Cooperative binding interaction of ethidium with allosteric DNA

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Abbreviations: CD, circular dichroism; SVD, singular value decomposition

Abstract

The specific association of drugs with deoxyoligonucleotides, containing a B-Z junction between left-handed Z-DNA and right-handed B-DNA, was examined by fluorescence and circular dichroism (CD) technique. Ethidium was chosen for a simple DNA binding compound because it binds to right-handed DNA and hybrid B-Z forms containing a B-Z junction in a highly cooperative manner. The binding isotherms were analyzed by an allosteric model in order to describe the cooperativity of association. Binding of ethidium to the DNA that are initially in the hybrid B-Z forms showed over an order of magnitude higher affinity than other DNA which were entirely in the B-form. The conformational transitions of deoxyoligonucleotides containing a B-Z junction as a result of ethidium binding were monitored by CD and the influence of NaCl on the complex formation was also determined by the CD spectra. The singular value decomposition (SVD) analysis was used to characterize a family of CD spectra of the species in binding equilibria. The results of SVD analysis showed a strikingly complex thermodynamic equilibria of cooperative binding of drugs to the allosterically converted DNA forms. The results also showed that these DNA forms in low- and high-salt were different in the absence or presence of drug. These results demonstrate that DNA-binding drugs can preferentially interact with specific DNA structures and that these interactions are accompanied by allosteric changes of DNA conformations.

Keywords: allosteric DNA, cooperativity, CD spectra, singular value decomposition, binding interactions

Introduction

DNA has been shown to adopt different conformations depending on its sequence and environment. This unique structural feature of drug binding specificity with unique DNA structures provide the potential targets for antibiotics or antitumor drugs Walker *et al.*, 1985; (Suh *et al.*, 1991; Suh, 1996). DNA is known to induce conformational changes in localized sites as well as in the entire secondary structures. In the localized conformational transitions, unusual DNA structures (bends, bulges, junction) may appear (Wells, 1988). Such structures may be recognized by proteins or antibiotics by unwinding or unstacking DNA double strand helix. These disruptions of stacked base pairs lower the energy barrier at the binding sites in the event of binding interaction. A detailed understanding of the DNA binding properties of DNA binding compounds is essential for the rational design of new DNA binding agents (Haq *et al.*, 1995; Suh & Chaires, 1995; Chaires *et al.*, 1996; Suh *et al.*, 1997). In order to describe thermodynamic effects in unusual DNA structures, several models requiring the presence of junction formation between two different secondary structures in contiguous DNA segments have been applied (Burd *et al.*, 1975; Crothers & Fried, 1983). Junctions can comprise only small regions of long DNA sequences because they are thermodynamically unstable. Since Z-DNA can exist *in vivo* (Wittig, 1989) and may be related to gene regulation (Jimenez-Ruiz *et al.*, 1991), the characteristics of the B-Z junction have been of interest to investigators.

The nucleotide sequence of the first of these synthesized, designated BZ-1, has been characterized to focus on the unusual localized conformational transitions which may occur infrequently in natural genomes. Recently, the structure, energetics, thermodynamics, and reactivity of B-Z junctions have been reported (Sheardy, 1988; Sheardy *et al.*, 1993; Sheardy *et al.*, 1994; Suh, 1996). From these studies, it was found that the formation of a junction requires more than 1 Kcal/mol and is dependent on sequence. The B-Z junction region spans 3 bp long and perturbs the structure of flanking right-handed helix regions. Ethidium was chosen as a simple DNA binding compound because it binds tight to the allosterically converted DNA form in high salt conditions which initially favors the hybrid B-Z form than any other forms (Suh *et al.*, 1991). In order to reveal the nature of highly reactive DNA forms, CD spectra of ethidium bound BZ-1 were collected for the conformational transition, and analyzed by singular

value decomposition. These results demonstrate that DNA binding drugs can preferentially interact with specific DNA structures and that these interactions are accompanied by allosteric changes of DNA conformations.

Materials and Methods

Materials

A deoxyoligonucleotide, which is designated BZ-1, is 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). The sequence of BZ-1 is as follows:

5'-CGCGCGCGACTGACTG
3'-GCGCGCGCTGACTGAC

where C in *italic* indicates 5-methyl-cytosine. Each strand of complementary deoxyoligonucleotide is mixed at equal concentration, heated up to 90°C and then slowly cooled down to 20°C, in order to anneal the non, self-complementary sequences. A complete annealing has been determined by the continuous variation method (Huang, 1982; Haq *et al.*, 1995), so called, Job plot (data not shown). The extinction coefficient has been obtained by calculating that of each deoxymononucleoside monophosphate (Fasman, 1975), and by measuring the percent of hyperchromicity of oligonucleotides. The measured extinction coefficient was used for concentration determinations. Ethidium was purchased from Sigma (St. Louis, MO). Ethidium concentrations were determined by absorbance, at 480 nm assuming $\epsilon = 5,600 \text{ M}^{-1}\text{cm}^{-1}$. 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 at 4.5 M NaCl.

Spectral Measurement

Absorbance was measured at 20°C, with a Cary-219 spectrophotometer. The cell path length of spectrophotometer was 1 cm. Circular dichroism was measured at 20°C with a JASCO J-500A spectropolarimeter, interfaced to and controlled by an PC. The cell path length of CD measurements was 1 cm.

Fluorescence Titrations

Ethidium binding was monitored by a steady-state fluorescence measurements on a Perkin-Elmer 650 spectrofluorometer, which was not equipped with a polarizer. The wavelength of excitation was 525 nm, and that of emission was 605 nm. Fluorescence titrations were described in detail before (Suh *et al.*, 1991; Suh, 1996). The bound ethidium concentration (C_b) was

calculated from the relation,

$$C_b = C_t (I - I_0) / (V - 1) I_0$$

where C_t is the total ethidium concentration, I is the observed fluorescence intensity, I_0 is the fluorescence intensity of the same concentration of ethidium in the absence of DNA, and V is the experimental coefficient determined in BPE buffer and in BPE at 4.5 M NaCl. Free ethidium concentration (C_f) were obtained from the conservation relationship, $C_t = C_f + C_b$. The binding ratio, r is defined as $r = C_b / [\text{DNA}]$. The fluorescence intensity of ethidium was calibrated for the inner-filter effect, particularly when the fluorescence titrations were performed at high concentration of total added ethidium (Lakowicz, 1983).

Monitoring the conformational changes of DNA

The conformational transition of BZ-1 by the effect of ethidium binding was monitored by circular dichroism measurements. Each 1 ml solution of BZ-1 in low- and high-salt, respectively, was prepared and was equilibrated for overnight in the dark place. Ethidium was added, ranging from 1.3 μM to 20.8 μM , to 9 separate solutions at the final concentration of 38 μM (bps) in low and high salt, respectively. Independently, CD spectra of NaCl induced conformational transition of ethidium bound BZ-1 were measured. Each 1 ml solution of 18 μM ethidium ranging from 0 to 4.5 M NaCl was prepared, separately. DNA was added to each solution at a concentration of 38 μM (bps) and equilibrated for overnight in the dark place. CD spectra of a family of samples were recorded, while some aliquots were taken out to measure the fluorescence intensity, independently, in order to estimate the bound ethidium concentration. Simultaneously, the fluorescence intensity of same ethidium concentration solution was measured in the absence of DNA.

A family of CD spectra was analyzed by singular value decomposition (SVD), which was available in PC-MATLAB (The Mathworks, Inc., Natick, MA). The wavelengths used for SVD analysis were spanned in the range of 220-360 nm at 2 nm intervals shown in Figure 3 and Figure 5.

Results and Discussions

Salt-dependent CD spectra of BZ-1

Representative CD spectra of BZ-1 collected as a function of NaCl concentration are shown in Figure 1. CD spectra of BZ-1 shows development of a hybrid structure at high salt and the magnitude of the molar ellipticity decreases with increasing NaCl. The G-C end of the molecule adopts the left-handed Z-DNA and the central 3 bp region forms a stable junction region as demonstrated by NMR. The rest of the molecule adopts

Figure 1. Circular dichroism spectra of BZ-1 as a function of NaCl concentration: solid line (—) represents a spectrum at low salt; dotted line (....) represents a CD spectrum at high salt.

the right-handed B conformation. Changes in the CD spectrum of BZ-1 are followed by the conformational transition of the duplex form, a right-handed B-form to a hybrid form containing a B-Z junction. The isoelliptic point can be observed near 260 nm. Although the isoelliptic point seems to be well defined the transition may not be a simple two-state process (Sheardy *et al.*, 1993)

Ethidium binding to BZ-1

We have earlier reported that fluorescence was strongly enhanced by binding of ethidium to DNA (Suh *et al.*, 1991). In this study, the binding of ethidium to BZ-1 examined by a steady state fluorescence titration was carried out at fixed DNA concentration with increasing amounts of ethidium concentrations. Scatchard plot of the binding data plotted by calculating the free and the bound ethidium concentrations is shown in Figure 2. The neighbor-exclusion model (McGhee & von Hippel, 1974) designed for the cooperative binding analysis was first used for estimation of the cooperative binding isotherms. However, this neighbor-exclusion model, showed poor fitting of the binding data of ethidium with BZ-1. Binding isotherms are dramatically deviated from the calculated lines by the neighbor-exclusion model shown in Figure 2. Thus, the allosteric binding model that have also been used for the quantitative analysis of binding data (Dattagupta *et al.*, 1980; Chaires, 1986), was tried. This model can be used to describe the association of ligand at the equilibrium state of the conformational transition between two different forms of DNA. This equilibrium requires two equilibrium constants, σ for a nucleation step and s for a propagation step. The ligand may bind to each DNA form. Neighbor exclusion parameters K_i , n_i , and ω_i , where $i = 1$ or 2 , represent each DNA form, respectively. The solid lines are the curves derived by an allosteric model (Figure 2). The parameter estimates of the allosteric interactions of

Figure 2. Binding isotherms of ethidium with BZ-1 in the form of Scatchard plot. (a) In BPE at 4.5 M NaCl. (b) In BPE buffer. Solid lines represent the calculated curves of an allosteric model. Dotted lines are the least-squares fits of data to the neighbor exclusion model. C is the free ethidium concentration and r is the binding ratio. Summary of the parameter estimates for an allosteric model was reported before (Suh, 1996).

ethidium with BZ-DNA were described previously (Suh, 1996). Ethidium was shown to bind 70 times tighter to the form-2 DNA in high salt, which initially favors formation of the hybrid B-Z form, than to the B form in low salt.

Conformational transition of BZ-1 by the effect of ethidium

CD spectra of BZ-1 were measured at two NaCl concentrations by varying the amount of ethidium shown in Figure 3. These spectra represent the conformational transition of BZ-1 by the effect of ethidium binding. Ethidium was added in increments of 0.06 mol per bp over the range of 0 to 0.24. In low salt, we observed the induced CD bands (see Figure 3a) in the 310-360 nm region. Similar results were observed with natural DNA (Chirico *et al.*, 1990) and with synthetic DNA polymers (Walker *et al.*, 1985). These induced CD bands were suggested as the result of direct interactions between ethidium molecules bound in neighboring binding sites (Aktipis & Kindelis, 1973). However, there were no significant induced CD bands of spectra in high salt (Figure 3b). These spectra were thoroughly examined by singular value decomposition (SVD) analysis that have been applied for obtaining the number of

Figure 3. Some representative CD spectra for the interaction of ethidium with BZ-1 at two NaCl concentrations. (a) In BPE buffer. (b) In BPE at 4.5 M NaCl. The spectra representing as solid lines were at low binding ratio, while those as dotted lines were at high binding ratio. Ethidium titration were obtained in increments of about 2.5 mM ethidium at each time.

significantly independent components in a set of spectra (Henry & Hofrichter, 1992; Johnson, 1992). A family of CD spectrum for NaCl-induced transition from the right-handed B form to a hybrid form containing a B-Z junction was analyzed by SVD (Sheardy *et al.*, 1993). The transition was noted: it is at least three-state process including the intermediate process (I), shown in the central region of Figure 7.

In brief, the data matrix A can be decomposed into the product of three matrices, $A = USV^T$, where the S matrix represents the singular value, the U and V matrices are called a matrix of basis vectors (U) and a matrix of amplitude coefficients (V), respectively. The V matrix contains coefficients for the least-squares fit the basis spectra which is the product, US , to the original CD spectra. The original CD spectra are in the range of 220-360 nm at 2 nm intervals. The CD spectra in Figure 3 and Figure 5 are composed to produce the data matrix A . Among the matrices, the number and the magnitude of significant singular values in the S matrix, can be used for selecting the most significant components. The two most significant basis CD spectra of BZ-1 by the effect of ethidium in low and high salt are shown in Figure 4a and b, respectively. The only two largest singular values represent these sets of spectra analyzed by SVD. Other basis spectra seem to be randomly distributed with noise of signals. The solid

lines represent the most significant basis CD spectra in low and high salt, which are different in their magnitudes of spectra particularly at 280 nm and in the range of 300-360 nm. The dashed lines represent the second most significant basis spectra in low and high salt which are very similar in their magnitudes and shapes as a function of wavelength. It has been discussed that the most significant basis spectrum shows the common features in a series of spectra under the same condition (Johnson, 1992). Thus, these most significant basis spectra can best describe for each spectrum in a series of CD spectra as the single shape under the same condition. The amplitude coefficients for the most significant components in low and high salts are shown in Figure 4c and d, respectively. The filled circles represent the coefficients for the most significant spectra and the empty circles represent those for the second most significant spectra. The amplitude coefficients for the most significant basis spectra changes little at the beginning, then increases slowly by adding ethidium and finally stays constant. Comparing these behaviors, the coefficients for the second most significant components change little at the beginning, but increases dramatically during the titration of ethidium and then finally stays constant. The amplitude coefficients of the rest corresponding basis spectra are randomly distributed like noise.

Another criterion for the selection of the significant components is to assess the signal to noise ratio of the columns of U and V . The autocorrelation function, $C(R_i)$ can be used to assess quantitatively the randomness of corresponding columns of the U and V matrix to columns of each singular value; $C(R_{ij}) = \sum (C(R_i)C(R_j))$, where R_{ji} represent the j -th elements of the i -th columns of U or V , respectively. Those column vectors are normalized to unity. If those vectors show slow variations from row to row, "signal", the autocorrelation values are close to 1 and if those show rapid variations, "noise", they are close to -1. The autocorrelation values of the first and second element of basic vectors stay above 0.8, but those values below third elements falls below 0.3. Similarly, the values of the first and second elements of amplitude vectors are above 0.5 and then values of other elements falls down to be negative as the values of 'noise'. Johnson suggested that if the autocorrelation values were greater than 0.3, those elements were significant, but if these were smaller than 0.1 or negative, those were not significant (Johnson, 1992). Thus, SVD analysis shows that the B and the hybrid B-Z form to ethidium bound forms transition in low and high salts, respectively is a simple two-state process. The similarity of induced CD bands also represents a close similarity of geometry of DNA-ethidium complexes (Aktipis & Martz, 1974). There is no such similarity between the induced CD bands in low and high salts.

Figure 4. The two most significant basis spectra and amplitude vectors for the interaction of ethidium with BZ-1 at two NaCl concentrations analyzed by singular value decomposition. Basis spectra (a) in BPE buffer, (b) in BPE buffer at 4.5 M NaCl, and amplitude vectors (c) in BPE buffer, and (d) in BPE buffer at 4.5 M NaCl. In both panels, the solid lines show the most significant basis spectra and the dashed lines show the second most significant basis spectra. Filled circles are amplitude vector coefficients of components-1 and open circles are those of components-2.

Monitoring the conformations of ethidium bound unusual DNA by NaCl

In an effort to understand these characteristics clearly, the saturating amount of ethidium was added to BZ-1 by maintaining the binding ratio of 0.25. Then, NaCl was added up to 4.5 M and compared changes in CD spectra (Figure 5). The magnitude of CD spectra decreases by increasing NaCl concentration particularly in the range of 300-360 nm. The CD band in a range of 290-360 nm is the representative characteristic of direct interaction between ethidium molecules bound in neighboring binding sites (Aktipis & Kindelis, 1973). The characteristics in this region of CD spectra clearly indicate the clustering of bound drugs. The binding of ethidium to the hybrid B-Z form in high salt shows difference where the bound molar ellipticity increases up to 8 at the saturated level of ethidium (Suh, 1996). Earlier report of Aktipis and Kindelis (1973) showed that salt had no effect on the dependency of bound ellipticity for ethidium with calf thymus DNA. The CD spectra analyzed by SVD indicated that first, three significant spectral species may exist as determined by the singular values. These three basis CD spectra are shown in

Figure 5. CD spectra represented for the interaction of saturating amount of ethidium with BZ-1 by varying NaCl concentration. The magnitude of CD spectra decreases with increasing NaCl concentration, especially in the range of 290-350 nm. The spectrum as a solid line is in BPE buffer, while the spectrum as a dotted (----) line were obtained in BPE buffer at 4.5 M NaCl.

Figure 6a and b. The solid line (see Figure 6a) represents the second most significant basis spectrum

Figure 6. The three most significant basis spectra and amplitude vectors for the interaction of saturating amount of ethidium with BZ-1 as a function of NaCl concentration analyzed by singular value decomposition. (a) The most significant basis spectrum. (b) The solid line shows the second and the dashed line shows the third most significant spectrum. (c) The amplitude vector of the most significant component. (d) The empty circles represent the amplitude vector of the second most significant component and empty triangle represent that of the third most significant component.

which is, however, very much different from that in Figure 6a. The dashed line shows the third basis spectrum in Figure 6b. In conjunction with these basis spectra, there are three significant amplitude vectors shown in Figure 6c and d. The first most important amplitude vector is decreased slightly initially but did not change much as a function of NaCl concentration in Figure 6c. The second, represented as empty-circles, increases sharply up to 1 M and then slowly decreases while the third, shown as empty-triangles decreases continually as a function of NaCl concentration. Other amplitude vectors are randomly distributed (data not shown). The autocorrelation values of both vector decreases sharply below 0.1. Therefore, SVD analysis data show that the transition of the ethidium-saturated DNA complex by salt seems to be a complex process involving sequential three-state process instead of a simple two-state process. The structure of ethidium bound BZ-1 in low and high salt, respectively, seems clearly not to be the same because of the dramatically different CD spectrum.

Thermodynamic pathways of binding equilibria exist

A minimal set of equilibria, which can describe binding data and CD results, is shown in Figure 7. There are

two major species; one in low salt (B-DNA & B'-DNA) and the other in high salt (BZ-DNA & B''-DNA) in the presence of ethidium as described earlier by the use of an allosteric model for fitting the binding data of ethidium with BZ-1 (Figure 2). The existence of two major species in the presence of a drug is consistent with two minimal species of equilibria by an allosteric model of binding data. Ethidium binds to both conformational forms. The nature of most of DNA forms is unknown, except that of form-1 DNA (B-DNA) in low salt and form-2 DNA (BZ-DNA) in high salt, respectively. Secondly, there are at least three major species of BZ-1 in the absence [results of SVD analysis were reported before (Sheardy *et al.*, 1993)] and the presence of drug (shown in Figure 5 & 6) and by the effect of NaCl. The nature of intermediate (I or I') may be an unfavored nucleated species for the B to B-Z transition. Similar behaviors were reported for the B to Z transition before (Jovin *et al.*, 1987). It is probably formed because the Watson-Crick hydrogen bonds are broken by an unwinding of the double helix, likely to happen during the B to Z transition (Manzini *et al.*, 1987). Finally, a minimal equilibria in Figure 7 can be explained for B to BZ transition and for the cooperative conversion of DNA with drugs as described above.

Figure 7. Minimal equilibria for describing the cooperative binding of drug to allosterically converted DNA forms. Details are described in the text.

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References

Aktipis, S. and Kindelis, A. (1973) Optical Properties of the Deoxyribonucleic acid - ethidium bromide complex. *Biochemistry* 12: 1213-1221.

Aktipis, S. and Martz, W. W. (1974) Circular Dichroism and Temperature-optical Density Studies on the Conformation of Polynucleotide-ethidium bromide Complex. *Biochemistry* 13: 112-118.

Burd, J. F., Wartell, R. M., Dodgson, J. B. and Wells, R. D. (1975) Transmission of Stability in Deoxynucleotides. *J. Biol. Chem.* 250: 5109-5113.

Caruthers, M. H. (1991) Chemical Synthesis of DNA and DNA Analogues. *Acc. Chem. Res.* 24: 278-284.

Chaires, J. B. (1986) Allosteric Conversion of Z-DNA to an Right-handed Conformation by Daunomycin. *J. Biol. Chem.*

261: 8899-8907.

Chaires, J. B., Saty, S., Suh, D., Fokt, I., Przewlorka, T. and Priebe, W. (1996) Parsing the Free Energy of Anthracycline Antibiotic Binding to DNA. *Biochemistry* 35: 2047-2053.

Chirico, G., Lunelli, L. and Baldini, G. (1990) Conformation of Intercalated DNA Plasmids Investigated by Circular Dichroism and Dynamic Light Scattering. *Biophys. Chem.* 38: 201-211.

Crothers, D. M. and Fried, M. G. (1983) Transmission of Long-range Effects in DNA. *Cold Spring Harbor Symp. Quant. Biol.* 47: 263-269.

Dattagupta, N., Hogan, M. and Crothers, D. M. (1980) Interaction of Netropsin and Distamycin with DNA. *Biochemistry* 19: 5998-6005.

Fasman, G. (1975) Measured and Calculated Extinction Coefficient at 260nm, 25C, and Neutral pH for Single-Strand RNA and DNA, in: *CRC Handbook of Biochemistry and Molecular Biology*, 3rd Ed., CRC Press, Cleveland.

Haq, I., Lincoln, P., Suh, D., Norden, B., Chowdhry, B. Z. and Chaires, J. B. (1995) Interaction of D- and L-Ru²⁺ with DNA: A Calorimetric and Equilibrium Binding Study. *J. Am. Chem. Soc.* 117: 4788-4796.

Henry, E. R. and Hofrichter, J. (1992) SVD Application to Analysis of Experimental Data in: *Methods in Enzymology*, vol. 210, (Brand, L. and Johnson, M.L., eds.), pp.129-192, Academic Press, Inc., San Diego.

Huang, C. Y. (1982) Determination of Binding Stoichiometry by the Continuous Variation Method: The Job Plot in: *Methods in Enzymology*, vol. 87, pp.509-525, Academic Press, Inc., San Diego.

Jimenez-Ruiz, A., Requena, J. M., Lopez, M. C. and Alonso, C. (1991) A Potential Z-forming DNA is Located Between Two Transcription Units Alternatively Expressed During Development of *Drosophila hydei*. *Proc. Natl. Acad. Sci. USA*, 88: 31-35.

Johnson, W. C., Jr. (1992) Analysis of CD Spectra in: *Methods in Enzymology*, vol 210, (Brad, L. & Johnson, M.L. eds.) pp.426-447, Academic Press, Inc., San Diego.

Jovin, T. M., Soumpasis, D. M. and McIntosh, L. P. (1987) The Transition Between B-DNA and Z-DNA. *Ann. Rev. Phys. Chem.* 38: 521-560.

Lakowicz, J. R. (1983) Chapt.2: Instrumentation for Fluorescence Spectroscopy, In: *Principles of Fluorescence Spectroscopy*, Plenum Press, New York.

Manzini, G., Xodo, L. E., Quadrioglio, F., van Boom, J. H. and van der Marel, G. A. (1987) dG-dC Alternating Oligonucleotides: Thermodynamics and Kinetic Aspects of the B-Z Transformation. *J. Biomol. Struct. Dyn.* 4: 651-662.

McGhee, J. D. and von Hippel, P. H. (1974) Theoretical Aspects of DNA-Protein Interaction. *J. Mol. Biol.* 86: 469-489.

Sheardy, R. D. (1988). Preliminary Spectroscopic Characterizations of a Synthetic DNA Oligomer Containing a B-Z Junction at High Salt. *Nucleic Acids Res.* 16: 1153-1167.

Sheardy, R. D., Suh, D., Kurzinsky, R., Doktycz, M. J., Benigh

A. S. and Chaires, J. B. (1993) Sequence Dependence of the Free Energy of B-Z Junction Formation in Deoxyoligonucleotides. *J. Mol. Biol.* 231: 475-488.

Sheardy, R. D., Levine, N., Marotta, S., Suh, D. and Chaires, J. B. (1994) A Thermodynamic Investigation of the Melting of B-Z Junction Forming DNA Oligomers. *Biochemistry* 33: 1385-1391.

Suh, D., Sheardy, R. D. and Chaires, J. B. (1991) Unusual Binding of Ethidium to a DNA Containing a B-Z Junction. *Biochemistry* 30: 8722-8726.

Suh, D. and Chaires, J. B. (1995) Criteria for the Mode of Binding of DNA Binding Agents. *Bioorg. Med. Chem.* 3: 723-728.

Suh, D. (1996) Specific Recognition of Unusual DNA Structures by Small Molecules: An Equilibrium Binding Study.

J. Biochem. & Mol. Biol. 29: 1-10.

Suh, D. and Povirk L. F. (1997) Mapping of the Cleavage-Associated Bleomycin Binding Site on DNA with a New Method Based on Site-Specific Blockage of the Minor Groove with N²-Isobutyrylguanine. *Biochemistry* 36: 4248-4257.

Walker, G. T., Stone, M. P. and Krugh, T. R. (1985) Ethidium Binding to Left-handed DNA Results in Regions of Right-handed DNA at the Intercalation Site. *Biochemistry* 24: 7462-7471.

Wells, R. D. (1988) Unusual DNA Structures. *J. Biol. Chem.* 263: 1095-1098.

Wittig, B., Dorbic, T. and Rich, A. (1989) The Level of Z DNA in Metabolically Active, Permeabilized Mammalian Cell Nuclei is Regulated by Torsional Strain. *J. Cell Biol.* 108: 755-764.