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Conformational Transitions in b-Structured Peptides Induced by Interaction with DNA

A. N. Surovaya[1], V. A. Nikolaev[1], S. L. Grokhovskii[1,2], and G. V. Gursky[1]

[1] *Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, 117984 Russia*
[2] *Oslo University, Medical Research Center, Oslo, Norway*

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Abstract — Conformational transitions of synthetic 16-mer linear and cyclic peptides capable of forming b-hairpins were studied in solution and upon interaction with DNA. In the cyclic peptide, the b-hairpin structure is stabilized by an S-S-bond between two cysteine residues. It was shown that both peptides in aqueous solution contain about 30-40% of a b-conformation along with a chaotic one. In 20 and 40% aqueous trifluoroethanol (TFE), the secondary structure of the protein is stabilized owing to an increase in the a - and b-conformation content. Upon interaction with DNA in 20% TFE, conformational changes are observed in both peptides whereas the DNA structure is not substantially affected. The conformational changes result in an increase in the content of the a -helical conformation. In complexes of the linear peptide with DNA in 20% TFE at a peptide-basepair ratio of 2:4, the content of the b-sheet increases considerably. It is shown that the transition from dis¬ ordered and a -helical conformations to a b-sheet upon linear peptide-DNA binding does not depend on the DNA nucleotide composition. This transition is weakly expressed in DNA-cyclic peptide complexes and is vir¬ tually absent in aqueous solution and 40% TFE.

Key words: b-sheets, DNA-binding proteins, double-stranded peptide motif, conformational transitions

INTRODUCTION

It is known that the optimum binding of regulatory proteins with DNA often requires mutual adjustment of the structures of the two macromolecules, resulting in conformational changes in the protein recognition site and in DNA. These structural changes can strongly affect the protein binding constant. They play an important role in the formation of a specific protein-DNA complex, which is largely stabilized by van der Waals contacts and hydrogen bonds between the protein and DNA reaction centers. For example, a sin¬ gle nucleotide substitution in the DNA bend in com¬ plexes of the CAP protein or repressor 434 with DNA operator sites can change the binding constant of the protein by one or even two orders of magnitude [1,2]. The study of conformational transitions in model pro¬ teins structurally resembling the constituents of DNA-binding domains of regulatory proteins is undoubt¬ edly interesting, because some model proteins are known to bind selectively to the same DNA sites as natural proteins do [3-10]. The advantage of the model approach is that DNA binding of comparatively small proteins rather than intricate macromolecules is considered.

It is known that the DNA-binding domains of some regulatory protein contain two antiparallel strands in b-conformation. In this context, the *Escherichia coli* host integration factor and the eukaryotic transcrip-tion activator TBP recognizing a TATA box in eukary-otic promoters should be mentioned [11-13]. A simi-lar structural motif is present in triostin antibiotics [14]. In a crystalline complex of triostin A with self-complementary oligonucleotide 5'-CGTACG-3' the depsipeptide ring of this antibiotic is inserted into the DNA minor groove [15, 16]. With all the structural resemblance of complexes of TBP and triostin A with DNA, there are also important differences. The DNA structure is much more distorted in the complex with TBP than with triostin A. In the complex with TBP, specific interactions with the bases involve the side chains of the amino acid-residues rather than the pro-tein backbone as in the complex with triostin A. The study of the structure of DNA complexes with pep-tides in b-conformation may shed light on the nature of these differences.

Here, the structure and DNA interaction of 16-mer linear and cyclic peptides I and II (Fig. 1) were stud-ied using CD spectroscopy. These peptides can form b-hairpins. The loop between two b-strands in the pro-posed b-hairpin contains four amino acid residues including a glycine residue, which has dihedral angles (f and j corresponding to a left-handed a -helical con-formation. Two other residues in the loop have dihe-dral angles f and j corresponding to an a -helical con-formation. All this provides for the abrupt change in the direction of the polypeptide strand that is required for the formation of a b-hairpin. The formation of the

Fig. 1. Chemical structures of the linear and cyclic 16-mer peptides I and II. Dns, fluorescent label dansyl. Chemical structures of the 26-mer linear and cyclic peptides are also given.

b-hairpin can be also promoted by a salt bridge between the lysine positively charged e-amino group at the N terminus of the protein and the negatively charged carboxyl group at the C terminus. The cyclic protein differs from the linear one by the presence of an S-S-bond between the cysteine residues. It is known that an S-S-bond can stabilize the b-structure in a short peptide *bis*(BocAlaCysOMe) [17].

EXPERIMENTAL

The fluorescently labeled linear and cyclic 16-mer peptides were synthesized by the solid-phase approach as described in [18]. The peptides synthesized were stored lyophilized and dissolved directly prior to the experiment in 1.0 mM sodium cacodylate, pH 7. The peptide concentration was determined spectrophotometrically using 4300 M^{-1} cm^{-1} as a molar absorbance value for Dns at 330 nm (e_{330}). Calf thymus DNA (e_{259} = 13 300) was obtained from Sigma, synthetic polynucleotides poly(dA)•poly(dT) (e_{259} = 12000) and poly(dG)•poly(dC) (e_{253} = 14800) were from PL Biochemical. All molar absorbances are given per DNA basepair. Poly(dG)•poly(dC) was dissolved in 0.1 N NaOH and dialyzed for 24 h against the same medium supplemented with 1.0 mM EDTA. Prior to the experiment, all polynucleotide solutions were dialyzed against three consecutive portions of 1.0 mM EDTA-free sodium cacodylate buffer. The CD spectra of the polynucleotides were measured and compared with the spectra described earlier. Slow changes in the shape of the CD spectrum of poly(dG)•poly(dC) were observed upon storing the polynucleotide solutions at 4°C. If the spectra were distorted, the pH value was increased to >11 and dialysis was repeated. CD spectra were registered on a Jasco 720 dichrograph (Japan).

RESULTS

CD spectra of linear and cyclic peptides in solution. Figure 2 presents the CD spectra of peptides I and II in the absence (7) and presence of 20 and 40% TFE (2 and 3). They have a negative band at 197 nm in the absence of TFE, which indicates that the majority of the peptide molecules are in a chaotic conformation. In the presence of 20% TFE, the negative shoulder at 220 nm grows markedly, the negative peak at 197 nm displays a long-wavelength shift, and a positive signal appears at 190 nm. All this indicates enhanced ordering in peptides I and II. In the presence of 40% TFE, a positive amplitude at 190 nm is also observed in solutions of the linear peptide. To quantitatively assess the content of various elements of the secondary structure of the linear and cyclic peptides as dependent on the TFE concentration, we followed the procedure described in [19]. The results of the calculations are presented in the table. As can be seen from these data, the conformations of the linear and cyclic peptides in aqueous solution in the absence and presence of 20% TFE vary considerably.

In the cyclic peptide, the content of the a-helical conformation is twice as high as in the linear peptide, whereas the content of the b-conformation is higher in the linear peptide. In the presence of 40% TFE, the contents of the a- and b-conformations in the two peptides are very close. Interestingly, the CD spectra of the linear and cyclic peptides in the presence of 40% TFE resemble the spectra of gramicidin S and its analogs, which, according to NMR spectral data, form covalently closed b-hairpins in solution [20-23]. These spectra differ considerably from the CD spectrum characteristic of an antiparallel b-structure, which is known to display a positive CD peak at 195 nm and a negative peak at 217 nm. The appearance of

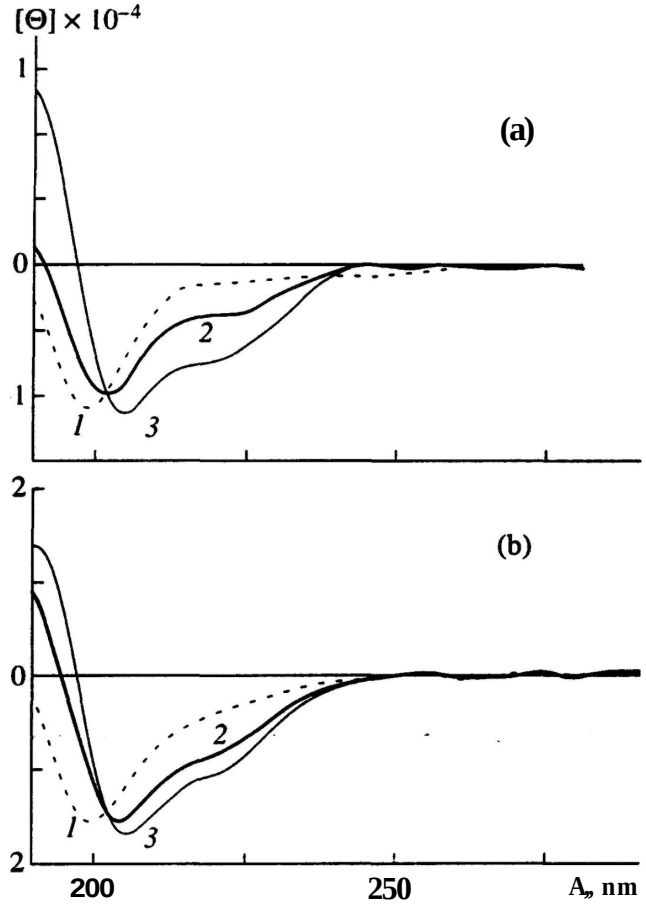

Fig. 2. CD spectra of linear (I) and cyclic (II) peptides in solution in the absence (curve *1)* and presence of 20% and 40% TFE (curves 2 and *3,* respectively). [O], ellipticity (deg cm^2 $dmol^{-1}$ of amino acid residues). Concentration of peptides I and II was $2.4\text{-}10^{-5}$ M. Conditions: 1.0 M cacodylate buffer, pH 7.0; 0.01 M NH_4F; 20°C.

a two-maximum negative CD band in the spectrum of gramicidin S and its analogs may be due to a contribu¬ tion into the CD amplitude on the part of two b-turns in the molecule of gramicidin S and its analogs fom¬ ing b-hairpins. The appearance of a gramicidin S-like CD spectrum supposedly indicates formation of a b-hairpin in the cyclic and linear peptides.

Conformational transitions in peptides induced by DNA binding. Figure 3 presents the CD spectra obtained upon titration of calf thymus DNA with the linear peptide (Fig. 3a) and the difference spectra obtained by subtracting the spectrum of nonbound DNA from the spectrum of a peptide-DNA mixture (Fig. 3b). In this figure (curve /), a CD spectrum of the nonbound peptide I measured under the same con¬ ditions is given for comparison. As Fig. 3 shows, low peptide filling of DNA (C/BP < 0.3; C, molar concen¬ tration of peptide; BP, molar DNA concentration per basepair) is accompanied with practically no changes in the shape of CD spectrum in the region of 250-300 nm. The peptide itself does not contribute to this region of the spectrum. As the long-wavelength band of CD spectrum is the most sensitive to conformational changes in the DNA [24], a conclusion suggests itself that the DNA structure is not essentially affected when the occupancy is below three molecules of pep¬ tide per ten base pairs. The binding is accompanied with considerable spectral changes in the region of 190-250 nm, where the peptide groups absorb. These changes may reflect conformational changes in the peptide molecule induced by the interaction with DNA. The comparison of shapes of the difference CD spectrum (Fig. 3b, curve 2) and CD spectrum of the nonbound peptide reveals a certain increase in the ordering in the peptide molecule. At C/BP = 0.5, a second conformational transition is observed, which is now accompanied with considerable changes in the DNA conformation as follows from spectral changes in the region of 250-300 nm. The shape of the differ¬ ence CD spectrum displays a resemblance to the CD spectrum characteristic for a (^-structure (a positive band at 197 nm and a negative band at 220 nm) whereas the two-maximum negative band in the region of 200-240 nm, characteristic for an a -helical conformation, disappears.

Figure 4 presents CD spectra for complexes of the cyclic peptide with thymus DNA. The analysis of dif¬ ference CD spectra implies that the proportion of the chaotic conformation decreases and the proportion of

Proportion of the a-, fi-, and chaotic conformations in peptides I and II in aqueous solution and water-trifluoroethanol mixtures

Peptide	TFE. %	Peptide conformation		
		a-helix	p-structure	chaotic
I	0	0.06	0.38	0.56
	20	0.10	0.35	0.51
	40	0.27	0.20	0.50
II	0	0.14	0.28	0.60
	20	0.27	0.21	0.53
	40	0.31	0.19	0.49

Note: According to the computing procedure, the total of the various conformations is within 1.00± 0.03.

the a-helix and b-structure increases upon interaction with DNA. In the presence of 20% TFE and the peptide filling of DNA with C/BP value equal to 0.5, the amplitude of the negative CD band at 205 nm in the difference CD spectrum decreases relative to the CD spectrum of the nonbound peptide whereas the amplitude at 225 nm remains unaffected. These spectral changes may correspond to an a-b-transition. However, unlike complexes with the linear peptide, the two-maximum negative band at the region of 200-250 nm is retained to show that a majority of the peptide molecules in the presence of DNA are in an a-helical conformation. It should be noted that difference CD spectra reveal a resemblance to the CD spectrum of gramicidin S and its analogs containing 6,10, or 14 residues [23]. These cyclic peptides, according to NMR data, form covalently closed b-hairpins [21,22]. At C/BP = 0.5, changes in the region of 250-300 nm are also observed, which apparently reflects structural changes in the DNA molecule upon peptide binding. In the difference CD spectrum, a negative CD band appears in this region whereas a positive band is observed for DNA complexes with the linear peptide. This difference may be caused by different structural changes in DNA upon binding of the linear and cyclic peptides. The difference in the shape of difference CD spectra for complexes of the linear and cyclic peptides with DNA may be caused by a contribution of the disulfide group of the cyclic peptide to the CD amplitude in this spectral region. On the other hand, in complexes of DNA with the linear peptide at C/BP = 0.5 unlike complexes with the cyclic peptide, dimers and associates of a higher order may form that are based on an antiparallel b-structure.

Influence of TFE on the conformation of peptides in complexes with DNA. Figure 5 presents difference CD spectra for the linear (a, c) and cyclic (b, d) peptides in the absence of TFE and in the presence of 40% TFE. In the absence of TFE, the shape of the difference CD spectrum for the complex of the linear peptide with DNA practically coincides with that of the nonbound peptide. We only observe an increase in the negative CD band at 197 nm. This indicates that the chaotic conformation of the peptide is retained upon peptide binding to DNA. In the presence of 40% TTE, the nonbound peptide contains elements of a- and b-conformations. However, their content is essentially unaffected upon the peptide-DNA complex formation, which follows from the semblance of the difference CD spectra of DNA-peptide complexes and the CD spectrum of nonbound peptide. Nor did we observe substantial spectral changes for complexes of the cyclic peptide with DNA as compared with the nonbound peptide. It should be noted that the DNA conformation is practically unchanged, which manifests itself in the absence of a CD signal in the difference CD spectra in the region of 250-300 nm. Thus, the conformational transitions induced in the linear and cyclic peptides by interaction with DNA are only observed in the presence of 20% TFE.

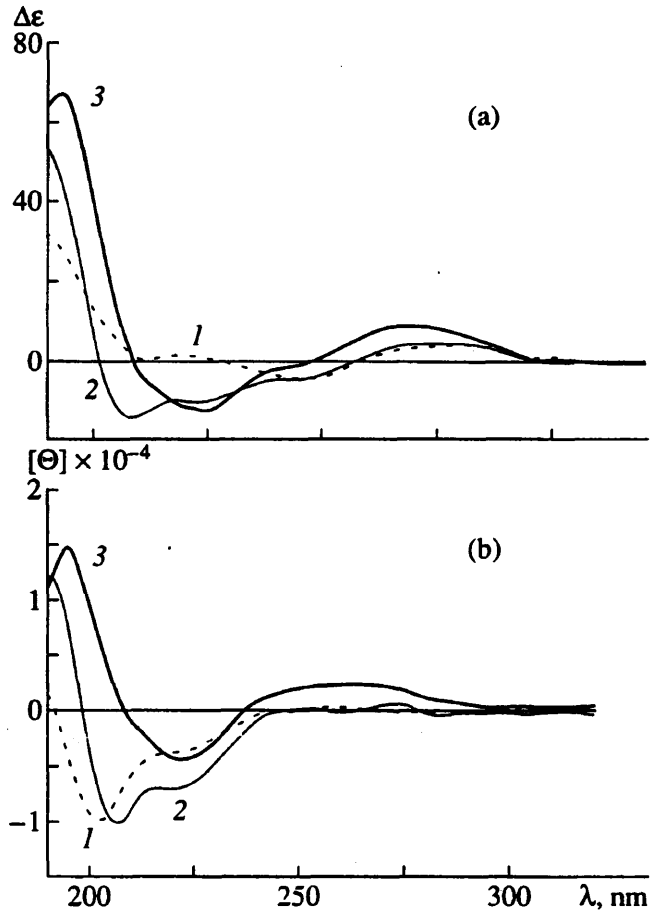

Fig. 3. Changes in CD spectra upon interaction of linear peptide (I) with thymus DNA. Panel (a): *1*, nonbound DNA; 2 and *3*, complexes of peptide I with DNA at C/BP = 0.25 and 0.5, respectively (C, peptide concentration; BP, DNA concentration in moles of basepairs). AE, molar dichroism computed per basepair. Panel (b): difference CD spectra obtained by subtracting the spectrum of nonbound DNA from the spectra of complexes of peptide I with DNA (2, C/BP = 0.25; *3*, C/BP = 0.50). *1*, *CD* spectrum of the nonbound peptide. 20% TFE. other conditions as in Fig. 2.

Interaction of the linear peptide with polynucleotides. Figure 6 presents CD spectra for complexes of the linear peptide with poly(dA)•poly(dT) and poly(dG)•poly(dC) and difference CD spectra obtained by subtracting spectra of the polynucleotides from the spectra of the corresponding complexes. It is known that the conformations of these polymers in solution differ strongly, which is reflected by the shape of CD spectra of the nonbound polynucleotides. However, difference CD spectra for complexes of the peptide with poly(dA)•poly(dT) and poly(dG)•poly(dC) are similar, which indicates that the conformational changes occurring upon peptide binding are the same. At C/BP = 0.5, a positive CD band at 197 nm and a negative CD band at 220 nm appear. The difference

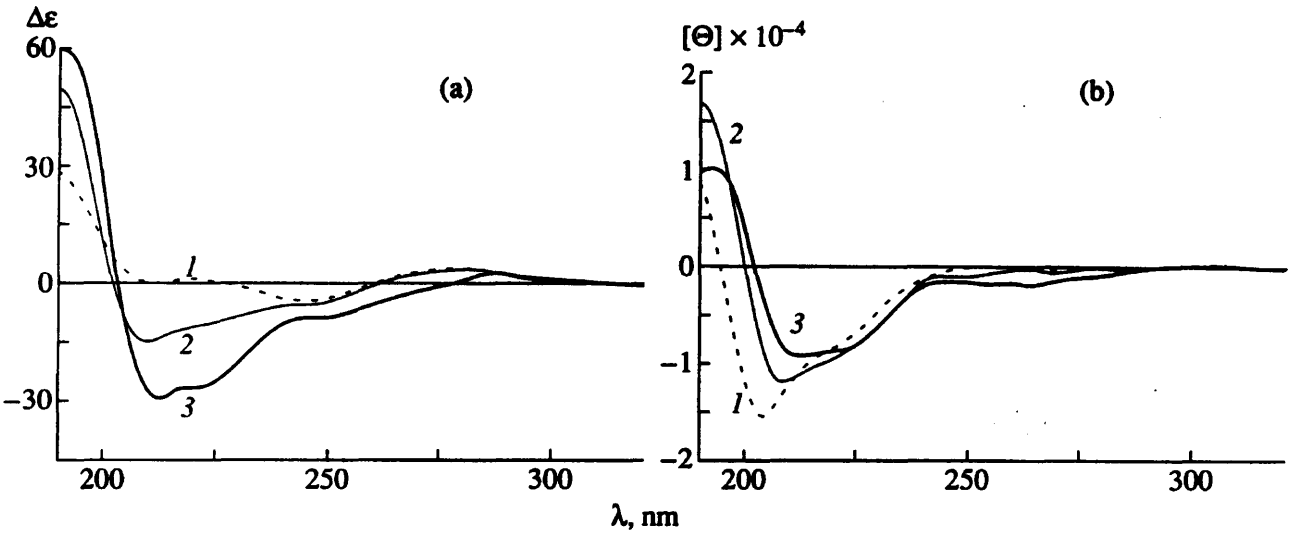

Fig. 4. CD spectra of complexes of peptide II with thymus DNA. Panel (a): *1*, nonbound DNA; *2* and *3*, complexes of peptide II with DNA at C/BP = 0.26 and 0.53, respectively; (b): difference CD spectra (2, C/BP = 0.26; *3*, C/BP = 0.53). *1*, CD spectrum of the nonbound peptide. 20% TFE, other conditions as in Fig. 2.

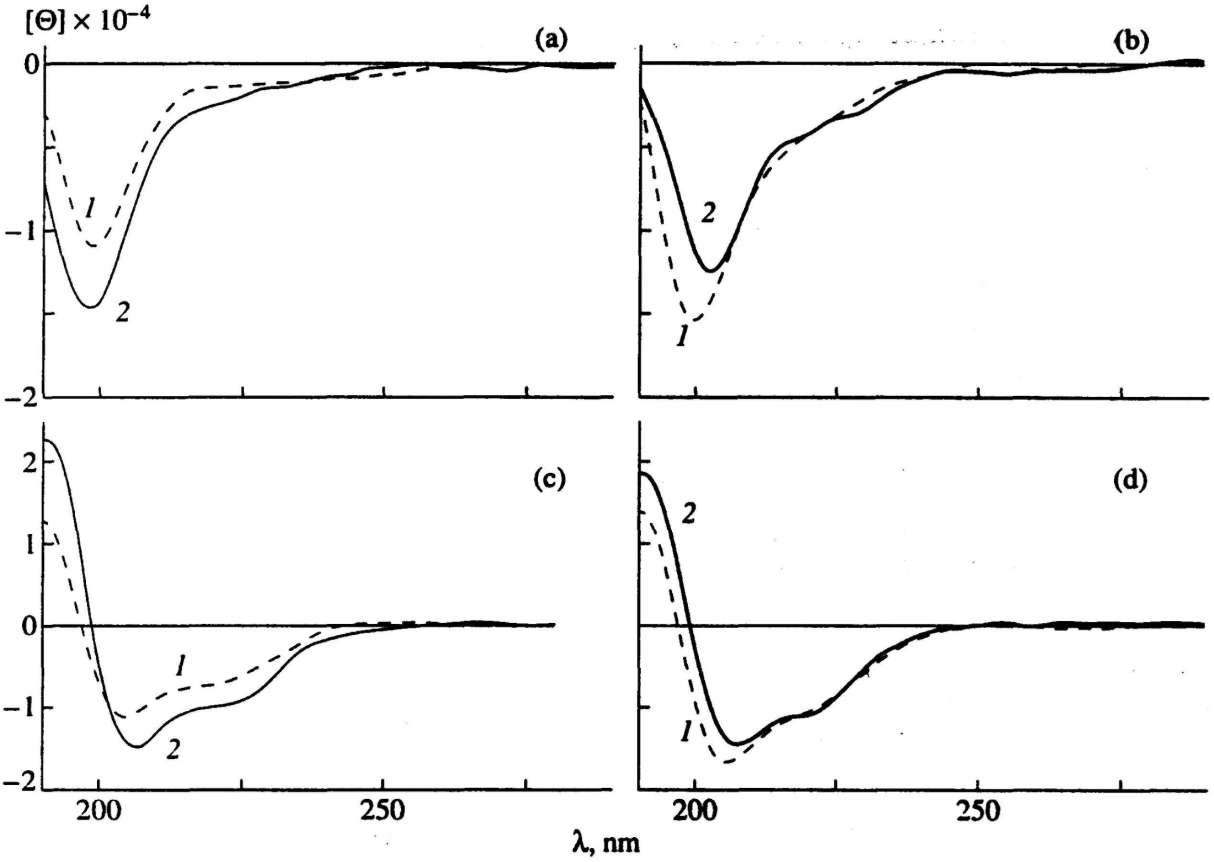

Fig. 5. Difference CD spectra obtained by subtracting the nonbound DNA spectrum from the spectrum of a mixture of peptide I (a, c) and II (b, d) with DNA in the absence (a, b) of TFE and in the presence (c, d) of 40% TFE.

observed in the shape of the difference CD spectra as compared with the nonbound peptide suggests that the linear peptide upon binding turns from a chaotic and a-conformations into a conformation close to an anti-parallel b-structure. In the difference CD spectrum, the positive band at 197 nm is more intensive ([O] = 2.3-2.5•10^4 deg cm^2 $dmol^{-1}$) than the negative band at 220 nm ([O] = 1.2-1.4-10^4 deg cm^2 $dmol^{-1}$), which, according to calculations by Woody *et al.*, is character¬ istic for CD spectrum of a right-helical b-sheet [20].

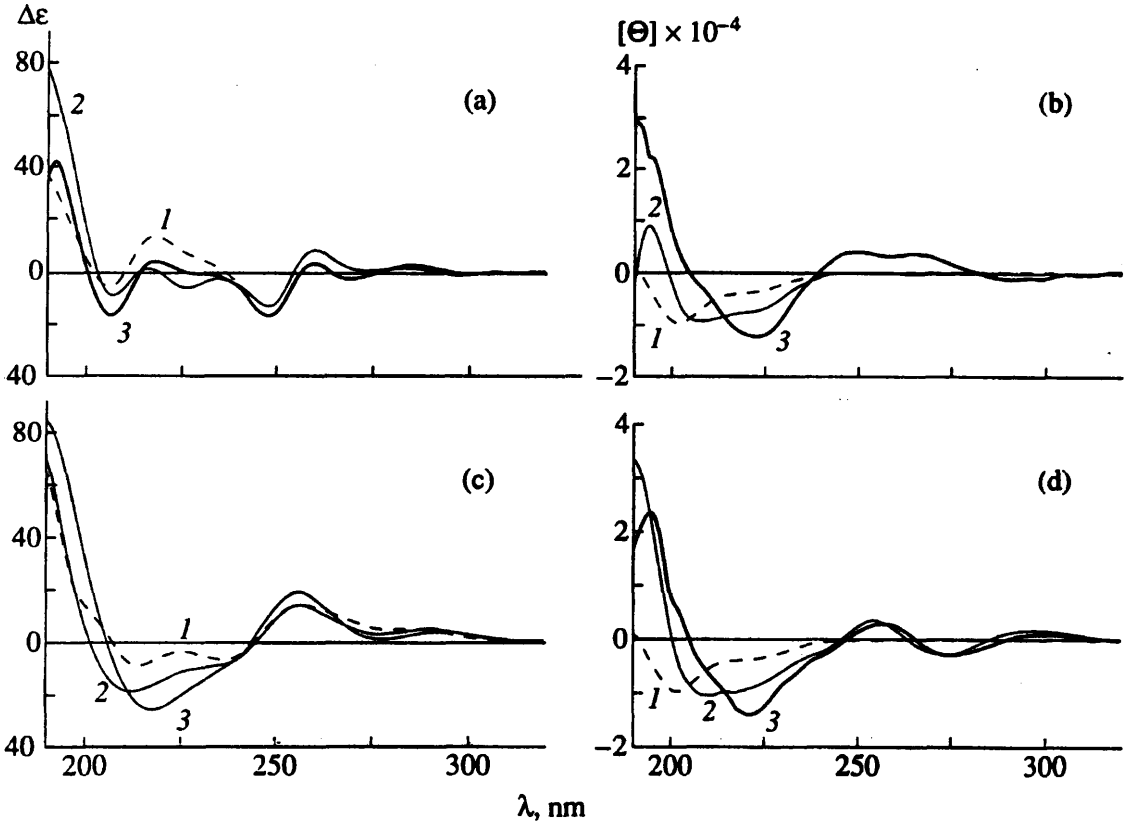

Fig. 6. Changes in CD spectra upon the interaction of linear peptide (I) with polynucleotides. Panel (a): *1,* nonbound poly(dA)•poly(dT): 2 and *3,* complexes of peptide / with poly(dA)•poly(dT) at C/BP = 0.24 and 0.48, respectively; (b): difference CD spectra obtained by subtracting the nonbound poly(dA)•poly(dT) spectrum from the spectrum of a mixture of poly(dA)•poly(dT) and peptide I *(2.* C/BP = 0.24; *3,* C/BP = 0.48). *1,* CD spectrum of the nonbound peptide. (c): nonbound poly(dG)•poly(dC); 2 and *3,* complexes of peptide I with poly(dG)•poly(dC) at C/BP 0.26 and 0.53, respectively; (d): difference CD spectra obtained by subtracting the nonbound poly(dG)•poly(dC)spectrum from the spectrum of a mixture of poly(dG)•poly(dC) with peptide I (2. C/BP = 0.26; 5, C/BP = 0.53). /, CD spectrum of the nonbound peptide. 20% TFE, other con¬ ditions as in Fig. 2.

DISCUSSION

Here, using CD spectroscopy. we studied conformational properties and DNA binding of linear and cyclic 16-mer peptides capable of forming b-hairpins with 4 aa in the loop between two b-strands. We showed that the conformations of the linear and cyclic peptides differ in aqueous solution in the absence of TFE and in the presence of 20% TFE. In the latter case, the content of the a-helical conformation is approximately twice as high as in the linear peptide. However, the linear peptide has a higher proportion of the b-structure than the cyclic peptide. In the presence of 40% TFE, the contents of the a- and b-conformations in both peptides become equal. It is noteworthy that earlier we studied the conformational properties and DNA binding of 26-mer linear and cyclic pep¬ tides, analogs of peptides I and II (Fig. 1) [25] and showed that the proportion of various types of second¬ ary structure elements computed from CD spectra is very similar for these peptides.

At C/BP < 0.3, we observed in peptides I and II conformational changes whose character depends on the TFE concentration. In the presence of 20% TFE. these changes are significant and imply an increase in the proportion of the secondary structure of the pep¬ tide upon DNA binding. It should be noted that the structure of the DNA molecule itself is not substan¬ tially affected. In the absence of TFE or in the pres¬ ence of 40% TFE, the spectral changes upon complex formation are insignificant.

At C/BP = 0.5 in the presence of 20% TFE, we observed a conformational transition resulting in the stabilization of the p-structure in a complex with the linear peptide. This transition does not occur if- the TFE content reaches 40%. In a complex of DNA with the cyclic peptide, this transition is weaker. It is known that addition of TFE to aqueous solution affects the peptide structure through promoting the a-helical conformation. Recently it was shown that TFE stabilizes the a-helical conformation if the amino acid sequence consists of residues predisposed to such a conformation [26]. On the other hand, in the case of b-philic amino acid residues (and our peptides do contain such residues), the strengthening of the

intramolecular hydrogen bonds, occurring in the presence of TFE, may result in the formation of a b-structure. It is known that TFE promotes also the formation of isolated b-hairpins [27]. Possibly, 20% TFE provides for the conformation of the linear peptide (which at the same time has some plasticity) necessary to be inserted into the DNA minor groove. In the case of the cyclic peptide, the disulfide bridge imparts rigidity to the b-hairpin. Possibly, the disulfide bond stabilizes the structure in which the mean distance between two b-strands in a short b-hairpin somewhat exceeds the corresponding distance in the absence of the disulfide bond. The disulfide group itself can contribute to the CD spectrum, which requires additional studies to assess. Earlier we showed that difference CD spectra for DNA complexes of a 26-aa cyclic peptide have a shape characteristic for a peptide in an antiparallel p-conformation [25]. The difference in the behavior of the 16- and 26-mers may be due to the fact that the region of the inter-b-strand turn and the disulfide group contribute more to the CD spectrum of the 16-mer peptide than it occurs in the 26-mer.

In our previous paper [18], the thermodynamic parameters for binding of peptides I and II to DNA in various solvents were determined. It was shown that in 20% TFE a cooperative binding of peptide I to DNA occurs to give two forms of the complex: monomeric binding (b-hairpin) and dimeric binding (b-sandwich). Both the monomer and the dimer occupy four to five basepairs upon binding. The monomer binds noncooperatively, whereas the dimer binding is a cooperative process for which interactions between b-sandwiches bound to adjacent binding sites are responsible. In the absence of TFE, the linear peptide binds only in a form of the monomer. The formation of the peptide secondary structure in 20% TFE may contribute to the formation of the sandwiches, whose cooperative binding to DNA promotes stabilization of the b-structure. The conformational transition takes place at a ratio of one sandwich per 4-5 basepairs. The interaction of the DNA-associated sandwiches may result in the formation of loops and DNA compaction as well as the formation of intermolecular aggregates such as tetraplexes [27, 28].

It is hardly probable that the changes observed in CD spectra upon peptide binding are caused only by conformational changes in the DNA molecule. DNA contributes little to the amplitude of CD at 220 nm, where the largest alterations of the CD spectra are observed. The fact that the difference CD spectra obtained for complexes of a peptide with poly(dA)•poly(dT) and poly(dG)•poly(dC), having different nucleotide composition and conformations in solution, practically coincided in the region of the peptide group adsorption confirms the conclusion that the difference CD spectra characterize the structure of the peptide backbone in bound peptide molecules, which is close to an antiparallel b-structure.

It is known that some natural and synthetic peptides are capable of a drastic conformational change to turn from a chaotic to an a-helical conformation or from an a-helical to a b-conformation [27,29]. These transitions occur in solution upon changing the peptide, TFE, and SDS concentrations, pH and ionic strength. Here, we showed that upon interaction of a peptide with a nucleic acid the peptide conformation changes. The insight into these conformational transitions is important for understanding the mechanisms of DNA-peptide interaction and processes of DNA compaction as well as the nature of bends and loop formation between distant DNA segments.

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