Design and Synthesis of a New Peptide Recognizing a Specific 16-Base-Pair Site of DNA

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Abstract: We designed a peptide to recognize a new 16-base-pair site (about 1.5 turns) of DNA by stitching together three peptides of the v-Jun basic region in a specified order. The binding site consists of three five-base-pair half-sites each of which is recognized by a different segment of the peptide. DNase I footprinting shows that the new peptide specifically recognizes the proposed site, and gel retardation shows that the dissociation constant is about 5 nM at 4 °C. Gel retardation shows that the new peptide does recognize the proposed trimer binding site about 10 times stronger than the dimer binding sites [having two half-sites for two arms]. These results also provide information about the relationship between specific and nonspecific binding in the recognition between protein and DNA.
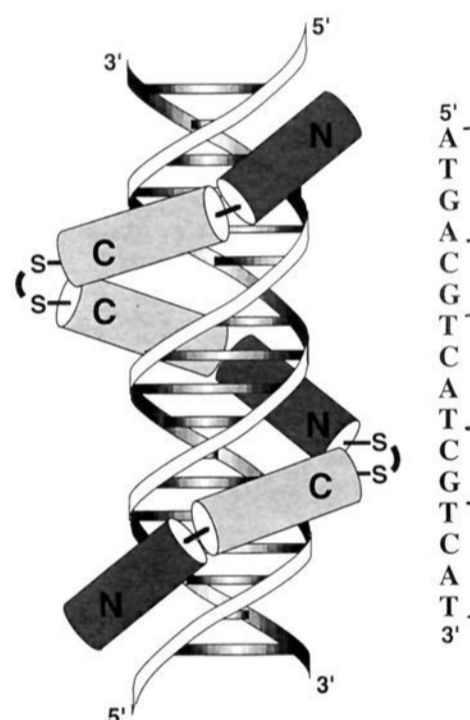
1. Introduction

Proteins that bind selectively to a specific DNA binding site play important roles in biological systems. Thus the regulation of cellular reactions (including replication, transcription, and translation) is mostly mediated by the specific interactions of DNA binding proteins with DNA.[1] As a result, design and synthesis of sequence-specific DNA binding proteins are of great interest in modern chemical biology.

Synthesis of peptides specifically recognizing long sequences (more than 10 base pairs (bp's)) of DNA is also important in mapping large genomes. Most known restriction enzymes recognize 4–8-bp sites, creating too many fragments to be handled when used to digest genomic DNA. Many attempts have been developed to recognize (and cleave) specific longer sites of DNA.[2–8] However, most of the current methods are indirect, requiring a series of steps (protection, chemical modification, and deprotection) to obtain the desired results.

We illustrate here the protein stitchery approach for designing a new protein to recognize a specific long site (16 bp's) of DNA. This is illustrated in Figure 1, which contains three fragments each corresponding to the basic region of v-Jun.

v-Jun is a member of the leucine zipper protein class of regulatory proteins for DNA transcription. It binds as a homodimer or as a heterodimer with Fos to a DNA site having

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(1) Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem.* **1984**, *53*, 293–321.
(2) McClelland, M.; Kessler, L.; Bittner, M. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 983–987.
(3) Pei, D.; Corey, D. R.; Schultz, P. G. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 9858–9862.
(4) Koob, M.; Grimes, E.; Szybalski, W. *Science* **1988**, *241*, 1084–1086.
(5) Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73–75.
(6) Ferrin, L. J.; Camerini-Otero, R. D. *Science* **1991**, *254*, 1494–1497.
(7) Sluka, J. P.; Hovath, S. J.; Bruist, M. F.; Simon, M. I.; Dervan, P. B. *Science* **1987**, *238*, 1129–1132.
(8) Mack, D. P.; Iverson, B. L.; Dervan, P. B. *J. Am. Chem. Soc.* **1988**, *110*, 7572–7574. Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73–75.
(9) Struhl, K. *Cell* **1987**, *50*, 841–846.

Figure 1. Schematic diagram for the complex between the peptide trimer pCC·NC and the trimer binding site o-CC·NC. The proposed binding site sequence for the top strand of DNA (see Figure 2b) is shown on the right of the diagram. The current and previous experimental results[13–15] suggest that the peptide wraps around the DNA along the major groove to recognize all three monomer binding sites.

dyad symmetry.[10,11] A recent X-ray crystal structure for the complex of GCN4 (another leucine zipper protein) and DNA[12,19] shows that the dimerization is mediated by the leucine zipper region and that each basic region forms an α-helix as it recognizes the half-site of the dimer binding site. The α-helix of the protein–DNA complex may bend depending on the nature of the binding site. In the absence of the specific DNA binding site, the basic region of the leucine zipper protein has a flexible structure in solution. However, it changes to α-helix when bound to the specific site of DNA.[22,23]

We use gel retardation and footprinting assays to show that the new peptide stitched together from three v-Jun basic regions

(10) Kaki, Y.; Bos, T. J.; Davis, C.; Starbuck, M.; Vogt, P. K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 2848–2852.
(11) Bos, T. J.; Bohmann, D.; Tsuchie, H.; Tjian, R.; Vogt, P. K. *Cell* **1988**, *52*, 705–712.
(12) Ellenberg, T. E.; Brandl, C. J.; Struhl, K.; Harrison, S. C. *Cell* **1992**, *71*, 1223–1237.

(a) Peptides (amino terminus on the left)

v-Jun-br:		S	QERIKAERKR	MRNRIAASKS	RKRKLERIAR	
v-Jun-N :	CGG	S	QERIKAERKR	MRNRIAASKS	RKRKLERIAR	
v-Jun-C :		S	QERIKAERKR	MRNRIAASKS	RKRKLERIAR	GGC
v-Jun-NC:	CGG	S	QERIKAERKR	MRNRIAASKS	RKRKLERIAR	GGC

(b) Oligonucleotides

o-CC·NC : 5'-ctcagatccggatcctaggttaaacgATGAcgTCATcgTCATcggtataggtcgagaattcggatcct-3'
           3'-gagtctaggcctaggatccaatttgcTACTgcAGTAgcAGTAgccatatccagctcttaagcctagga-5'

o-CC   : 5'-ctcagatccggatcctaggttaaacgATGAcgTCATcggtataggtcgagaattcggatcct-3'
           3'-gagtctaggcctaggatccaatttgcTACTgcAGTAgccatatccagctcttaagcctagga-5'

o-CN   : 5'-ctcagatccggatcctaggttaaacgATGAcgATGAcggtataggtcgagaattcggatcct-3'
           3'-gagtctaggcctaggatccaatttgcTACTgcTACTgccatatccagctcttaagcctagga-5'

(c) Procedure to make pCC·NC
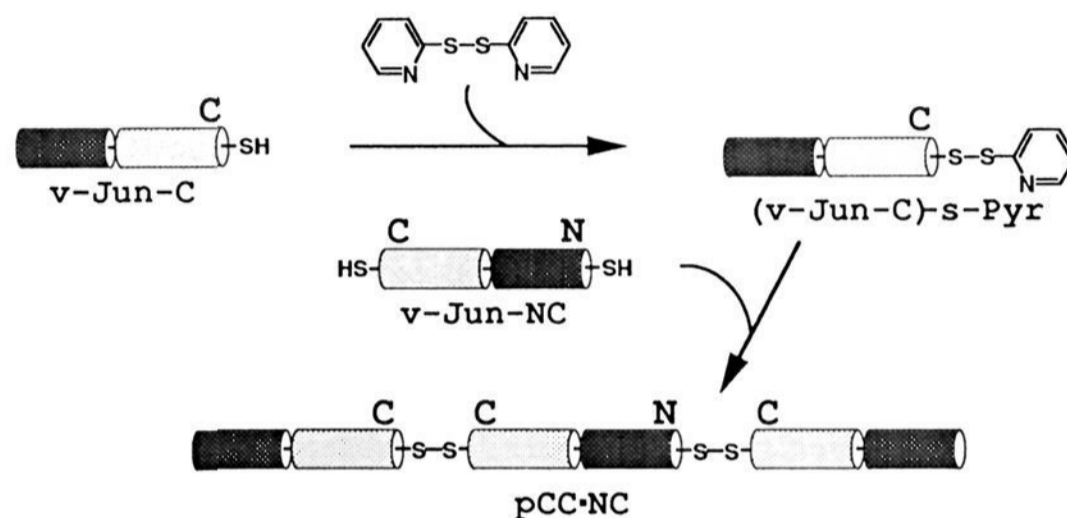
Figure 2. Sequences of protein (a) and oligonucleotides (b) used in the gel retardation and footprinting studies. The total length of oligonucleotides 'o-CC, o-CN, and o-CC·NC are 62, 62, and 68, respectively. The peptide v-Jun-br contains the basic region of v-Jun (amino acids 214–244).[9] v-Jun-N and v-Jun-C were prepared as described previously.[13–15] v-Jun-CN (which is equivalent to v-JUN-NC) was chemically synthesized and purified, and the purity was checked by mass spectroscopy at the Biopolymer Synthesis Center at the California Institute of Technology as described previously:[13,15] calcd, 4256.4; exptl, 4259.1. (c) Strategy for making the pCC·NC trimer.

binds selectively to the 16-bp site of DNA composed of three half-sites (appropriately oriented) for the v-Jun dimer. These results provide further insight on the interaction of leucine zipper proteins with DNA.

2. Materials and Experiments

2.1. Peptides and Oligonucleotide Synthesis. The peptide monomers v-Jun-N, v-Jun-C, and v-Jun-CN (see Figure 2a) were prepared as described previously.[13–15] The automated stepwise syntheses were done on an Applied Biosystems model 430A peptide synthesizer with an optimized synthetic protocol of the *N-tert*-butoxycarbonyl (*t*-Boc) chemistry. The peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) on a Vydac C18 column. A linear gradient of 0–50% aqueous/acetonitrile/0.1% trifluoroacetic acid was run over 120 min.

The oligonucleotides o-CC, o-CN, and o-CC·NC (indicating an oligonucleotide containing the proposed binding site of pCC·NC, see below for the notation) were used to mimic various DNA binding sites as shown in Figure 2b. These were synthesized using the facilities at the Biopolymer Synthesis Center at Caltech. o-CC has the binding site (ATGAcgTCAT) of the v-Jun dimer while the others are formed with various rearrangements of the half-site. The synthesized oligonucleotides were purified by using 10% denaturing polyacrylamide gel, and duplexes were made between complementary oligonucleotides if needed.

2.2. Synthesis of the Peptide Dimer and Peptide Trimer. The procedure to synthesize homodimer pCC is straightforward. In oxidizing conditions (5 mM oxidized dithiothreitol) v-Jun-C dimerizes to form pCC. However, to synthesize heterodimer pCN requires additional steps. In order to form pCN without also forming pCC and pNN, we activated the thiol group of v-Jun-C using 2,2'-dithiodipyridine (see Figure 2c) and purified the resulting thiopyridyl-(v-Jun-C) with HPLC.[14–17] This was reacted with purified v-Jun-N to form pCN.

To form the trimer pCC·NC (indicating a peptide trimer consisting of three monomer arms connected by two disulfide bonds; one is made between two C-termini of the first and second arms, and the other one is made between the N-terminus of the second arm and the C-terminus of the third arm), we used a similar procedure in which purified monomer v-Jun-NC was reacted with excess (3 equiv) thiopyridyl-(v-Jun-C) to make the trimer product pCC·NC (see Figure 2c), which was purified by HPLC.[14,15] To verify the formation of peptide heterodimer and peptide heterotrimer, HPLC analyses were done with the purified pCC·NC and pCN and with pCC·NC and pCN reduced by 20 mM dithiothreitol (DTT). The HPLC analysis (Figure 3) showed that reduction of pCC·NC yields only the two peaks corresponding to v-Jun-NC and v-Jun-C in the expected 1:2 ratio, while pCN shows two peaks corresponding to v-Jun-C and v-Jun-N in the expected 1:1 ratio. This HPLC analysis confirms the formation of heterotrimer pCC·NC and heterodimer pCN because each of v-Jun-C and v-Jun-N has only one thiol group on one terminus, while v-Jun-CN has two thiol groups on

(13) Park, C.; Campbell, J. L.; Goddard, W. A., III. *Proc. Natl. Acad. Sci. U.S.A* **1992**, *89*, 9094–9096.

(14) Park, C.; Campbell, J. L.; Goddard, W. A., III. *Proc. Natl. Acad. Sci. U.S.A* **1993**, *90*, 4892–4896.

(15) Park, C. Ph.D. Thesis, Caltech, May 1993.

(16) Corey, D.; Schultz, P. G. *Science* **1987**, *238*, 1401–1403. Zuckermann, R.; Corey, D.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 1614–1615.

(17) Zuckermann, R.; Corey, D.; Schultz, P. G. *Nucleic Acids Res.* **1987**, *15*, 5305–5321.

(a)

Heterotrimer
pCC·NC

(b)

Reduced
v-Jun-C

Reduced
v-Jun-CN

(c)

Heterodimer
pCN

(d)

Reduced
v-Jun-N
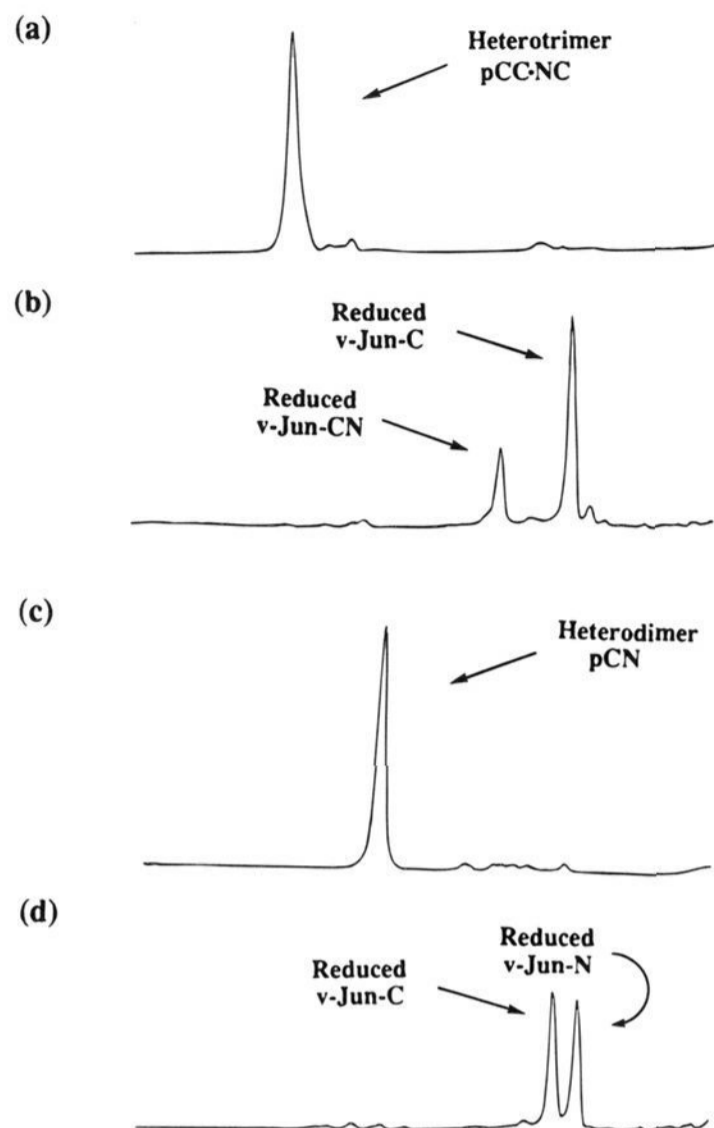
Reduced
v-Jun-C

Figure 3. HPLC analysis of peptide dimer and trimer. (a) Purified heterotrimer pCC·NC and (b) reduced pCC·NC with 20 mM DTT for 4 h at 25 °C. (c) Purified heterodimer pCN and (d) reduced pCN as in b. The results of HPLC analysis show that the heterotrimer pCC·NC consists of 1 equiv of v-Jun-CN and 2 equiv of v-Jun C, while pCN consists of 1 equiv of v-Jun-C and 1 equiv of v-Jun-N as expected.

both termini, each of which is eligible to make a disulfide bond with another thiol group.

2.3. Gel Retardation and Footprinting Assays. Gel retardation and footprinting assays were carried out as described previously.[13,15] The binding solution of gel retardation contains bovine serum albumin at 50 mg/mL, 10% (v/v) glycerol, 20 mM Tris-HCl (pH 7.5), 4 mM KCl, 2 mM MgCl$_2$, and 3 nM of appropriate peptides in 10 μL reaction volume. After adding 5000 cpm of each 5'-^{32}P-labeled probe DNA as indicated, the solutions were stored at 4 °C for 1 h and loaded directly on an 8% nondenaturing polyacrylamide gel in TE buffer at 4 °C. The gel was equilibrated for 2 h at 20 mA before the samples were loaded, and electrophoreses were performed for 3 h at 100 V at 4 °C after the samples were loaded.

The gel was dried and exposed to Kodak storage phosphor screen SO 230 (from Molecular Dynamics) in the dark room for 2 h. A Molecular Dynamics 400S PhosphoImager and IMAGEQUANT version 3.0 were used to integrate the volume of each rectangle drawn around the free and bound bands in the same dimension (see Table 1).

The footprinting assay solution (in 50 L) contains bovine serum albumin at 50 mg/mL, 5% glycerol, 20 mM Tris-HCl (pH 7.5), 4 mM
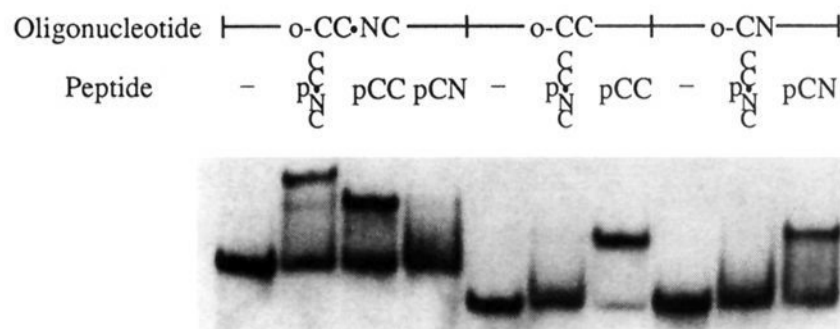
Figure 4. Gel retardation assays for binding of pCC·NC, pCC, and pCN to o-CC·NC, o-CC, and o-CN. These studies were carried out as described in the text. A 3 nM solution of each peptide was used in a 10 μL reaction volume containing 5000 cpm of the appropriate oligonucleotide.

KCl, 2 mM MgCl$_2$, 1 mM CaCl$_2$, and 20 000 cpm of each 5'-^{32}P-labeled probe DNA (60–62 bp) and 50 nM v-Jun-NN. This solution was stored at 4 °C for 1 h. After adding 5 μL of DNase I diluted in 1× footprinting assay buffer, the solutions were stored 1 min more at 4 °C. The DNase I digestion was stopped by addition of 100 μL of DNase I stop solution containing 15 mM EDTA (pH 8.0), 100 mM NaCl, 25 μg/mL sonicated salmon sperm DNA, and 25 μg/mL yeast tRNA. This was phenol/chloroform extracted, ethanol precipitated, and washed with 70% ethanol. The pellet was resuspended in 5 μL of formamide loading buffer, denatured at 90 °C for 4 min, and analyzed on 10% denaturing polyacrylamide sequencing gel (50% urea).

3. Results

3.1. Specificity of pCC·NC for o-CC·NC. The gel retardation assays (Figure 4) show that pCC·NC binds to o-CC·NC, which has the exact site designed to simultaneously bind all three arms of pCC·NC. On the basis of gel shift titrations the binding constant is about 5 nM (see Table 1). However, the gel retardation assays show very weak binding (40–50 nM) of pCC·NC to o-CC or o-CN, each of which has a site for two arms of pCC·NC. Combined with the results for v-Jun homodimers, this indicates that pCC·NC makes contact with about 16 bp's of DNA (about 1.5 turns of duplex DNA) along the major groove (see Figure 1).
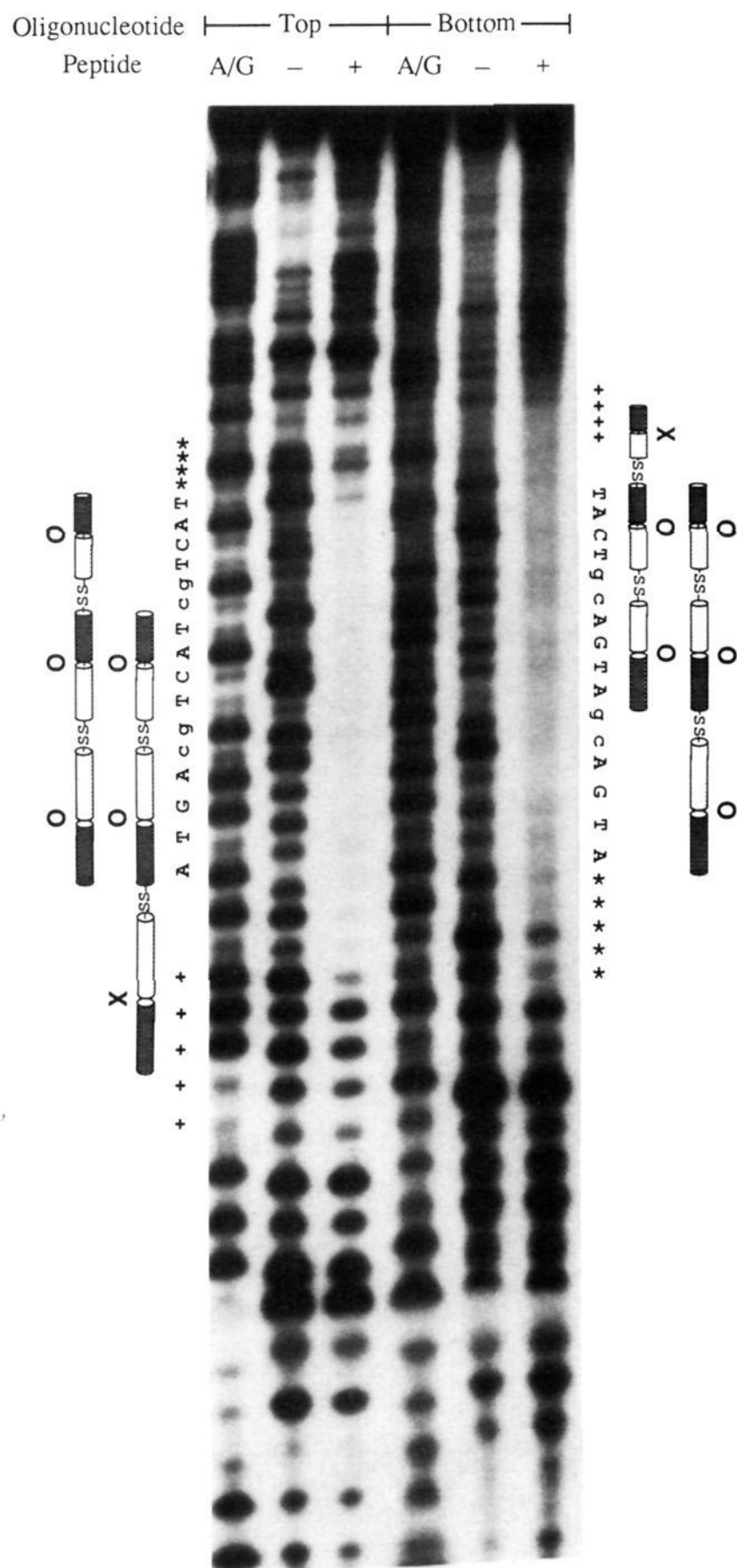
The DNase I footprinting assays (Figure 5) show that the new peptide pCC·NC protects the full proposed binding site, confirming the results from gel retardation assays. These results indicate that each of the three arms of pCC·NC binds to the proposed half-site, protecting each of the three half-sites from DNase I digestion (see Figure 1).

3.2. Binding of pCC and pCN to the Dimer and Trimer Binding Site. The results of gel retardation show that pCC and pCN bind to their proposed binding site with dissociation constants of about 2 and 6 nM, respectively. This in good agreement with our previous experiments.[14] pCC and pCN bind to the trimer binding site, o-CC·NC, about three times more weakly than to the dimer binding sites, o-CC and o-CN, respectively. This indicates that the additional monomer binding site in o-CC·NC compared to the dimer binding site interferes with the dimers in binding to their dimer binding sites. This

Table 1. Results of Titration of the Gel Shift Using a Molecular Dynamics 400S PhosphorImager

	o-CC·NC				o-CC			o-CN		
peptide	no	pCC·NC	pCC	pCN	no	pCC·NC	pCC	no	pCC·NC	pCN
bound	3682[a]	35469	35522	12434	2479[a]	5802	38385	3056[a]	10243	46431
free	72854	47297	52981	61928	61515	58087	20701	89452	88586	86518
ratio[b]		0.645	0.601	0.141		0.057	1.735		0.081	0.501
K_d[c] (nM)		4.7	5.0	21.3		52.6	1.7		37.0	6.0
ΔG_a[d] (kcal/mol)		10.6	10.5	9.7		9.2	11.1		9.4	10.4

[a] These values are used to correct the background for the bound band, used as (background) below. [b] Ratio = (bound)*/(free), where (bound)* = (bound) − (background) as described in a. [c] $K_d = 1/K_a = $ [P][D]/[PD] = [P](free)/(bound), where [P], [D], and [PD] indicate the concentrations of peptide, DNA binding site, and peptide/DNA complex, respectively. [d] $\Delta G_a = -RT \ln K_a = RT \ln K_d$ at $T = 277.15$ K (4 °C).

Figure 5. DNase I footprinting assays of pCC·NC with o-CC·NC were performed as described in the text. A 50 nM solution of pCC·NC was used with 50 000 cpm of o-CC·NC in a 50 μL reaction volume. The first column to the left and right shows the sequence of the o-CC·NC active site, and the outer column on each side shows the p-CC·NC protein bonded to this site. Clearly this entire region is protected. In addition the two sites next to the active site will generally show protection. However the observations show additional protection on the 5′ side of the top strand and the 5′ side of the bottom strand. Additional protection is given next to the last column on each side which shows the semispecific binding of the pCC-NC protein to the o-CC site. This leads to exactly the additional protection of the sites labeled as ++++ (5′ side of site) but not to protection of the sites labeled **** on the 3′ side.

implies that there might be some direct interaction between the dimer and the added monomer binding site or that the added monomer binding site affects the binding of dimer to the neighboring dimer binding site indirectly in an unknown way (for example, by changing the conformation of DNA).

3.3. Binding of Heterotrimer to Dimer Binding Site. The

heterotrimer binds specifically to the proposed trimer binding site. However, the gel retardation results show that there is also a weak binding to the dimer binding sites. Gel titration (Table 1) shows that the heterotrimer pCC·NC binds to the proposed trimer binding site, o-CC·NC, about 10 times more strongly than to the dimer binding sites, o-CC and o-CN. This is equivalent to a free energy difference of about 1.3 kcal/mol. In another words, the third arm of the heterotrimer stabilizes the trimer by about 1.3 kcal/mol when bound to the trimer binding sites compared to the dimer binding site. However, for binding to o-CC, the third arm destabilizes the binding relative to pCC by 1.9 kcal/mol and, for binding to o-CN, the third arm destabilizes the binding relative to pCN by 1.0 kcal/mol. This results in destabilizing the binding of the other two monomers to the dimer binding site by about 30 times for o-CC and about six times for o-CN. Therefore, compared to the dimers, the additional arm of pCC·NC trimer destabilizes the binding of the trimer to the imperfect binding site while it stabilizes the binding of trimer to the trimer binding site.

3.4. Semispecific Binding of pCC·NC. The footprinting studies also provide some evidence for *semispecific binding* in which the pCC·CN protein is reversed so that it recognizes only the o-CC binding site of o-CC·NC. The location of the p-CC·CN protein on the full o-CC·NC binding site is indicated by the outer columns of Figure 5 (where O indicates specific binding). The reversed p-CC·NC protein can also recognize the o-CC region as indicated in the next to the last column of Figure 5 (here X indicates nonspecific binding), which is about 10 times weaker than the specific binding.

In such semispecific binding the nonspecifically bound arm would create partial protection on the bases beyond the 5′ end of the protein site for the top strand and on the bases beyond the 3′ end of the protein binding site for the bottom strand. At the same time the semispecific binding would lead to incomplete protection on the 3′ end of the protein binding site on the top strand and of the 5′ end on the bottom strand. Therefore, such semispecific binding would result in a quite asymmetric protection pattern around the binding site.

The results of DNase I footprinting (Figure 5) show this expected asymmetry. For the top strand of DNA, the partially protected region is expanded far beyond the protein binding site (up to the seventh base) in the 5′ region, whereas for bottom strand of DNA, the last two base pairs in the 5′ region of protein binding site are not completely protected. The reverse situation occurs for the 3′ regions, where extra protection occurs for the bottom strand and less occurs for the top strand.

4. Discussion

Polypeptides can recognize more than one turn of DNA (that is, more than 10 bp's of DNA) in two ways: (1) by wrapping around the DNA along the major groove and (2) by approaching the binding site from one face of the DNA. Case 2 requires the polypeptide to also interact with the minor groove of DNA, while case 1 allows binding to only the major groove. Case 1 is much easier to design than case 2 because an α-helix fits nicely into the major groove of DNA but not into the minor groove. However, to wrap around the DNA, the polypeptide must be sufficiently flexible to follow the major groove of DNA along its helical pathway. If the structure of the polypeptide is too rigid, it cannot wrap around the DNA to recognize an additional turn of the DNA. The basic region of the leucine zipper protein is an ideal candidate to satisfy all these criteria. It has no fixed structure in solution in the absence of its specific DNA binding site, but it changes into an α-helix when bound to the specific DNA binding site. From our previous experi-

ments,[14] each one of the v-Jun basic regions exactly recognizes its monomer binding site independently of the relative orientation of the additional basic regions (connected through a disulfide bond between the thiol groups of cysteines added on the terminus of the peptide monomer).

The new results show that the new peptide trimer pCC·NC specifically binds to the proposed trimer binding site of o-CC·NC (see Figure 1) but also binds about 10 times more weakly to the dimer binding sites, o-CC or o-CN. This protein stitchery strategy can be used to design other new peptides for recognizing new or longer sites. Thus we would decompose the target site in terms of segments (three to five base pairs) each of which is recognized by a portion of a DNA binding protein. The DNA binding regions would then be stitched together to form the full protein for selectively recognizing the new site.

In order to measure accurate free energy differences for a peptide to a different DNA binding site, direct competition assays between the DNA binding sites are required. However, we can estimate the free energy difference (see Table 1) from the free energies calculated using the intensity of the bound and free bands in Figure 4. Our current results show that the third peptide arm of pCC·NC compared to the dimer (pCC or pCN) (1) stabilizes the binding of pCC·NC when it finds a perfect trimer binding site, o-CC·NC, and (2) destabilizes the binding of pCC·NC to the incomplete binding site (o-CC or o-CN), as compared with the dimers binding to the dimer binding sites. This provides an explanation for the results of our previous experiments[13–15] where each peptide dimer (pCC, pCN, and pNN) selectively recognized the proposed dimer binding sites (o-CC, o-CN, and o-NN, respectively) but not the binding sites selectively recognized by the other peptide dimers.

These studies provide additional observations that should be useful in elucidating the details of protein-DNA recognition. Thus pCC shows a binding affinity for the o-CC·NC site of about one-third of the affinity for o-CC even though o-CC·NC contains a binding site for pCC (Table 1). Similarly pCN shows a binding affinity[19] for the o-CC·NC site of about one-fourth of the affinity for o-CN even though o-CC·NC has a binding site for pCN. This implies that the half-site added next to the binding site of pCC (or pCN) to make the binding site of pCC·NC interferes with pCC (or pNN) in binding to the dimer binding site. Additional recent results[18] show that the basic region of v-Jun by itself recognizes the dimer binding site specifically without dimerization. This implies that the interaction between the monomer of basic region of v-Jun and the monomer binding site is strong enough to retain the complex. Therefore, it is reasonable to propose that a direct interaction between the dimer and the added monomer binding site in o-CC·NC compared to the o-CC (or o-CN) interferes with the

dimer in binding to the neighboring dimer binding site. However, it may also be that other indirect effects interfere with the dimer binding site.

For the top strand of o-CC·NC there is partial protection on the 3′ end bases flanking the binding site. The reason for this partial protection is that p-CC·NC also exhibits semispecific binding to the o-CC portion of the site. Such semispecific binding is supported by the observation that the glucocorticoid receptor recognizes the incorrect spaced binding site semispecifically, with one subunit binding specifically with the correct half-site and the other nonspecifically with a noncognate site.[20] Similarly the basic region of GCN4 shows a relatively strong binding affinity for the randomized sequence of DNA,[21] indicating it is possible for the basic region to have a nonspecific interaction with the nonspecific sequence of DNA. Our results do not indicate if the affinity of nonspecific binding depends on the DNA sequence.

Comparing the binding of pCC·NC to o-CC·NC and pCC to o·CC, there is no gain in binding energy from dimer to trimer even though the trimer binds to the trimer binding site 10 times stronger than to the dimer binding site. These results suggest that the added linker on the terminus of the peptide monomer to replace the leucine zipper region is not flexible enough (or long enough) to wrap around 1.5 turns of DNA, resulting in strain on the trimer. This does not happen in the case of dimers because they need only to wrap around about one turn of DNA. Therefore a more flexible (or longer) linker than the present one (Gly-Gly-Cys) may improve the binding affinity of the trimer to the trimer binding site.

We are now in the process of using molecular modeling, molecular dynamics, and thermodynamic perturbation theory to determine the details concerning the protein DNA recognition and to explain the origins of the above results.

(18) Park, C.; Campbell, J. L.; Goddard, W. A., III. The monomer of the DNA binding region of the v-Jun leucine zipper protein recognizes the dimer binding site without dimerization. To be submitted for publication.

(19) Konig, P.; Richmond, T. J. *J. Mol. Biol.* **1993**, *233*, 139–154.

(20) Luisi, B. F.; Xu, W. X.; Otwinowski, Z.; Freedman, L. P.; Yamamoto, K. R.; Sigler, P. B. *Nature* **1991**, *352*, 497–505.

(21) Cuenond, B.; Schepartz, A. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 1154–1159.

(22) Weiss, M. A. *Biochemistry* **1990**, *29*, 8020–8024.

(23) Patel, L.; Abate, C.; Curran, T. *Nature (London)* **1990**, *347*, 572–575.