols in the form of Scatchard plots where r is the binding ratio and C is the free ethidium concentration. The dashed lines are the least-squares fits of data to the neighbor exclusion model and the solid lines are the calculated curves using an allosteric model.

the B to Z transition (Behe and Felsenfeld, 1981), there is little red-shift and a smaller difference of absorbance at 295 nm between a B-form and a hybrid DNA. The ratio of the absorbance A_{260}/A_{295} changes from 5.6 in low salt to 4.4 in high salt. This ratio contrasts with that for a B to Z transition, which varies from 8.5 at low salt to 3.2 at high salt concentration (Pohl and Jovin, 1972). The result of u.v. absorption spectrum measurements may not be suitable to determine the transition of B form to hybrid B-Z form. CD spectra of BZ-1 were measured for the B to B-Z transition in low and in high salt condition, shown as an example in Fig. 3(b). The CD spectrum of BZ-1 in low salt is similar to that of the B form but the spectrum in high salt is a slightly inverted shape which can represent that of a hybrid B-Z form (Suh *et al.*, 1991). The CD spectra of the BZ series of DNA look like the combined spectra of AT-8 and CG-8 in high salt, except for the region below 210 nm. Especially, the region between 270~310 nm is characteristic of CD bands of hybrid B-Z DNA containing a B-Z junction, which are, so called, a trough. Here, it may be emphasized that the CD band below 200 nm is considerably more intense in terms of the magnitude and the shape (Johnson, 1978). Both the magnitude and shape at about 195 nm is strikingly different for low salt and high salt concentrations. The CD band shows a higher intensity in low salt than in high salt at ~195 nm even though it shows a positive peak at ~195 nm in contrast to the negative peak of Z-DNA at ~195 nm (Sutherland *et al.*, 1981).

Ethidium binding to a series of deoxyoligonucleotides

The binding of ethidium to deoxyoligonucleotides was examined by a steady state fluorescence titration. The titration has been done at fixed DNA concentration with increasing amounts of ethidium concentrations. As we reported before (Suh *et al.*, 1991), fluorescence was strongly enhanced when ethidium binds to DNA. The binding data are represented in the form of a Scatchard plot by calculating the free and the bound ethidium concentrations as shown in Fig. 4. Binding data are represented as symbols which are the average values of 2 or 3 sets of independent titrations. The dashed lines are the least-squares fits to the cooperative neighbor exclusion model and the solid lines are the calculated curves using an allosteric model. There are 10-fold differences of scale on the y-axis for both panels in each row. Binding isotherms show positive slopes which indicate cooperative binding in

a positive manner. For both panels, there is a 10-fold difference of scale in the y-axis for showing the binding isotherm in BPE buffer at 4.5 M NaCl. The initial positive slope of these plots indicates the cooperative binding in a positive manner. The binding data were also produced independently by using the "model-free analysis" method for fluorescence titration data. The results and the details of our approach have already been reported (Chaires *et al.*, 1992). The data obtained by this method agreed with the data obtained by titration at fixed DNA concentration, particularly in the initial rising regions of the binding isotherms. The similar results obtained by two independent methods clearly indicated the cooperativity of these binding isotherms. The cooperative binding isotherms were tentatively described by the extended system of the neighbor exclusion model (McGhee and von Hippel, 1974). For the binding data of the hybrid B-Z form in high salt, the neighbor exclusion model does not well describe these binding isotherms, even though this model is designed for a cooperative binding systems. Particularly, the initial regions of binding isotherms dramatically deviated from the calculated lines of the neighbor exclusion model as shown in Fig. 4 (a), (c) & (e). An allosteric binding model then could be used for the quantitative analysis of binding data as described before (Dattagupta *et al.*, 1980; Chaires, 1986). The application of an allosteric binding model to BZ-1 has been reported before (Suh *et al.*, 1991). For a simple explanation for this model, there are two different forms of DNA in equilibrium during the conformational transition. This equilibrium requires two equilibrium constants which are the constant s for a nucleation step and the constant s for a propagation step. Ligands may bind to each DNA form with neighbor exclusion parameters K_i, n_i, and w_i, where $i=1$ or 2, representing each DNA form, respectively. The solid lines are the calculated curves by an allosteric model shown in Fig. 4. Binding data are well described graphically and statistically by the calculated curves of an allosteric model. The best values of parameters described by this model are summarized in Table 2 and 3. The results of statistically fitting the binding data to an allosteric model will be discussed later. It is interesting that the ethidium binding is ~70-times tighter to the form-2 DNA in high salt, which initially favors the hybrid B-Z form, than to the B form in low salt.

In order to determine the best fitting curves statistically rather than graphically, the binding data and the corresponding points on the lines were calculated by changing one parameter each time. Among six critical parameters, actual binding data and the corresponding calculated values are digitized one by one, while other

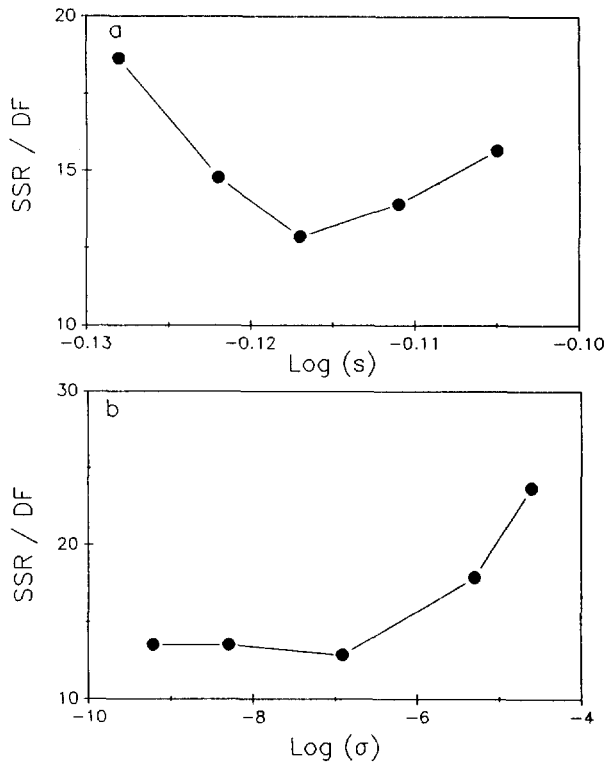

Fig. 5. The assessment of the "Goodness of Fit" for BZ-5, as an example, in BPE buffer at 4.5 M NaCl using an allosteric model. (a) The statistics of fit as a function of s which is described in the text. (b) The statistics of fit as a function of s which is described in the text. It is undefinable when is below 0.001. Passing through the minimum of the "Goodness of Fit", the statistics of fit is getting worse.

parameters are held constant, mainly because an allosteric binding model is not one of the nonlinear least-squares models. The difference between binding data and corresponding points of calculated lines are obtained as residuals and sums of residuals are calculated to estimate "the goodness of fit". The concept of "the goodness of fit" is used for a quantitative analysis of statistics of better fits, in addition to the graphical selection by superimposing the calculated curves on the data points (Motulsky and Ransnas, 1987). Goodness of fit can be assessed from the relation:

$$\text{Goodness of fit} = SS/df$$

where SS represents the sum of squares of residuals and df the degrees of freedom. The comparison of two models with a different number of parameters is less straightforward but still can be assessed statistically by performing an F-test by using the following equation

$$F=[(SS_2-SS_1)/(df_2-df_1)/(SS_1/df_1)]$$

where the subscript 1 refers to the simpler model which has fewer parameters, such as the neighbor exclusion

Table 1. Summary of the statistical analysis of interaction of ethidium with BZ-1, BZ-2, BZ-5, and BZ-6 in BPE buffer and in BPE at 4.5 M NaCl by fitting to a neighbor-exclusion model and to an allosteric binding model

	BZ-1	BZ-2	BZ-5	BZ-6
LS : F-value[a]	21.6	2.8	1.0	4.3
p[b]	0.01	0.05	0.19	0.01
HS : F-value	54.5	37.1	67.9	38.6
p	0.01	0.01	0.01	0.01

[a] F-test is performed to obtain the improved fit, statistically using following equation:

$$F=[(SS_1-SS_2)/(df_1-df_2)]/[SS_2/df_2]$$

where SS refers to sums of squares of the residuals, df refers to the number of degrees of freedom, and subscript 1 refers to the fit with a fewer parameters, which is neighbor-exclusion model in this case.

[b] P-value is obtained from F-value by consulting a standard table using (df_1-df_2) and df_2. A small p-value indicates that the more complex model (with more parameters), which is an allosteric model in this case, fits the data significantly better than the simpler model.

model, and the subscript 2 refers to the complex model which has more parameters, such as an allosteric model. A p-value is obtained from the F-value by using a standard table, which is estimated at (df_2-df_1) and df (Bevington, 1969). The assessment of goodness of fit for BZ-5 in high salt is shown, as an example, in Fig. 5. The statistics of better fitting using an allosteric model is assessed by changing s while other parameters are set constant shown in Fig. 5(a). The magnitude of goodness of fit is related to the distance between binding data and calculated values of residuals. Thus, the higher the magnitude of these, the worse the fitting of data to an allosteric model. The minimum of sums of residuals may be the best value of this parameter s for describing the binding isotherms of BZ-5 in high salt shown in Fig. 5(a). Similarly, σ was changed while other parameters were held constant. The minimum was found to be 1×10^3. It was, however, undefinable below this value, clearly shown in Fig. 5(b). The values of parameters were obtained by statistical analysis. The results of statistical comparison of the goodness of between the neighbor exclusion model and an allosteric model were summarized in Table 1. The values of parameters from the best description of BZ-DNA by an allosteric model statistically were also summarized in Table 2 and 3. By the quantitative estimation of binding data, it was found that ethidium bound much tighter to any one of BZ-1, BZ-2, BZ-5, and BZ-6 in high salt which favored initially to form the hybrid B-Z form. There seems to be a dependency of sequence effect on the binding affinity of species involved in the

Table 2. Summary of the parameter estimates for the allosteric interaction of ethidium with BZ-1, BZ-2, BZ-5, and BZ-6 in BPE at 4.5 M NaCl

	BZ-1[a]	BZ-2	BZ-5	BZ-6
s	0.890	0.925	0.890	0.950
$K_1(\times10^{-4})$	8.8	2.7	4.2	8.0
$K_2(\times10^{-6})$	2.8	1.7	3.3	1.0
ω_2	2.0	0.3	0.3	2.3
K_2/K_1	32	64	79	13

[a] Data are taken from Suh, D. (1993). The parameters are defined for an allosteric binding model (Dattagupta *et al.*, 1980). K_1 and K_2 are the binding constants for the interaction of ethidium with form-1 and form-2 DNA, respectively. w is the cooperative constant referring to ethidium binding to form-2. The parameter s is the nucleation constant for the formation of a base pair of form-2 within a stretch of form-1 DNA, and the parameter s is the propagation constant for the conversion of a base pair at a preexisting interface from form-1 to form-2. The remaining parameters for an allosteric model were found to be constant, as follows: $\omega=1.0$, $n_1=2$, $n_2=4$, and $\sigma=0.001$.

Table 3. Summary of the parameter estimates for the allosteric interaction of ethidium with BZ-1, BZ-2, BZ-5, and BZ-6 in BPE buffer

	BZ-1[a]	BZ-2	BZ-5	BZ-6
s	0.970	0.995	0.990	0.990
K_1 $(\times10^{-4})$	4.0	2.2	6.8	5.0
K_2 $(\times10^{-5})$	1.2	4.4	1.4	1.0
ω_2	13	13	6	17
K_2/K_1	3	2	2	2

[a] Data are taken from Suh, D. (1993). The parameters are defined for an allosteric model. The remaining parameters were found to be constant, as follows: $\omega_1=1.0$, $n_1=2$, $n_2=4$, and $\sigma=0.001$.

binding equilibria of ethidium, particularly in high salt, which can be expressed as the ratio K_2/K_1 as in Table 2. The binding isotherms of ethidium to deoxyoligonucleotides in low salt, which favors to form initially the B-form are shown in Fig. 4(b), (d) & (f). The corresponding values of parameters are summarized in Table 3. It can be observed that the requirement of the allosteric conversion between the two DNA forms seems to be very small based on the binding constants of the propagation process, which are close to 1, and also the binding affinity with ethidium is similar between these two forms in terms of the ratio K_2/K_1 as shown in Table 3. It should be noted that the cooperativity decreases from ~10 in low salt to ~1 in high salt. The induced CD effects in conjunction with the ligand-ligand interactions will be discussed later. They are, however, also observed for the changes of CD spectra of ethidium-saturated BZ-DNA in the range of 300~360 nm. The requirements of energy for the allosteric

conversion ot the hybrid B-Z form by ethidium in high salt is different from those of the B form in low salt because the propagation constants are smaller than those in low salt. The sequence effect on ethidium binding is discussed later on.

The characteristics of the CD spectra of deoxyoligonucleotides showing the effect of ethidium binding

The nature of DNA forms bound by ethidium is unknown. To further understand their nature, CD spectra of the conformational changes of BZ-1, as an example, showing the effect of ethidium were measured. The dependence of the bound molar ellipticity as a function of the binding ratio is shown in Figure 5. The bound molar ellipticity at 320 nm was calculated by using the following equation

$$\theta_b = [\theta]/(33\ C_b\ l)$$

where $[\theta]$ is ellipticity in degrees, C_b the bound ethidium concentration in moles and l is the path length in centimeters (Walker *et al.*, 1985). The bound ellipticity increases as the binding ratio increases to ~0.3. This induced CD band behaves in a cooperative manner under low and high salt conditions. The magnitude of bound ellipticity reaches a maximum at r-values higher than 0.25 as the oligonucleotides are saturated and then undergo allosterical changes. However, the magnitude in low and high salts is different, particularly as the helix is saturated with ethidium. The maximum value of bound ellipticity in low salt for the allosteric conversion of B-form is ~22, which is a little higher than that of native DNA (Chirico *et al.*, 1990) and synthetic polymers (Walker *et al.*, 1985). The maximum value of the hybrid B-Z form in high salt is ~6 for this process, which is completely different from that of Z-DNA (Walker *et al.*, 1985). The dependence of the bound molar ellipticity on the binding ratio indicates the interactions between bound ethidium molecules. The similarity of induced CD bands also indicates a close similarity in the geometry of DNA-ethidium complexes (Aktipis and Martz, 1974). There is no such similarity between the induced CD bands in low and high salts, even though they are both ethidium-bound B-form DNA under an allosteric conversion.

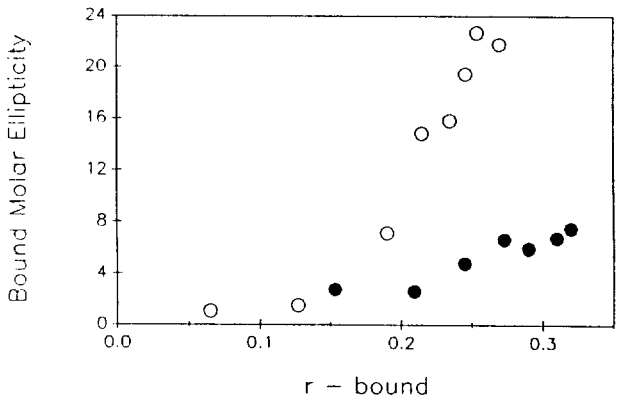

Fig. 6. The dependence of bound molar ellipticity at 320 nm on the binding ratio for the binding interaction of ethidium with BZ-1 at two NaCl concentrations, as an example. Open circles represent the values of bound ellipticity in BPE buffer and filled circles are those in BPE buffer at 4.5 M NaCl as a function of the binding ratio. The bound molar ellipticity is calculated by bound ethidium concentration.

Discussion

Conformation of deoxyoligonucleotides in low and high salt condition

The CD spectra of the methylated CG-8 in low salt are similar to those of the methylated and unmethylated poly (dG-dC), referring to the B conformation. The CD spectra of the methylated CG-8 in high salt, however, are different from those of the above synthetic polymers, which are all in the Z conformation (Behe and Felsenfeld, 1981). The spectrum of the methylated CG-8 in high salt has a flat positive peak in the range of 250~280 nm. The CD spectrum of AT-8 in high salt shows an unusual optical property in that it has lower magnitude over the range of 260~300 nm than that of the B conformation. This spectrum, which may be called a shrinked spectrum, is still parallel to that in low salt over the range, and it overlaps the CD spectrum of the B conformation below 260 nm. It has been reported that the CD band at 275 nm is positively correlated to the base pair twist and is negatively correlated to the helix winding angle if all conformations are right-handed helices (Johnson *et al.*, 1981). Considering the random sequence of AT-8, it is presumably non Z-form DNA even in high salt. Thus, it can be interpreted that this unusual conformation in high salt by this spectrum has a higher helix winding angle and a lower base pair twist angle than those of AT-8 in the B conformation. Another aspect could be related to this structural transition. It was suggested that the alkali metal salts could convert the typical conservative spectrum to a nonconservative spectrum of native calf thymus DNA with a decrease in the CD bands in the range of 260~300 nm by increasing the concentration of these salts concentration (Hanlon *et al.*, 1975). There was a linear relationship between the fractional B conformation and the net hydration, such that 18 mol H_2O bound to mol of nucleotide at 100% B- and 4 mol H_2O bound at 0% B-conformation. Thus, the changes of CD bands in the range of 260~300 nm were positively correlated to the net hydration of DNA

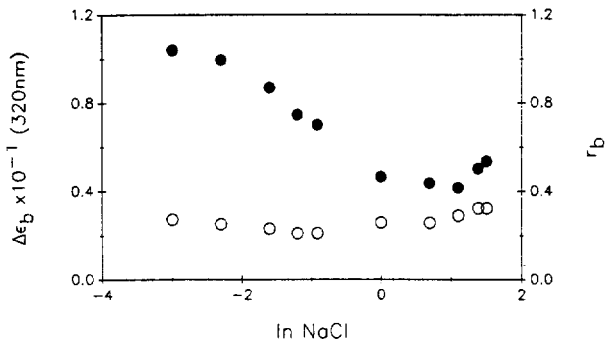

Fig. 7. The relationship between the bound ellipticity and NaCl concentration for the ethidium interaction of BZ-1 by varing NaCl concentration, as an example. The bound molar ellipticity is calculated by bound ethidium concentration.

leaving the CD bands below 260 nm constant (Wolf and Hanlon, 1975). The exact conformation of AT-8 in high salt, however, is not known. In company with those CD spectra of the Z conformation, u.v. absorbance spectra of this with the methylated and unmehtylated poly(dG-dC) has been well characterized (Pohl and Jovin, 1972; Behe and Felsenheld, 1981).

However, it was observed that the optical characteristics for the B to B-Z form was significantly different. The ratio of A_{260}/A_{295} for the B conformation is ~2.7 times higher than that for the Z conformation. This ratio for the B conformation is only ~1.3 times higher than that for the hybrid B-Z form, which is half of the ratio for the B to Z transition. A significant absorbance difference for the B form and for the hybrid B-Z form can be still found at 295 nm. It should be noted that this behaviour is definitely different from the melting process in which the absorbance at 260nm and at 295nm increases with increasing temperature (Pohl and Jovin, 1972). Below 200 nm, the CD band of the B-form presents a large positive peak at 187 nm and that of the Z-form presents a large negative peak at 194 nm. It has been observed that the CD band of the Z-form is an inverted spectrum of the B-form and the difference of magnitude below 200 nm of the B- and the Z-form is ~10-times greater than that measured in the range of 260~310 nm (Sutherland *et al.*, 1981). Also, the CD bands below 200 nm are known to have a relation to base-base interactions. Heat-denatured DNA shows diminished intensity below 200 nm though there is little change above 200 nm (Johnson, 1978). I was observed interesting CD bands of the hybrid B-Z form of BZ-1 below 200 nm. This CD band shows a positive peak at 187 nm which is strikingly different from that of Z-form DNA, and is a much smaller peak than that of B-form DNA. Some of base-base interactions may be lost, considering the decreased intensity in these wavelengths, such as has been shown with denatured DNA.

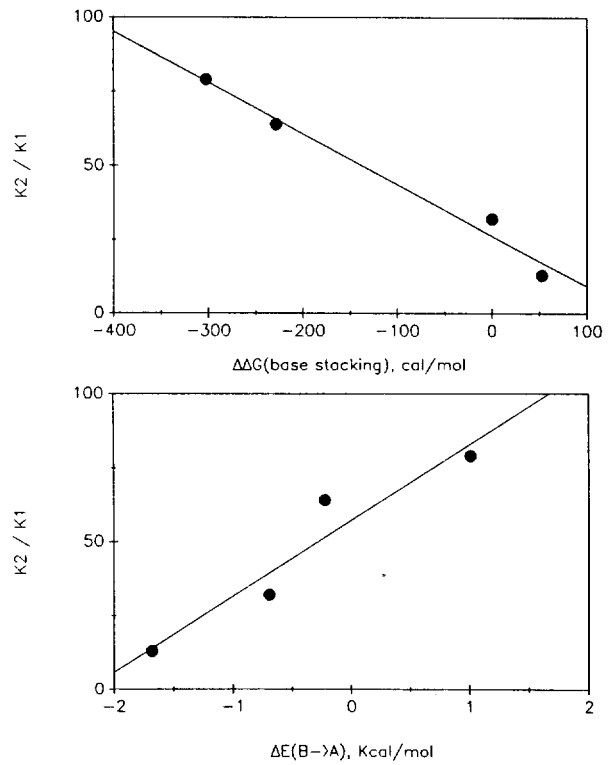

Fig. 8. Correlation of the quantity K_2/K_1 in high-salt to (a) the difference of base stacking energy of dinucleotides adjacent to a B-Z junction based on that of BZ-1 and (b) to the conformational transition energy for B to A form DNA (Peticolas *et al.*, 1988). The lines represent the linear least-squares fits.

The sequence effect on the binding interaction of ethidium with deoxyoligonucleotides containing a B-Z junction

The statistical analysis of the fitting of data of BZ-1 to an allosteric model improves the fitting of the tailing region of the binding isotherm at a high binding ratio of ~0.2 in high salt. Ethidium binds much tighter to oligonucleotides of all BZ DNA in high salt, which favors initially to form the hybrid B-Z form, than it does in low salt. Another DNA binding ligand, actinomycin D, also binds tighter to the hybrid B-Z form of BZ-1 in high salt than to the B form in low salt (Suh *et al.*, 1992). It was reported that 7-amino-actinomycin D bound to single-strand DNA enhanced fluorescence intensity with a binding constant above 1×10^7 (Wadkins and Jovin, 1991). It is also shown that the cleavage of the B-Z junction is dramatically enhanced by dynemicin A, which may be used as a probe of the existence of a B-Z junction *in vivo* (Ichikawa *et al.*, 1992). A series of oligonucleotides were designed and synthesized by changing systematically the dinucleotide step adjacent to the junction region, based on the information which showed the location of the junction in

BZ-1. These oligonucleotides are designated BZ-2, BZ-5, and BZ-6, shown in Fig. 1. The binding isotherms of these oligonucleotides indicate a positive cooperative binding, which has been already confirmed for those of BZ-1 by two independent approaches. The rising portion of the binding isotherms of BZ-1 were also observed, particularly at the region of a lower binding ratio, which represented a positive cooperative binding according to a model free analysis (Chaires *et al.*, 1992). One interesting feature is that the ratio K_2/K_1 in high salt negatively correlates to the difference in the base stacking energy of dinucleotides (Doktycz *et al.*, 1992) and positively correlates to the conformational energy of the B- to A-form transition (Peticolas *et al.*, 1985) as shown in Fig. 8. The constant K_1 represents a binding constant of ethidium with the hybrid B-Z form and K_2 represents that of ethidium with an allosterically converted form-2 in high salt, described by an allosteric model in Table 2. The lower the base stacking energy, the more easily ethidium drives the conversion from the hybrid B-Z form to form-2 (B″) for a preferential binding, while the harder it is converting the B″-form to the B-Z form, and therefore the more ethidium favors the stability of this B″-form in high salt. In conjunction with the positive correlation with the conformational energy of the B- to A-form, the smaller propagation constant for BZ-5 is in line with a high requirement of energy for the allosteric conversion and its high positive conformational energy, while BZ-6 shows the opposite behaviour and a high propagation constant. Thus, the sequence located nearby the junction region effects significantly the binding interaction of ethidium to deoxyoligonucleotides.

Spectroscopic evidence of the conformational transition of BZ-1 under the effect of ethidium

It has been suggested that the CD band in the range of 290~360 nm shows the representative characteristics of a direct interaction between ethidium molecules intercalated among neighboring binding sites (Aktipis and Kindelis, 1973). The bound molar ellipticity of the Z-form showed a constant value of ~12 as a function of the binding ratio (Walker *et al.*, 1985). This result implies that ethidium is bound in clustered regions of the binding sites of the conformation equivalent to a fully saturated helix. Thus, they suggested that this behavior clearly indicated a clustering with ethidium. The binding of ethidium to the hybrid B-Z form in high salt shows a difference in that the bound molar ellipticity increases up to ~8 at the saturated level of ethidium. It has been reported that there is no salt effect on the dependency of bound ellipticity for ethidium-calf thymus DNA. The bound ellipticity of ethidium-bound calf thymus DNA remains constant between concentrations from 0.04 M to 5 M NaCl (Aktipis and Kindelis, 1973). In contrast, the induced CD bands of ethidium bound calf thymus DNA between concentrations of 0.45 mM and 85 mM NaCl shows variation indicating that there is salt effect over that range (Dahl *et al.*, 1982). The cooperative constant (ω) decreases from ~10 in low salt to ~1 in high salt still indicating that ethidium prefers to bind to adjacent sites more in low salt than in high salt. Thus, ethidium can specifically recognize an unusual structure not by the salt effect but by the structural specificity.

Finally, it should be noted that the mode of binding of ethidium with these unusual DNA structures might be intercalation. The results of fluorescence contact energy transfer measurement indicate that ethidium binds to DNA forms which were initially B-Z hybrid forms by intercalation, even though these are unusual DNA forms (data not shown). The details of this technique was described previously (Suh and Chaires, 1995; Haq *et al.*, 1995).

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