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Structure–Function Relationship in a β-Sheet Peptide Inhibitor of E47 Dimerization and DNA Binding

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Abstract—A β-sheet peptide inhibitor, 2H10, has been developed that inhibits the dimerization of the transcription factor E47. Inhibition of E47 dimerization has been demonstrated to also inhibit the DNA binding of this transcription factor. Truncated peptides based on 2H10 have demonstrated that the β-sheet content of these peptides directly correlates with their inhibitory properties. Individual residues within 2H10 were identified that were responsible for the β-sheet secondary structure by employing an alanine replacement strategy. The β-sheet character of the alanine mutants also correlated well with their inhibition of E47 DNA binding. These results provide further evidence that interactions between the interfacial peptide inhibitors of E47 and the transcription factor itself are mediated by a β-sheet structure.

Introduction

Protein regulation of DNA transcription through sequence specific DNA binding is essential for normal cell growth and differentiation. There are examples, however, of transcription factors that are implicated in abnormal cellular proliferation. Oncogenic transcription factors of viral or cellular origin, for instance, which are no longer regulated by normal signal transduction processes or whose production is deregulated play significant roles in cell proliferation.[1–3] Chromosomal translocations have also been documented wherein immunoglobulin genes come into close proximity to transcription factor protooncogenes resulting in activation, or translocations occur in the middle of transcription factor genes resulting in fusion proteins with aberrant DNA binding properties.[4] There is great interest, therefore, in developing strategies to regulate the DNA binding of transcription factors.

Since transcription factors often exist as oligomers, interference with the assembly of active oligomeric structures could lead to transcriptional regulation. The goal of our work has been the inhibition of transcription factor–DNA interactions by specifically disrupting the protein–protein interactions of dimeric transcription factors, in this case the immunoglobulin enhancer binding protein E47 (E47). This strategy is utilized in the cellular mechanism for transcription factor inhibition, and relies on the formation of heterodimers between the active transcription factor and an inactive protein variant. Heterodimers of E47 with counterparts such as Id[5–7] and Twist,[8] HLH proteins lacking DNA binding basic regions, are incapable of DNA binding and gene activation. However, this strategy can be taken a step further by the design of therapeutic agents that interfere specifically with the assembly of the proper quaternary structure of a target transcription factor. This strategy has been called 'dimerization-inhibition' and has been recently reviewed in the context of oligomeric enzymes and receptor–subunit interactions.[9] However, the use of small molecules, such as peptides, to inhibit the assembly of dimeric transcriptional factors is still in its infancy.[10–12] It was our goal to test the concepts of dimerization inhibition in the context of the dimeric transcription factor E47.

E47 as a model system for testing dimerization inhibition

E47 is one of the class of dimeric basic-helix-loop-helix (bHLH) proteins.[13] The co-crystal structure of the dimeric bHLH region of E47 with DNA has been solved, and the basic region of E47 is found in the major groove of DNA in a helical conformation with two amphiphilic helices of each monomer forming the dimeric, four-helix bundle interface (Fig. 1).[14] E47 plays an important role in activating expression of the immunoglobulin light chain gene through binding at the κE2 enhancer site,[15] and also binds the μE2 and μE5 enhancer sites in a pre-T-cell line resulting in immunoglobulin heavy chain transcription.[16] The gene for E47 (E2A) is involved in chromosomal translocations that have been found in patients with pre-B-cell acute lymphoblastic leukemia.[17]

Dimerization of E47 is crucial for its cellular activity; thus, the identification of small peptides that can interfere with

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the dimerization behavior of E47 would serve to regulate DNA binding as well (Fig. 1). We have recently shown that interfacial peptides that correspond to the dimerization domain of E47 could inhibit E47 dimerization, and thereby prevent the DNA binding activity of E47.[10] This strategy allowed us to identify a 20 amino acid peptide, helixII (Fig. 2), corresponding to the second helix of the bHLH transcription factor E47. HelixII was found to completely inhibit E47 DNA binding at equimolar concentrations and displayed a β-sheet secondary structure by circular dichroism (CD) spectroscopy. Furthermore, CD studies indicated that helixII induced a β-sheet structure in the normally helical E47 protein.

Experimental design

The further observation that the 10 residue peptide, 2H10 (Fig. 2), adopted a β-sheet secondary structure and abrogated E47 DNA binding,[10] provided the impetus for deconvoluting the determinants for its β-sheet formation and inhibitory properties. Truncating the 2H10 peptide from the C-terminus would provide 2H9, 2H8 and 2H7 (Fig. 2), peptides that were predicted to have a high β-sheet propensity.[18–21] These peptides would allow us to determine if a correlation existed between their ability to form a β-sheet secondary structures and their inhibitory potential. A control peptide, 2C10 (Fig. 2), corresponding to the C-terminus of helixII would allow us to test our hypothesis that the β-sheet propensity of helixII mapped to its N-terminus.

In concert with the C-truncation studies of 2H10, we wished to identify the individual residues within 2H10 that were responsible for its β-sheet secondary structure. To this end, an alanine replacement strategy was employed. In this approach, every residue in the peptide would be individually replaced by an alanine, as a means to address the specific role of the substituted residue (Fig. 3). We hypothesized that residues that were important for the stability of the β-sheet structure within the peptides would also be necessary for effective inhibition of the DNA binding of E47.

Results and Discussion

Structural studies on truncated peptides

Initial structural studies with the 2H10 peptide inhibitor of E47 demonstrated that 2H10 adopted a β-sheet conformation that was not dependent on concentration or temperature.[10] This type of behavior for a β-sheet containing peptide usually indicates that the peptide is forming an aggregate at low concentration that persists at high concentration. Furthermore, analytical ultracentrifugation studies (8000 and 12000 rpm rotor speeds) confirmed that 2H10 (25 and 100 μM) is in an oligomeric state. This peptide sedimented as a species with a molecular weight of 57,200, a value that corresponds to approximately 50 peptide units in an aggregate, as was previously found with the full length peptide helixII.[10]

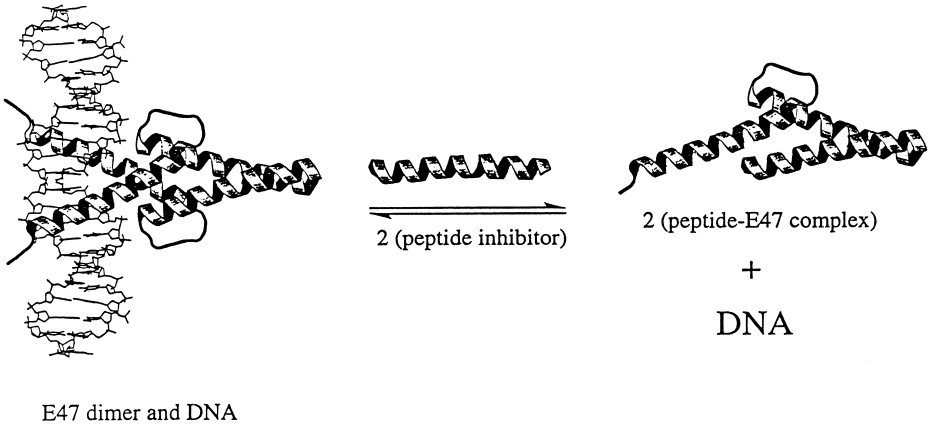

Figure 1. Schematic representation of the strategy for inhibition of E47 dimerization and DNA binding.

	BASIC / HELIX I / LOOP / HELIX II
E47	ERRMANNARERVRVRDINEAFRELGRMCQMHLKSDKAQTKLLILQQAVQVILGLEQQVR-H
helixII	KLLILQQAVQVILGLEQQVR-NH_2
2H10	KLLILQQAVQ-NH_2
2H9	KLLILQQAV-NH_2
2H8	KLLILQQA-NH_2
2H7	KLLILQQ-NH_2
2C10	VILGLEQQVR-NH_2

Figure 2. The sequences of E47, helixII, and truncated peptides.

CD spectroscopy was performed to investigate whether or not the truncated peptides (2H9, 2H8, 2H7, and 2C10) also adopted a β-sheet conformation (Fig. 4). The CD spectrum of 2H9 (50 μM) was found to exhibit β-sheet character, as seen by the minima at 218 nm, but its relative β-sheet content was only 58% that of the parent peptide 2H10.[10] The peptides 2H8, 2H7, and 2C10, however, exhibited random-coil CD signatures even after prolonged incubation at room temperature and up to concentrations of 150 μM.

IR spectroscopy also provided secondary structural assignments for 2H10, 2H9, 2H8, and 2H7 at concentrations of 1 mM in D_2O (Fig. 5). The second derivative spectrum of 2H10 was similar to the spectrum obtained with helixII, and showed a large band at 1620 cm^{-1} and a smaller band at 1664 cm^{-1} which indicates that this peptide has β-sheet content (1620 cm^{-1}) and possible β-turn character as evidenced by the band at 1664 cm^{-1}.[22,23] Peptide 2H9 showed prominent bands at 1618 cm^{-1} and 1637 cm^{-1}, indicating a mixture of β-sheet structure and random-coil structure (1637 cm^{-1}) even at high concentrations. The IR spectrum of peptide 2H8, on the other hand, showed weak bands of equivalent intensity at 1637 cm^{-1} and 1618 cm^{-1}. Thus, at concentrations of 1 mM, 2H8 has some β-character (1618 cm^{-1}) not observed by CD experiments, suggesting that this truncated peptide aggregates as well. In contrast, the IR spectrum of 2H7 clearly showed the loss of the β-sheet structure as no signal was observed at approximately 1618 cm^{-1}, but instead bands were observed at 1650 cm^{-1} and 1637 cm^{-1}, which can be attributed to a random-coil secondary structure.

helixII	KLLILQQAVQVILGLEQQVR-NH_2
2H10	KLLILQQAVQ-NH_2
2H10Q10A	KLLILQQAV**A**-NH_2
2H10V9A	KLLILQQA**A**Q-NH_2
2H10Q7A	KLLILQ**A**AVQ-NH_2
2H10Q6A	KLLIL**A**QAVQ-NH_2
2H10L5A	KLLI**A**QQAVQ-NH_2
2H10I4A	KLL**A**LQQAVQ-NH_2
2H10L3A	KL**A**ILQQAVQ-NH_2
2H10L2A	K**A**LILQQAVQ-NH_2
2H10K1A	**A**LLILQQAVQ-NH_2

Figure 3. The sequences of alanine mutants of 2H10.

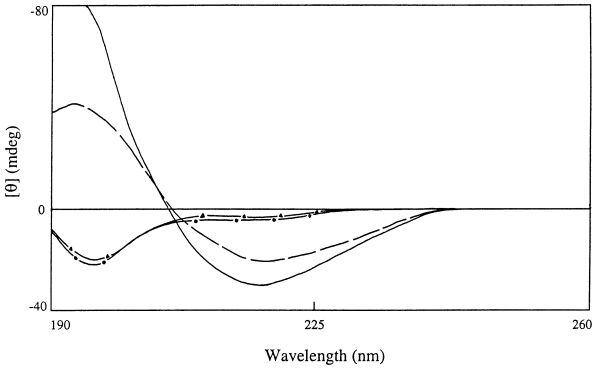

Figure 4. Circular dichroism spectra for 2H10 (—), 2H9 (—), 2H8 (-●-), and 2H7 (-▲-) at 50 μM.

DNA binding inhibition assays with truncated peptides

The inhibition of E47 DNA binding by the interfacial peptides, 2H9, 2H8, 2H7, and 2C10, was monitored by a gel mobility shift assay. The concentration of E47 (8 μM) and the ^{32}P-labeled oligonucleotide containing the κE2 enhancer sequence (CAGGTG) (10 nM) were kept constant. The degree of inhibition by the truncated peptides was measured by titrating in a necessary amount of peptide until the E47 DNA binding was abolished. IC_{50} values were obtained by the densitometric analysis of the relevant autoradiograms. Sigmoidal curves were obtained by plotting the decrease in intensity of the protein–DNA band as a function of inhibitor concentration. The IC_{50} values were determined by a least squares analysis of the linear portion of the sigmoidal curves. It was found that 2H9, 2H8, and 2H7 had IC_{50} values of 32 ± 3 μM, 53 ± 5 μM, and 71 ± 7 μM, respectively, compared to an IC_{50} value of 19 ± 2 μM for 2H10.[10] The control peptide 2C10, however, had no measurable inhibitory effect on E47 inhibition up to a concentration of 150 μM.

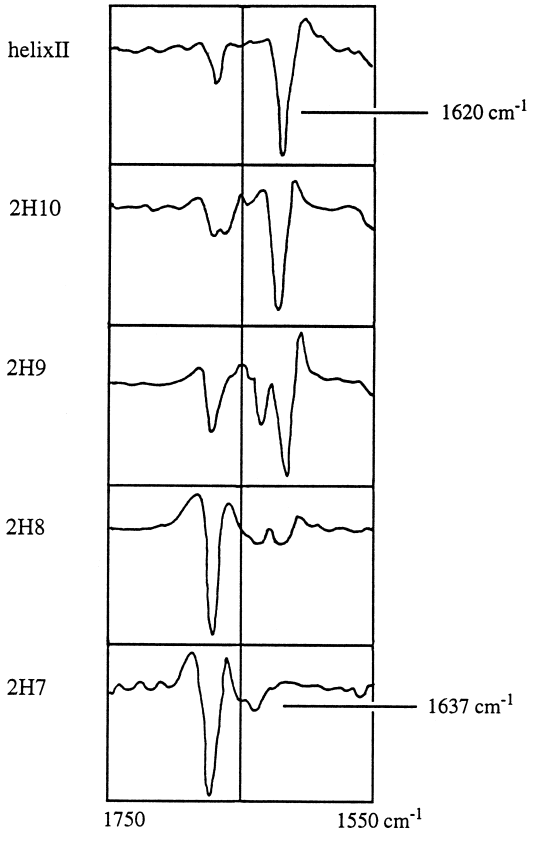

Figure 5. The second derivative of the IR spectra for 2H10 and truncated peptides. The prominent peak at 1673 cm^{-1} was assigned to trace amounts of salts of trifluoroacetic acid.

Structure–function comparisons with truncated peptides

The trend in the inhibition of E47 DNA binding agrees well with the observed β-sheet secondary structural characteristics of the truncated peptides from the CD and IR experiments. A sharp transition was observed both structurally and functionally from the deletion of a single glutamine residue from 2H10 ($IC_{50} = 19\,\mu M$) to 2H9 ($IC_{50} = 32\,\mu M$). The subsequent deletion of a valine residue results in a peptide, 2H8, that has β-sheet character only at high concentrations and an IC_{50} value of 54 μM, which upon further truncation to 2H7, displays no β-sheet character by CD and IR and a correspondingly low IC_{50} value of 71 μM. Thus it appears that the mechanism for effective inhibition of E47 with these peptides relies on their ability to form stable β-sheet structures.

Circular dichroism studies on alanine mutants of 2H10

CD spectroscopy was performed to investigate the secondary structure of the alanine mutants of 2H10. CD spectra recorded in the absence of any added NaCl indicated that only two peptides, 2H10A1K and 2H10A6Q adopted β-sheet structures displaying a single minima at 218 nm at 50 μM concentrations. However, the addition of 200 μM NaCl was sufficient to induce a random-coil to β-sheet transition in many of the alanine mutants of 2H10, implying that aggregation is taking place, an effect that is well documented for various β-sheet forming peptides.[24,25] In order to compare the relative β-sheet forming propensities of the alanine mutants, the ellipticity at 218 nm was measured in a buffer containing 200 mM NaCl (50 μM peptide) and compared to the β-sheet content of the parent 2H10 peptide (50 μM). This provided a measure of the relative percentage β-sheet content for each of the alanine mutants (Table 1).

The relative β-sheet content for peptides 2H10I4A (42%) and 2H10V9A (38%) were found to be the lowest, thus implying that Ile at position 4 and Val at position 9 are essential for β-sheet formation, which agrees well with β-sheet propensity scales.[18–21] The next set of important residues that were identified were Leu 2, Leu 3, Gln 7, Leu 9, and Gln 10 as their corresponding alanine mutants displayed 72%, 84%, 68%, and 70% of the relative β-sheet content of the parent peptide 2H10. The importance of the Leu residues in the formation of a β-sheet in 2H10 may at first be surprising, but Leu residues have been used in the design of β-sheet forming peptides despite having an average β-sheet propensity.[26] The importance of the Gln residues at position 7 and 10 was also not surprising as Gln residues have been used in designed β-sheets.[27] The least important residues were found to be Lys1, Leu5 and Gln6, as their corresponding mutants 2H10K1A, 2H10L5A, and 2H10Q5A displayed identical CD spectra when compared to the parent 2H10 peptide. Thus, a combination of hydrophobic residues, such as Ile, Val, and Leu, along with polar residues such as Gln contribute to the stability of the β-sheet structure of 2H10.

Table 1. Comparison between β-sheet content and E47 inhibition with alanine mutants of 2H10

Peptide	IC_{50} (μM)[a]	% β-sheet[b]
2H10	19	
2H10Q6A	21	100
2H10L5A	24	100
2H10L2A	35	72
2H10L3A	36	84
2H10Q10A	36	70
2H10Q7A	42	68
2H10V9A	47	38
2H10I4A	53	42
2H10K1A	54	100

[a]Values are ± 10%.
[b]As compared to 2H10

Gel mobility shift assay with alanine mutants of 2H10

IC_{50} values for the alanine mutants were determined in a similar manner as that of the truncated peptides, and the values are tabulated in Table 1 for comparison with the relative β-sheet content of each of the peptides. It was found that the IC_{50} values were all lower than that of the parent 2H10 peptide, and that the IC_{50} values correlated well with the observed β-sheet content for these peptides in 200 μM NaCl, with the exception of the 2H10K1A mutant. The 2H10L5A and 2H10Q6A mutants, which displayed identical β-sheet character as the parent 2H10 ($IC_{50} = 19\,\mu M$), also had similar inhibitory capabilities with IC_{50} values of 24 and 21 μM respectively. In addition, 2H10I4A and 2H10V9A, the two least stable β-sheet forming peptides, were also the worst inhibitors of E47 DNA with IC_{50} values of 53 and 47 μM, respectively. The only exception to this trend was the Lys1 to Ala mutant, 2H10K1A, which had a strong propensity for forming a β-sheet, but had a high IC_{50} value of 54 μM. This Lys residue has been shown to be critical for dimerization by Baltimore and coworkers, and may be necessary for mediating specific contacts between the peptide inhibitors and E47.[16]

Conclusion

By using truncation and alanine replacement approaches an intriguing peptide–protein interaction has been deconvoluted. Through these studies an α-helical segment (2H10) of a larger protein (E47) has been identified that forms a β-sheet secondary structure outside the framework of the parent protein, but retains the ability to interact and disrupt the dimeric E47 structure. Overall, the correlation between β-sheet formation and inhibitory potential within the truncated and mutated peptides reflects the essential nature of this β-sheet within the peptide–protein complex.

Experimental

Peptide synthesis

The truncated peptides, 2H9, 2H8, 2H7, and 2C10, were synthesized by solid phase methodology on a Rink amide resin[28] using a fluorenylmethyloxy-carbonyl (Fmoc)-based strategy on a Biosearch 9500 Peptide

Table 2. Characterization of truncated and mutated derivatives of 2H10

Peptide	MS	MW calcd	Amino acid analysis
2H10	1155.9	1154	Glx(3)3.1,Ala(1)1.0,Val(1)1.0,Ile(1)0.96,Leu(3)2.9,Lys(1)1.0
2H9	1027.5	1026	Glx(2)1.9,Ala(1)1.0,Val(1)1.0,Ile(1)1.0,Leu(3)2.7,Lys(1)1.1
2H8	925.8	926	Glx(2)2.0,Ala(1)0.94,Ile(1)1.1,Leu(3)3.1,Lys(1)1.0
2H7	854.8	853	Glx(2)1.9,Ile(1)1.0,Leu(3)2.9,Lys(1)1.0
2H10Q10A	1097.3	1097	Glx(2)2.2,Ala(2)2.0,Val(1)1.0,Ile(1)0.96,Leu(3)2.9,Lys(1)1.0
2H10V9A	1127	1126	Glx(3)2.9,Ala(2)2.2,Ile(1)1.2,Leu(3)2.7,Lys(1)0.7
2H10Q7A	1100	1097	Glx(2)1.9,Ala(2)2.0,Val(1)1.1,Ile(1)0.96,Leu(3)2.9,Lys(1)0.8
2H10Q6A	1098	1097	Glx(2)2.1,Ala(2)1.9,Val(1)1.2,Ile(1)1.0,Leu(3)2.7,Lys(1)1.1
2H10L5A	1113.2	1112	Glx(3)3.2,Ala(2)1.8,Val(1)1.0,Ile(1)0.96,Leu(2)1.8,Lys(1)0.9
2H10I4A	1114.2	1112	Glx(3)2.8,Ala(2)1.9,Val(1)1.0,Leu(3)2.9,Lys(1)1.1
2H10L3A	1111.3	1112	Glx(3)2.9,Ala(2)2.0,Val(1)1.2,Ile(1)0.96,Leu(2)1.8,Lys(1)1.0
2H10L2A	1112.8	1112	Glx(3)3.2,Ala(2)2.1,Val(1)0.8,Ile(1)0.96,Leu(2)1.8,Lys(1)1.0
2H10K1A	1098	1097	Glx(3)3.1,Ala(2)2.0,Val(1)1.2,Ile(1)1.1,Leu(3)3.2

Synthesizer. The 9 mutant peptides for the alanine scanning experiment were synthesized by a split-synthesis protocol where 10% of the resins was removed at each step of the synthesis and alanine was incorporated in place of the natural residue from the 2H10 sequence. The peptides were cleaved from the resin with a cocktail comprising of 90% TFA, 5% thioanisole, 3% ethanedithiol and 2% anisole for 4 h at room temperature. Following cleavage from the resins, the peptides were precipitated in cold anhydrous ether and stored at −20 °C overnight. The peptides were recovered from the ether solution by centrifugation and dried in vacuo. All peptides were purified to homogeneity using high performance liquid chromatography (HPLC) on a Vydac C8 reverse phase semipreparative column, 2.2 × 25 cm, 8.0 mL min^{-1}, mobile phase A (100% CH_3CN, 0.1% TFA) and mobile phase B (100% H_2O, 0.1% TFA). Peptides were purified with linear gradients over 60 min on a Waters Delta Prep 4000. The gradients for the C-truncated peptides were 10–45% A, 10–30% A, and 10–30% A for 2H9, 2H8, 2H7, and 2C10, respectively. All alanine mutants of 2H10 were purified at a gradient of 25–45% A. The eluent in all cases was monitored at 214 nm. The peptides were characterized by plasma desorption mass spectrometry, and amino acid analysis (Table 2).

Circular dichroism spectroscopy

CD spectra were recorded on a Jasco J600 spectropolarimeter at 24 °C in a buffer containing 0.5 mM phosphate at pH 7 with no NaCl added unless otherwise mentioned. The peptide concentrations were 50 μM as determined by amino acid analysis. Cells of path length 1 or 2 mm were employed.

Infrared spectroscopy

IR spectra were recorded on a Perkin–Elmer Model 1800 FT IR at room temperature in a 0.5 mM phosphate buffer at pH 7.0 in D_2O using a 0.05 mm path length CaF_2 cell. All spectra were acquired as an average of 10 scans with a resolution of 4 cm^{-1} from 1500 to 1800 cm^{-1}. The concentration of peptides was 1 mM for these experiments. A background spectra of D_2O was obtained before each scan and subsequently subtracted. The spectra were processed by taking the second derivative. A prominent peak at 1673 cm^{-1} was assigned to trace amounts of trifluoroacetic acid salts which was present in all our peptide samples stemming from the HPLC purification step.

Electrophoretic mobility shift assay (EMSA)

EMSA's were performed with a ^{32}P-labelled 29 bp double stranded oligonucleotide probe containing a CAGGTG DNA-binding sequence. The 5′ sense strand of the DNA probe was as follows: 5′-AATTCGCGGCCGCAGAA-CAGGTGGTCCAG-3′. The DNA was radiolabelled with the Klenow fragment of DNA Polymerase (New England Biolabs) in the presence of dCTP, dGTP, dTTP and 5′-α-^{32}P-dATP (Amersham) and purified through a Nick column (Pharmacia-Biotech) containing Sephadex G-25. Typical inhibition assays involved the incubation of E47 (10 μM) with or without a given concentration of the peptide inhibitor for 1 h in Buffer A (0.5 mM phosphate, 0.1 mM EDTA, 0.1 mM DTT, at pH 7.0). Incubation was followed by the addition of 10 nM labelled DNA probe, 10% glycerol and 1 mg poly-dIdC and further incubation for 30 min, in a total volume of 20 μL at room temperature. The reactions were analyzed by 8% nondenaturing PAGE. Densitometric analysis of the relevant autoradiograms allowed the measurement of the relative intensities of the protein–DNA bands. Sigmoidal curves were obtained by plotting the decrease in intensity of the protein–DNA band as a function of inhibitor concentration. The IC_{50} values were determined by a least squares analysis of the linear portion of the sigmoidal curves.

Acknowledgements

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References

1. Bishop, J. M. *Cell* **1991**, *64*, 235.
2. Hunter, T. *Cell* **1991**, *64*, 249.
3. Lewin, B. *Cell* **1991**, *64*, 303.
4. Rabbitts, T. H. *Nature* **1994**, *372*, 143.
5. Benezra, R.; Davis, R. L.; Lockshon, D.; Turner, D. L.; Weintraub, H. *Cell* **1990**, *61*, 49.
6. Sun, X.; Copeland, N. G.; Jenkins, N. A.; Baltimore, D. *Mol. Cell. Biol.* **1991**, *11*, 5603.

7. Fairman, R.; Beran-Steed, R.; Anthony-Cahill, S.; Lear, J.; Stafford, W.; DeGrado, W. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10429.
8. Spicer, D. B.; Rhee, J.; Cheung, W. L.; Lassar, A. B. *Science* **1996**, *272*, 1476.
9. Zutshi, R.; Brickner, M.; Chmielewski, J. *Curr. Opin. Chem. Biol.* **1998**, *2*, 62.
10. Ghosh, I.; Chmielewski, J. *Chem. Biol.* **1998**, *5*, 439.
11. Yao, S.; Brickner, M.; Pares-Matos, E.; Chmielewski, J. *Biopolymers: Peptide Science*, **1998**, in press.
12. Draeger, L. J.; Mullen, G. P. *J. Biol. Chem.* **1994**, *269*, 1785.
13. Murre, C.; McCaw, P. S.; Baltimore, D. *Cell* **1989**, *56*, 777.
14. Ellenberger, T.; Fass, D.; Arnaud, M.; Harrison, S. C. *Genes Dev.* **1994**, *8*, 970.
15. Lenardo, M.; Pierce, J. W.; Baltimore, D. *Science* **1987**, *236*, 1573.
16. Schlissel, M.; Voronova, A.; Baltimore, D. *Genes Dev.* **1991**, *5*, 1367.
17. Look, A. T. *Science* **1997**, *278*, 1059.
18. Chou, P. Y.; Fasman, G. D. *Biochemistry* **1974**, *13*, 211.
19. Kim, C. A.; Berg, J. M. *Nature* **1993**, *362*, 267.
20. Minor, D. L.; Kim, P. S. *Nature* **1994**, *367*, 660.
21. Smith, C. K.; Withka, J. M.; Regan, L. *Biochemistry* **1994**, *33*, 5510.
22. Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181.
23. Jackson, M.; Mantsch, H. H. *Crit. Rev. Biochem. Mol. Biol.* **1995**, *30*, 95.
24. Baumruck, V.; Huo, D.; Dukor, R. K.; Keiderling, T. A.; Lelievre, D.; Brack, A. *Biopolymers* **1994**, *34*, 1115.
25. Bertrand, M.; Sy, D.; Brack, A. *J. Peptide Res.* **1997**, *49*, 269.
26. Degrado, W. F.; Lear, J. D. *J. Am. Chem. Soc.* **1985**, *107*, 7684.
27. Aggeli, A.; Bell, M.; Boden, N.; Keen, J. N.; Knowles, P. F.; McLeish, T. C. B.; Pitkeathly, M.; Radford, S. E. *Nature* **1997**, *386*, 259.
28. Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787.