

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/247384278>

# Inhibition of DNA Binding by NF- $\kappa$ B with Pyrrole-Imidazole Polyamides †

ARTICLE *in* BIOCHEMISTRY · JUNE 2002

Impact Factor: 3.02 · DOI: 10.1021/bi020114i

---

CITATIONS

41

---

READS

18

4 AUTHORS, INCLUDING:



Joel Pomerantz

Johns Hopkins Medicine

27 PUBLICATIONS 1,612 CITATIONS

SEE PROFILE

Inhibition of DNA Binding by NF- $\kappa$ B with Pyrrole-Imidazole Polyamides<sup>†</sup>

Nicholas R. Wurtz, Joel L. Pomerantz, David Baltimore, and Peter B. Dervan\*

*Division of Chemistry and Chemical Engineering and Division of Biology, California Institute of Technology, Pasadena, California 91125**Received February 7, 2002; Revised Manuscript Received April 3, 2002*

**ABSTRACT:** Synthetic ligands that bind to predetermined DNA sequences will offer a chemical approach to gene regulation if inhibition of a broad range of transcription factors can be achieved. NF- $\kappa$ B is a transcription factor that regulates a multitude of genes, including those involved in immune, inflammatory, and anti-apoptotic responses. NF- $\kappa$ B binds as heterodimer predominantly in the major groove. We report the design of polyamides that bind in the minor groove and target overlapping portions of an NF- $\kappa$ B binding site (5'-GGGACTTTCC-3'). We find that compounds that target the 5'-GGGACT-3' portion of the site can inhibit DNA binding by NF- $\kappa$ B while those that target the 5'-ACTTTCC-3' portion do not. Addition of NF- $\kappa$ B to the list of protein–DNA complexes that can be disrupted by minor groove binding ligands potentially increases the utility of polyamides as regulators of gene expression.

NF- $\kappa$ B is a heterodimeric transcription factor activated by a variety of signal transduction pathways in many different cell types to effect rapid changes in gene expression (1). A diverse group of stimuli activate NF- $\kappa$ B, including bacterial and viral products, antigen receptors on immune cells, proinflammatory cytokines, and UV and  $\gamma$ -irradiation. NF- $\kappa$ B is bound in a latent form in the cytoplasm of cells by inhibitory I $\kappa$ B proteins. Many signaling pathways activate NF- $\kappa$ B by inducing the phosphorylation of I $\kappa$ B by the I $\kappa$ B kinase complex. After its phosphorylation, I $\kappa$ B is ubiquitinated, then degraded by the 26S proteasome, leaving NF- $\kappa$ B to translocate to the nucleus and bind to regulatory regions of target genes [for review see (2)].

Mice deficient in NF- $\kappa$ B subunits or in components of NF- $\kappa$ B-inducing signaling pathways have revealed that the proper regulation of NF- $\kappa$ B activity is critical for the normal development of certain tissues (e.g., fetal liver, osteoclasts, limbs, skin) and for normal innate and adaptive immune responses to several pathogens (3). NF- $\kappa$ B regulation is also clinically important. NF- $\kappa$ B is used by several disease-causing viruses to regulate viral gene expression, including HIV-1, HTLV-1, HSV, and CMV (1). In inflammatory conditions such as inflammatory bowel disease, arthritis, and asthma, inappropriately activated NF- $\kappa$ B is thought to induce expression of several mediators of the inflammation (4). Furthermore, the resistance of some tumors to chemotherapeutic agents has been attributed to an anti-apoptotic response mediated by NF- $\kappa$ B (5).

Agents that could inhibit NF- $\kappa$ B function would be useful both as research tools to dissect the particular roles of NF- $\kappa$ B in specific biological contexts and as potential therapeutic agents for the disease states mentioned above. One approach

to the inhibition of NF- $\kappa$ B function would target the protein–DNA interaction by which activated NF- $\kappa$ B recognizes binding sites in the regulatory regions of its target genes.

The most prevalent form of NF- $\kappa$ B is a heterodimer of p50 and p65 subunits. The DNA-binding domains of both p50 and p65 are located near their N-termini and are members of the Rel homology domain (RHD) family of DNA-binding domains. Structural studies have revealed that the RHD consists of two immunoglobulin-like  $\beta$ -sandwich domains joined by a linker region with the C-terminal domain mediating dimerization contacts (6, 7). Cococrystallization of p50/p65 heterodimer with the  $\kappa$ B site 5'-GGGACTTTCC-3' has shown that DNA contacts are formed by loops joining  $\beta$ -strands in both the N- and C-terminal domains. The p65 monomer derives its specificity from contacts in the major groove for the four-base-pair subsite (5'-TTCC-3'), while the p50 monomer recognizes a five-base-pair subsite (5'-GGGAC-3') in the major groove. The heterodimer also makes several phosphate contacts throughout the binding site (7) (see Figure 1).

An attractive strategy for inhibition of NF- $\kappa$ B DNA binding would use small molecules that could be readily synthesized and designed to target specific DNA sequences. Hairpin polyamides containing pyrrole (Py), imidazole (Im), and hydroxypyrrole (Hp) amino acids are synthetic ligands that bind predetermined DNA sequences in the minor groove with affinities and specificities comparable to many DNA-binding proteins (8, 9). Rules have been developed that allow for sequence-specific recognition of the DNA minor groove by relating each Watson Crick base pair with a particular pairing of the aromatic rings, Py, Im, and Hp (8–11). The crescent-shaped polyamides bind in the minor groove of DNA with pairs of aromatic rings stacked against each other and the walls of the groove, allowing the backbone amide hydrogens and the substituents at the 3-position of the Py, Im, and Hp residues to make specific contacts with the edges of the intact base pairs. An  $\gamma$ -aminobutyric acid residue ( $\gamma$ ) connects the polyamide subunits in a “hairpin” motif that

<sup>†</sup> This research was supported by NIH Grant GM57148. N.R.W. was supported by predoctoral fellowships from Bristol-Myers Squibb and the Ralph M. Parsons Foundation. J.L.P. was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation and is currently a Special Fellow of the Leukemia and Lymphoma Society of America.

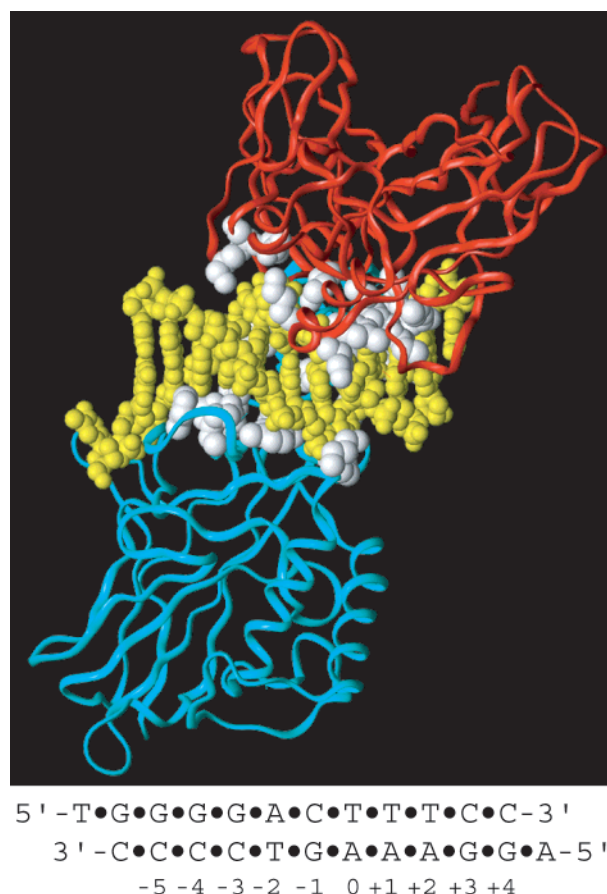


FIGURE 1: (Top) Crystal structure of p50/p65 NF- $\kappa$ B heterodimer bound to the DNA duplex 5'-TGGGACTTCC-3' (7). The p50 and p65 monomers are represented as blue and red ribbons, respectively. Side chains that contact DNA are shown as white space-filling models, while the DNA is yellow. (Bottom) Sequence of DNA bound by NF- $\kappa$ B, shown above.

enhances affinity and unambiguously locks the desired ring pairings in register (8, 9).

Polyamides have been found to interfere with DNA binding for several classes of proteins (12). An examination of the available crystal structures of these proteins reveals that inhibition is observed when polyamides are targeted to key protein-DNA contacts in the minor groove. Inhibition of protein binding to DNA has been observed when polyamides were targeted to the binding site of minor groove-binding proteins such as the TATA Box-binding protein and HMG-domain protein, LEF-1 (13), or to sites where a major groove-binding protein has important recognition elements such as a loop in the minor groove, as in the case of ETS-1 (13, 14). In contrast, polyamides have been shown to bind simultaneously with some proteins such as bZIP protein, GCN4, that exclusively occupy the DNA major groove, unless key phosphate contacts are targeted with a ligand with multiple positive charges displayed on a polyamide (15-17). Each new class of proteins presents a different challenge for designing minor groove-binding polyamides that can inhibit protein-DNA binding, especially proteins that predominantly dock to the major groove of DNA, such as NF- $\kappa$ B.

We report here the synthesis and DNA-binding properties of a series of hairpin polyamides targeted to the NF- $\kappa$ B-binding site and their ability to inhibit DNA binding by NF- $\kappa$ B. Polyamides were designed to bind overlapping sites

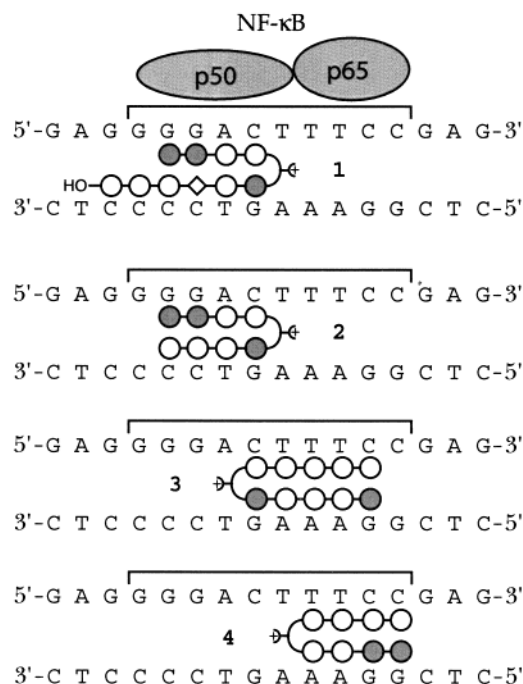


FIGURE 2: Ball-and-stick representation of polyamides bound to an NF- $\kappa$ B DNA-binding site. Filled circles represent imidazole rings while open circles denote pyrrole rings. The diamond represents  $\beta$ -alanine, OH represents a propanol amide, and (R)-diaminobutyric acid is depicted as a curved line and a plus sign.

along the protein-binding site to determine which sites provided optimal inhibition. The DNA-binding affinity and specificity of each of these compounds for their target DNA site were evaluated using quantitative DNase I footprinting. Gel mobility shift assays were employed to investigate the ability of these polyamides to inhibit the interaction of NF- $\kappa$ B with DNA.

## MATERIALS AND METHODS

Polyamides 1-4 were prepared by solid-phase methods as previously reported (18, 19) and characterized by HPLC and MALDI-TOF mass spectrometry. ImImPyPy- $H_2N\gamma$ -ImPyPy- $\beta$ -PyPyPy-NHCOPrOH (1) [M+H] 1333.60, 1333.58 calculated for [M+H]. ImImPyPy- $H_2N\gamma$ -ImPyPyPy-NHCOMe (2) [M+H] 1095.61, 1095.47 calculated for [M+H]. ImPyPyPyIm- $H_2N\gamma$ -PyPyPyPyPy-NHCOMe (3) [M+H] 1996.58, 1096.46 calculated for [M+H]. ImImPyPy- $H_2N\gamma$ -PyPyPyPy-NHCOMe (4) [M+H] 1339.63, 1339.57 calculated for [M+H].

**DNase Footprinting.** Experiments were performed as previously reported (20).

**Nuclear Extract Preparation.** Approximately  $2 \times 10^7$  confluent 293T cells were treated with TNF $\alpha$  (recombinant human TNF $\alpha$ , R&D Systems) at 25 ng/mL for 15 min at 37 °C. Cells were then washed with PBS, scraped, pelleted, resuspended in 0.4 mL of buffer A [10 mM Hepes, pH 7.9, 1.5 mM MgCl<sub>2</sub>, 10 mM KCl, 0.5 mM DTT, 0.2 mM PMSF, 1 mg/mL aprotinin], and incubated for 10 min at 4 °C. Cells were vortexed for 10 s, and nuclei were pelleted at 5000 rpm for 5 min at 4 °C. Nuclear proteins were extracted by incubation in 0.1 mL of buffer C [20 mM Hepes, pH 7.9, 1.5 mM MgCl<sub>2</sub>, 420 mM KCl, 0.5 mM DTT, 0.2 mM EDTA, 25% glycerol, 0.2 mM PMSF, 1  $\mu$ g/mL aprotinin] for 20 min at 4 °C with intermittent vortexing. Debris was removed

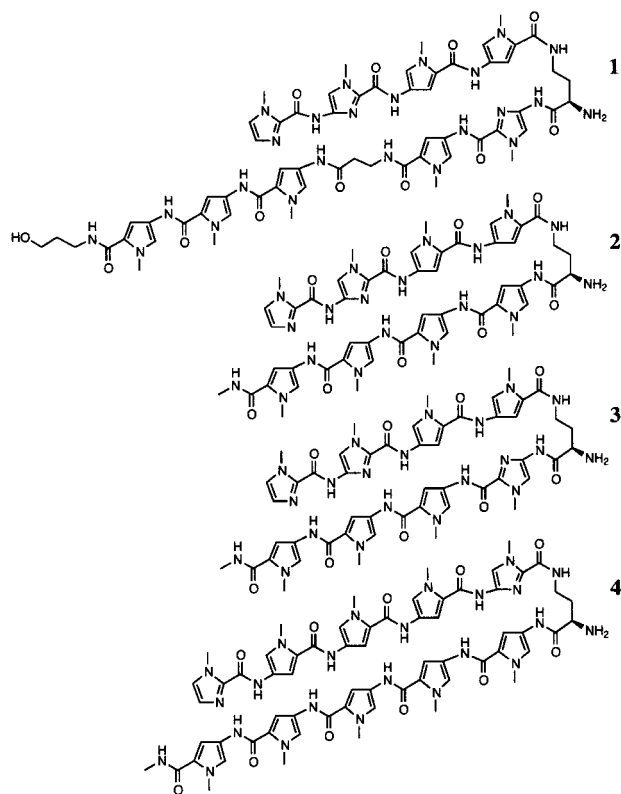


FIGURE 3: Structures of polyamides **1–4** targeted to the NF- $\kappa$ B-binding site.

by spinning at 12 000 rpm for 2 min. Protein concentration in the nuclear extract was determined to be 1.5 mg/mL by Bradford assay.

**Electrophoretic Mobility Shift Assay.** The probe used for gel shift (5'-AATTCATGCAGTTGAGGGGACTTTCCAG-GCATGCAAGCT-3') was 3'-end-labeled. Binding reactions contained 0.1 ng of DNA probe, 1  $\mu$ g of poly[d(I-C)]/poly-[d(I-C)], 15 mM Tris, pH 7.5, 1.5 mM EDTA, 5% glycerol, 1.5 mM DTT, 20  $\mu$ g/mL BSA, and 1  $\mu$ L of polyamide (10 $\times$ ) to give final concentrations of 1 nM–1  $\mu$ M in a total volume of 9  $\mu$ L. After equilibration for 4 h, nuclear extract (1.5  $\mu$ g) was added, and the reactions were incubated for an additional 30 min at 30  $^{\circ}$ C and then resolved on 4% nondenaturing polyacrylamide gels using 0.25 $\times$  TBE electrophoresis buffer.

## RESULTS AND DISCUSSION

We designed and synthesized several polyamides with the goal of being able to scan the entire NF- $\kappa$ B-binding site (see Figures 2 and 3). Compounds **1** and **2** were targeted to match sites 5'-GGGACT-3' and 5'-GGACT-3', respectively, while compounds **3** and **4** were targeted to match sites 5'-ACTTTC-3' and 5'-TTTCC-3', respectively. All four of these compounds were found to specifically bind their targets with high

Table 1: Equilibrium Association Constants ( $M^{-1}$ )<sup>a</sup>

polyamide	match site	$K_a$
<b>1</b>	5'-gAGGGGACTt-3'	$9.3 (\pm 1.6) \times 10^9$
<b>2</b>	5'-gGGACTt-3'	$2.2 (\pm 0.3) \times 10^{10}$
<b>3</b>	5'-gACTTTCc-3'	$6.4 (\pm 1.6) \times 10^9$
<b>4</b>	5'-cTTTCCg-3'	$1.3 (\pm 0.2) \times 10^9$

<sup>a</sup> Values reported are the mean values obtained from three DNase I footprint titration experiments. The assays were carried out at 22  $^{\circ}$ C, 10 mM Tris $\cdot$ HCl (pH 7.0), 10 mM KCl, 10 mM MgCl<sub>2</sub>, and 5 mM CaCl<sub>2</sub>.

affinity in quantitative DNase I footprint titrations (see Table 1 and Figures 4 and 5).

The unpaired pyrrole rings in polyamide **1** were placed over two G,C base pairs since previous attempts to bind four consecutive G's with ring pairs result in low-affinity ligands (21). As evidenced by the high affinity of **1**, the unpaired pyrrole ring in each of these compounds is well tolerated by the G,C base pair. This motif allows exploitation of the binding site immediately adjacent to the four G's for specificity, while still binding the G-rich site with high affinity. Polyamides **2–4** were synthesized on oxime resin and cleaved with methylamine to yield methyl amide tails, which allow the binding of sequences with G,C base pairs flanking the paired rings at the tail position (19). This targeting of match sites with polyamides with methylamine tails demonstrates the utility of the truncated tail for recognition of G,C-rich sequences without serious energetic penalty.

Polyamides **1–4** were tested in gel mobility shift assays for the ability to inhibit DNA binding by NF- $\kappa$ B. Our source of NF- $\kappa$ B was a nuclear extract made from 293T cells activated by TNF $\alpha$ . Antibody supershift analyses confirmed that the NF- $\kappa$ B complex in this nuclear extract was a p50:p65 heterodimer (data not shown). Interestingly, polyamides **1** and **2** inhibited protein–DNA binding at nanomolar concentrations [50% inhibition of protein was observed at  $\sim$ 5 and  $\sim$ 30 nM for compounds **1** and **2**, respectively] (see Figure 6). Polyamides **3** and **4** did not inhibit NF- $\kappa$ B binding, even at a concentration of 1  $\mu$ M (Figure 6). Thus, in order for a polyamide to inhibit binding by NF- $\kappa$ B, it must target G<sub>-4</sub>G<sub>-3</sub>A<sub>-2</sub> in the minor groove. The concentrations of polyamides **1** and **2** that were required for 50% inhibition of protein binding were higher than their respective dissociation constants for polyamide–DNA binding (calculated from the equilibrium association constants determined in the footprinting assays). This difference may result from the different binding conditions used in the footprinting and gel mobility shift assays.

The p50/p65: $\kappa$ B site cocrystal structure (7) suggests two possible mechanisms for inhibition by polyamides. p65-K221 is positioned over the minor groove and contacts the phosphate between G<sub>-3</sub> and A<sub>-4</sub> of GGG\*ACTTTC. The

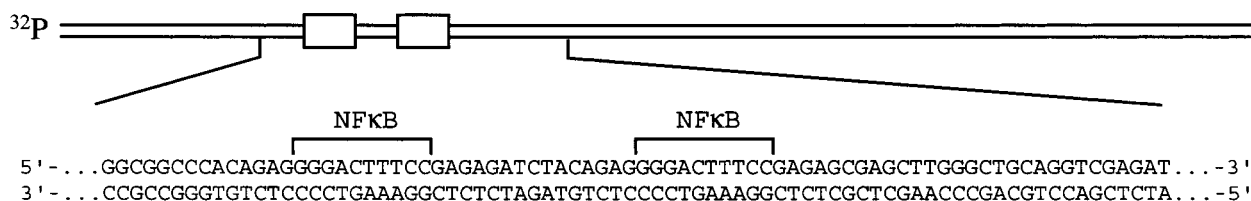


FIGURE 4: DNA sequence of PCR product used for DNase I footprinting with the NF- $\kappa$ B-binding sites depicted as boxes.

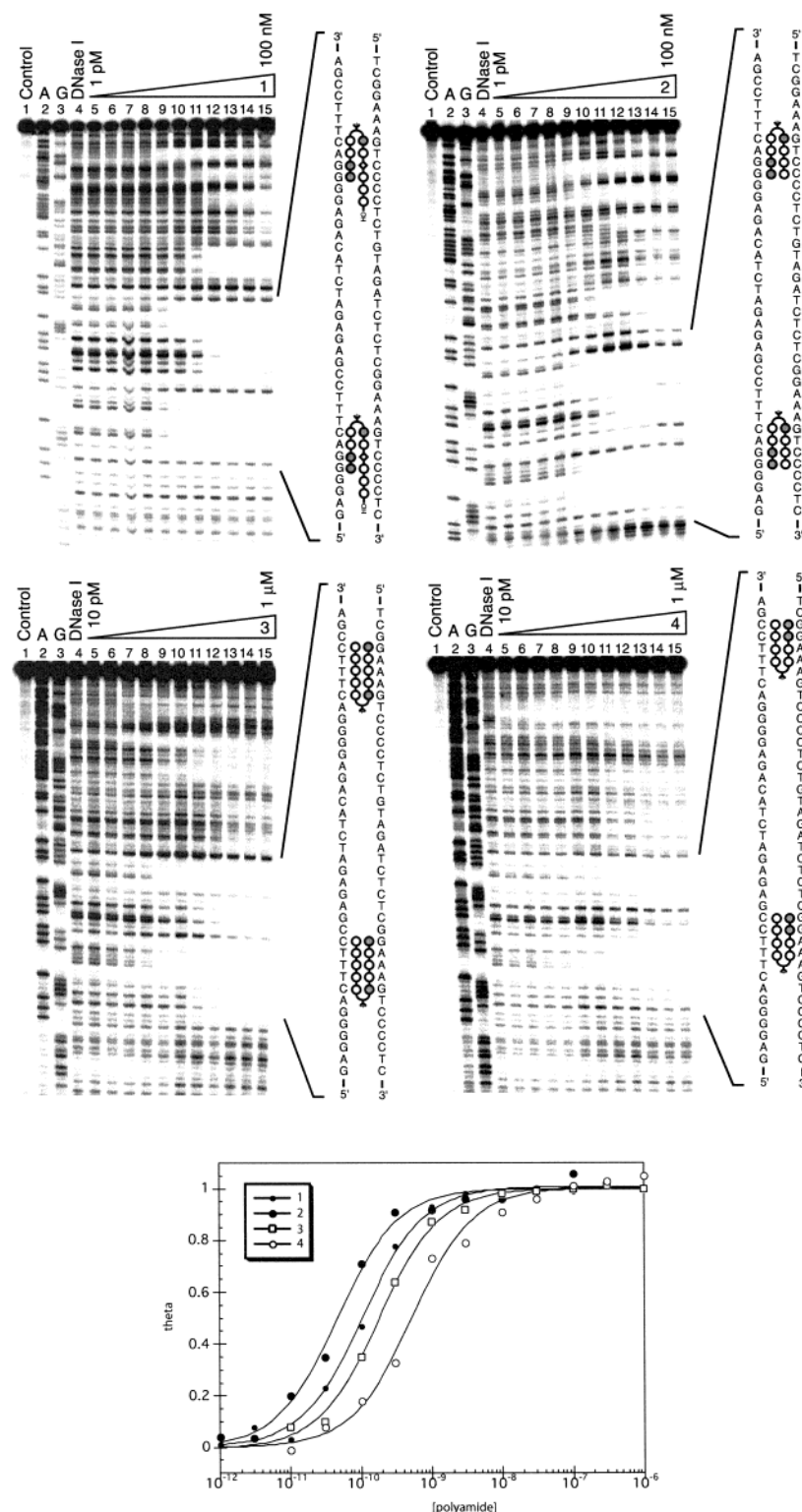


FIGURE 5: Storage phosphor autoradiograms of quantitative DNase I footprint titration experiments with ImImPyPy-<sup>H<sub>2</sub>N</sup>-ImPy- $\beta$ -PyPyPyPy-NHCOPROH (1), ImImPyPy-<sup>H<sub>2</sub>N</sup>-ImPyPyPyPy-NHCOMe (2), ImPyPyPyIm-<sup>H<sub>2</sub>N</sup>-PyPyPyPyPy-NHCOMe (3), and ImImPyPy-<sup>H<sub>2</sub>N</sup>-PyPyPyPyPy-NHCOMe (4) on the 5'-<sup>32</sup>P-end-labeled PCR product. All reactions contained 10 kcpm of restriction fragment, 10 mM Tris·HCl (pH 7.0), 10 mM KCl, 10 mM MgCl<sub>2</sub>, and 5 mM CaCl<sub>2</sub> and were performed at 22 °C. For compounds 1 and 2: lane 1, intact DNA; lane 2, A-specific reaction; lane 3, G-specific reaction; lane 4, DNase I standard; lanes 5–15, 1 pM, 3 pM, 10 pM, 30 pM, 100 pM, 300 pM, 1 nM, 30 nM, 100 nM. Each gel contains two match binding sites that are bracketed. For compounds 3 and 4: lane 1, intact DNA; lane 2, A-specific reaction; lane 3, G-specific reaction; lane 4, DNase I standard; lanes 5–15, 10 pM, 30 pM, 100 pM, 300 pM, 1 nM, 30 nM, 100 nM, 300 nM, 1 mM. Polyamides are shown bound to their match sites to the right of the gels. Polyamides are depicted as in Figure 2. (Bottom) Binding constants were obtained from Langmuir-binding isotherms using the modified Hill equation (20). Footprints observed outside the NF- $\kappa$ B-binding sites occurred at sequences closely related to the match sites at higher concentrations of polyamide than what was required to footprint within the NF- $\kappa$ B sites.



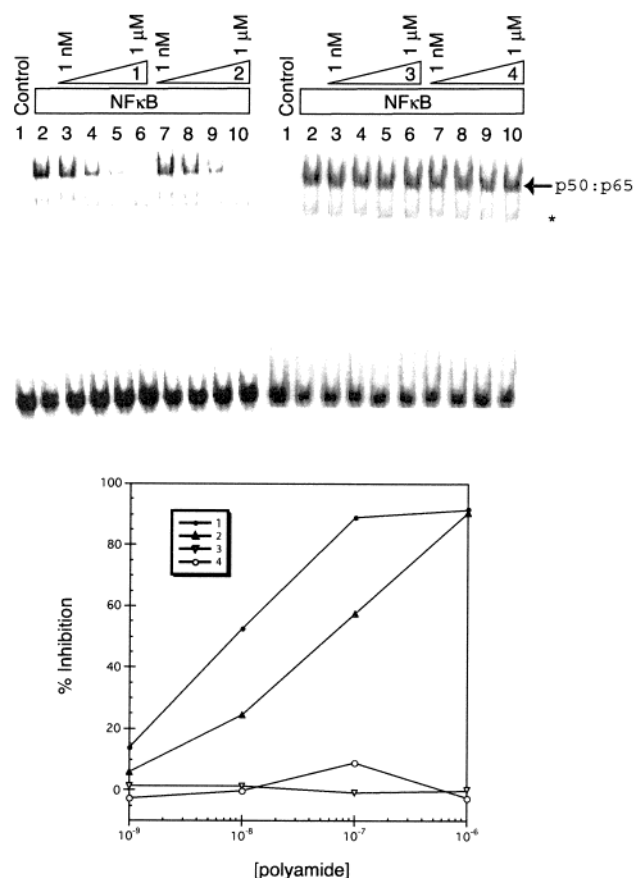


FIGURE 6: (Top) NF- $\kappa$ B gel mobility shift assay with polyamides 1–4. The asterisk denotes nonspecific protein interactions with the DNA duplex. (Bottom) Percent inhibition by polyamides 1–4 of the formation of the NF- $\kappa$ B:DNA complex.

*N*-methyl groups on the *N*-terminal imidazole of polyamides 1 and 2 might interfere with this Lys:phosphate contact. Since an equivalent contact does not exist for p50 on the 3' end of the binding site, this could explain the inhibitory activity of 1 and 2 bound on the 5' end of the protein-binding site.

A second possibility is that the binding of compounds 1 and 2 in the minor groove perturbs the DNA conformation such that protein–DNA interactions occurring in the major groove are disfavored. Presumably, these compounds would then affect major groove recognition by the p50 monomer, which binds in the major groove opposite of polyamides 1 and 2. The minor groove of the DNA bound by NF- $\kappa$ B has a narrow minor groove of approximately 4 Å throughout the binding site, except for a widening at the four consecutive guanines. Polyamides are known to enlarge the minor groove from 6 Å for typical B-form DNA to approximately 7.9 Å (22). Compounds 3 and 4 might correspondingly target p65-mediated major groove interactions but do not inhibit and therefore do not interfere with any critical interactions. The p50-binding consensus is conserved as 5'-GGGRN-3', and this is explained by contacts observed in the cocrystal structure. The p50 monomer recognizes the G<sub>-5</sub>G<sub>-4</sub>G<sub>-3</sub>A<sub>-2</sub>C<sub>-1</sub> portion of the site using His64, which contacts G<sub>-5</sub>, Arg56, which contacts G<sub>-4</sub>, Arg54, which contacts G<sub>-3</sub>, and Glu60, which hydrogen-bonds to Arg54 and Arg56 and contacts C<sub>-5</sub> and C<sub>-4</sub>. In addition, Tyr57 makes van der Waals contacts with C<sub>-3</sub> and T<sub>-2</sub>. Several similar protein–DNA interactions are observed in the recognition of the T<sub>+1</sub>T<sub>+2</sub>C<sub>+3</sub>C<sub>+4</sub> portion of the site by p65. The G base-paired to C<sub>+4</sub> is contacted by

p65 Arg35, the G base-paired to C<sub>+3</sub> is contacted by Arg33, and Glu39 contacts C<sub>+3</sub> and C<sub>+4</sub>. In addition, Arg187 bonds to T<sub>+2</sub>, and Tyr36 interacts with T<sub>+1</sub> and T<sub>+2</sub>. These contacts result in a less stringent binding consensus for p65, 5'-TTCC-3'. It is possible that contacts made by p50 in the major groove are more susceptible than those made by p65 to the effects of polyamides binding in the opposite minor groove space. The His64:G<sub>-5</sub> interaction is not mimicked by p65 (the corresponding residue in p65 is Ala43) and may serve as a p50-specific target for compounds 1–2.

In conclusion, we have successfully designed hairpin polyamides that bind to an NF- $\kappa$ B target sequence and inhibit DNA binding by NF- $\kappa$ B. Interestingly, inhibition was observed only with polyamides targeted to the GGGAC portion of the canonical  $\kappa$ B site. This unexpected specificity of action suggests that there may be the potential for future compounds to selectively inhibit certain Rel heterodimers and not others. Such compounds may be useful for research aimed at elucidating specific biological roles of NF- $\kappa$ B heterodimers. There is also the potential to use the design capabilities of polyamides to target gene-specific NF- $\kappa$ B-binding sites. While the NF- $\kappa$ B consensus "GGGRN-YYYCC" includes many sites found in the genome, one might be able to target individual sites by designing specific polyamide–DNA recognition which involves binding to the GGGRN portion of the site but which depends on site-specific contacts either within the  $\kappa$ B core or immediately 5' to the  $\kappa$ B core. We note that we have successfully designed polyamides that can specifically recognize base pairs 5' to the GGGACTTTCC sequence while inhibiting NF- $\kappa$ B binding (data not shown). Gene-specific polyamides would potentially have much higher specificity of action than those targeted to multiple NF- $\kappa$ B-binding sites. For promoters or enhancers at which NF- $\kappa$ B binds cooperatively with other transcription factors, multiprotein complex assembly will likely be inhibited by interference with individual protein–DNA interactions. This has been observed for a polyamide targeted to an Ets-1-binding site in the HIV-1 enhancer, which interferes with the cooperative assembly of Ets-1:NF- $\kappa$ B:DNA ternary complex formation (23).

## REFERENCES

- Pahl, H. L. (1999) *Oncogene* 18, 6853–6866.
- Karin, M., and Ben-Neriah, Y. (2000) *Annu. Rev. Immunol.* 18, 621–663.
- Gerondakis, S., Grossmann, M., Nakamura, Y., Pohl, T., and Grumont, R. (1999) *Oncogene* 18, 6888–6895.
- Barnes, P. J., and Karin, M. (1997) *N. Engl. J. Med.* 336, 1066–1071.
- Cusack, J. C., Liu, R., Houston, M., Abendroth, K., Elliott, P. J., Adams, J., and Baldwin, A. S. (2001) *Cancer Res.* 61, 3535–3540.
- Ghosh, S., Gifford, A. M., Riviere, L. R., Tempst, P., Nolan, G. P., and Baltimore, D. (1990) *Cell* 62, 1019–1029.
- Chen, F. E., Huang, D. B., Chen, Y. Q., and Ghosh, G. (1998) *Nature* 391, 410–413.
- Dervan, P. B. (2001) *Bioorg. Med. Chem.* 9, 2215–2235.
- Dervan, P. B., and Bürli, R. W. (1999) *Curr. Opin. Chem. Biol.* 3, 688–693.
- White, S., Baird, E. E., and Dervan, P. B. (1997) *Chem. Biol.* 4, 569–578.
- White, S., Szewczyk, J. W., Turner, J. M., Baird, E. E., and Dervan, P. B. (1998) *Nature* 391, 468–471.

12. Gottesfeld, J. M., Turner, J. M., and Dervan, P. B. (2000) *Gene Expression Purif.* 9, 77–91.
13. Dickinson, L. A., Gulizia, R. J., Trauger, J. W., Baird, E. E., Mosier, D. E., Gottesfeld, J. M., and Dervan, P. B. (1998) *Proc. Natl. Acad. Sci. U.S.A.* 95, 12890–12895.
14. Chiang, S. Y., Burli, R. W., Benz, C. C., Gawron, L., Scott, G. K., Dervan, P. B., and Beerman, T. A. (2000) *J. Biol. Chem.* 275, 24246–24254.
15. Oakley, M. G., Mrksich, M., and Dervan, P. B. (1992) *Biochemistry* 31, 10969–10975.
16. Bremer, R. E., Baird, E. E., and Dervan, P. B. (1998) *Chem. Biol.* 5, 119–133.
17. Bremer, R. E., Wurtz, N. R., Szewczyk, J. W., and Dervan, P. B. (2001) *Bioorg. Med. Chem.* 9, 2093–2103.
18. Baird, E. E., and Dervan, P. B. (1996) *J. Am. Chem. Soc.* 118, 6141–6146.
19. Belitsky, J. B., Nguyen, D. H., and Wurtz, N. R. (2001) *Bioorg. Med. Chem.* (in press).
20. Trauger, J. W., Dervan, P. B. (2001) *Methods Enzymol.* 340, 450–466.
21. Swalley, S. E., Baird, E. E., and Dervan, P. B. (1997) *J. Am. Chem. Soc.* 119, 6953–6961.
22. Kielkopf, C. L., Baird, E. E., Dervan, P. D., and Rees, D. C. (1998) *Nat. Struct. Biol.* 5, 104–109.
23. Dickinson, L. A., Trauger, J. W., Baird, E. E., Dervan, P. B., Graves, B. J., and Gottesfeld, J. M. (1999) *J. Biol. Chem.* 274, 12765–12773.

BI020114I