mediate, we note the well-precedented migrations of phenyl and other carbon substituents from coordinated tertiary phosphorus centers to transition metals.[9,10] A recent example of a phenyl migration from silicon to platinum has also been reported.[11] These results demonstrate for the first time that analogous migrations from ligated carbon atoms may also be feasible.

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Supplementary Material Available: Full experimental methodology and spectroscopic characterization of all compounds reported herein; crystallographic summary for **4a**; tables of fractional coordinates, bond lengths, bond angles, anisotropic displacement coefficients, H atom coordinates, and isotropic displacement coefficients (16 pages); tables of observed and calculated structure factors (23 pages). Ordering information is given on any current masthead page.

(9) Garrou, P. E. *Chem. Rev.* **1985**, *85*, 171-185. Dubois, R. A.; Garrou, P. E.; Lavin, K. D.; Allcock, H. R. *Organometallics* **1984**, *3*, 649-650; *Ibid.* **1986**, *5*, 460-466. Dubois, R. A.; Garrou, P. E. *Ibid.* **1986**, *5*, 466-473. Jung, C. W.; Fellmann, J. D.; Garrou, P. E. *Ibid.* **1983**, *2*, 1042-1044. A theoretical treatment appears in: Ortiz, J. V.; Havlas, Z.; Hoffmann, R. *Helv. Chim. Acta* **1984**, *67*, 1-17.

(10) α-Elimination of H or Ph from carbon to iridium requires that either dissociation of a PMe_3 ligand or of one arm of the acac occurs in order to provide the required coordination site on the metal.

(11) Chang, L. S.; Johnson, M. P.; Fink, M. J. *Organometallics* **1991**, *10*, 1219-1221.

Evidence for Induced Fit in Antibody·DNA Complexes

Shawn Y. Stevens,[†] Patrick C. Swanson,[†]
Edward W. Voss, Jr.,[‡] and Gary D. Glick*,[†]

Department of Chemistry, University of Michigan
Ann Arbor, Michigan 48109-1055
Department of Microbiology
University of Illinois at Urbana-Champaign
Urbana, Illinois 61801

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Anti-DNA autoantibodies (anti-DNA) are a hallmark of the autoimmune disorder systemic lupus erythematosus (SLE). Most subjects with active SLE spontaneously produce antibodies that bind both double-stranded and single-stranded DNA (dsDNA and ssDNA, respectively).[1,2] Deposition of these anti-DNA·DNA complexes in the kidneys is thought to mediate the tissue injury associated with SLE. However, the elements of DNA that are targeted by anti-DNA have not been identified.[3] One problem in trying to correlate anti-DNA specificity with DNA structure is that binding may be accompanied by conformational changes in polynucleotide ligands, facilitating a better fit to the antibody combining site ("induced fit").[4] For example, studies of antibody-protein,[5] antibody-peptide,[6] protein-DNA,[7] and protein-small molecule complexes[8] show that structural reorganization of ligands can help stabilize biomolecular complexes.[9] We have examined the importance of induced fit in anti-DNA·DNA binding and report that monoclonal anti-ssDNA BV04-01,[10] which is typical of anti-DNA in lupus-prone mice, forces structural changes in DNA ligands upon binding.

X-ray analysis of an F(ab) fragment from BV04-01 bound to $d(pT)_3$ and $d(pT)_6$ has provided insight into the molecular basis of anti-DNA·DNA recognition.[11] In these complexes the DNA conformation is very different from the extended stacked geometry of oligo(dT),[12] which suggests that the antibody might drive this conformational change.[13] To explore this hypothesis we studied BV04-01 recognition of four DNA hairpin sequences[14] (**1-4**) along with disulfide cross-linked analogs of these molecules (**5-8**).[15] If conformational reorganization of the hairpins is required for binding, then BV04-01 should possess a *lower* affinity for the modified sequences, since the disulfide bond renders them resistant to structural changes.[16] However, if preorganization is important for complexation, then the more rigid oligomers should bind with equal or greater affinity than the unmodified ligands.[17]

In preliminary experiments an enzyme-linked immunosorbant assay demonstrated that the hairpin loops were recognized by the antibody.[18] The affinity of BV04-01 for **1-4** was then measured by gel shift assay (Figure 1, top).[19] Hairpin **1** binds more tightly than the other three ligands, which is consistent with solid-phase binding data that indicate a preference for thymidine (Figure 2). When binding to cross-linked hairpins **5-8** was studied, a substantial increase in K_d relative to the unmodified hairpins was observed (Figure 1, bottom). Moreover, the relative affinity of the antibody for the unmodified ligands is different from the specificity displayed toward the cross-linked molecules. This suggests that the mode of binding to the wild-type and cross-linked hairpins may be different.

If the weaker affinity of BV04-01 for **5-8** results from the constraint imposed by the cross-link, then removing this constraint by reduction of the disulfide bond should afford a set of ligands that bind with roughly the same affinity as the unmodified hairpins. Indeed, BV04-01 recognition of **9-12** is nearly indis-

[†] University of Michigan.
[‡] University of Illinois.

(1) *Anti-DNA Antibodies in SLE*; Voss, E. W., Jr., Ed.; CRC Press: Boca Raton, FL, 1988.

(2) Koffler, D.; Miller, T. E.; Faiferman, I. *Human Pathol.* **1983**, *14*, 406-418. Tan, E. M. *Adv. Immunol.* **1989**, *44*, 93-115.

(3) Koffler, D.; Schur, P. H.; Kunkel, H. G. *J. Exp. Med.* **1967**, *126*, 607-617. Schwartz, R. S.; Stollar, B. D. *J. Clin. Invest.* **1985**, *75*, 321-327.

(4) Pollard, K. M.; Jones, J. E.; Tan, E. M.; Theofilopoulos, A. N.; Dixon, F. E.; Rubin, R. L. *Clin. Immunol. Immunopathol.* **1986**, *40*, 197-208.

(5) Getzoff, E. D.; Tainer, J. A.; Lerner, R. A.; Geyson, H. M. *Adv. Immunol.* **1988**, *43*, 1-98.

(6) Stanfield, R. L.; Fieser, T. M.; Lerner, R. A.; Wilson, I. A. *Science* **1990**, *248*, 712-719. Rini, J. M.; Schulze-Gahmen, U.; Wilson, I. A. *Science* **1992**, *255*, 959-965.

(7) Frederick, C. A.; Grable, J.; Melia, M.; Samudzi, C.; Jen-Jacobson, L.; Wang, B.-C.; Greene, P.; Boyer, H. W.; Rosenberg, J. M. *Nature* **1984**, *309*, 327-331. Schultz, S. C.; Shields, G. C.; Steitz, T. A. *Science* **1991**, *253*, 1001-1007.

(8) For a recent example, see: Van Duyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *252*, 839-832.

(9) Koshland, D. E., Jr.; Neet, K. E. *Annu. Rev. Biochem.* **1968**, *37*, 359-410. Herschlag, D. *Bioorg. Chem.* **1988**, *16*, 62-96. Wüthrich, K.; von Freyberg, B.; Weber, C.; Wider, G.; Traber, R.; Widmer, H.; Braun, W. *Science* **1991**, *254*, 953-954. Jorgensen, W. L. *Science* **1991**, *254*, 954-955. Davies, D. R.; Padlan, E. A. *Curr. Biol.* **1992**, *2*, 254-256.

(10) Ballard, D. W.; Lynn, S. P.; Gardner, J. E.; Voss, E. W., Jr. *J. Biol. Chem.* **1984**, *259*, 3492-3498. Ballard, D. W.; Voss, E. W., Jr. *J. Immunol.* **1985**, *135*, 3372-3386.

(11) Herron, J. N.; He, X. M.; Ballard, D. W.; Blier, P. R.; Pace, P. R.; Bothwell, A. L. M.; Voss, E. W., Jr.; Edmundson, A. B. *Proteins* **1991**, *11*, 159-175.

(12) Camerman, N.; Fawcett, J. K.; Camerman, A. *J. Mol. Biol.* **1976**, *107*, 601-621.

(13) Structural changes in BV04-01 were also observed upon binding.[11]

(14) The single-stranded and double-stranded regions on DNA hairpins can model the ssDNA and dsDNA molecules that are recognized by anti-DNA. Swanson, P. C.; Glick, G. D., manuscript in preparation.

(15) Glick, G. D. *J. Org. Chem.* **1991**, *56*, 6746-6747. Glick, G. D.; Osborne, S. E.; Knitt, D. S.; Marino, J. P., Jr. *J. Am. Chem. Soc.* **1992**, *114*, 5447-5448.

(16) **5-8** begin to denature at 84 °C, which is >30 °C above the T_m of **1-4** in 1 mM NaCl buffer.

(17) Cram, D. J. *Angew. Chem.* **1986**, *98*, 1041-1060.

(18) The hairpins were covalently linked to BSA and then coated onto microtiter plates. The plates were treated with S1 nuclease to degrade the loops, which decreased BV04-01 binding by >90%. The resulting duplex is still recognized by anti-dsDNA.

(19) Carey, J. *Methods Enzymol.* **1991**, *208*, 103-118, and references therein.

(20) Chrambach, A. In *The Practice of Quantitative Gel Electrophoresis*; Neuhoff, V., Maelicke, A., Eds.; VCH Press: New York, 1988; pp 63-78.

0002-7863/93/1515-1585$04.00/0

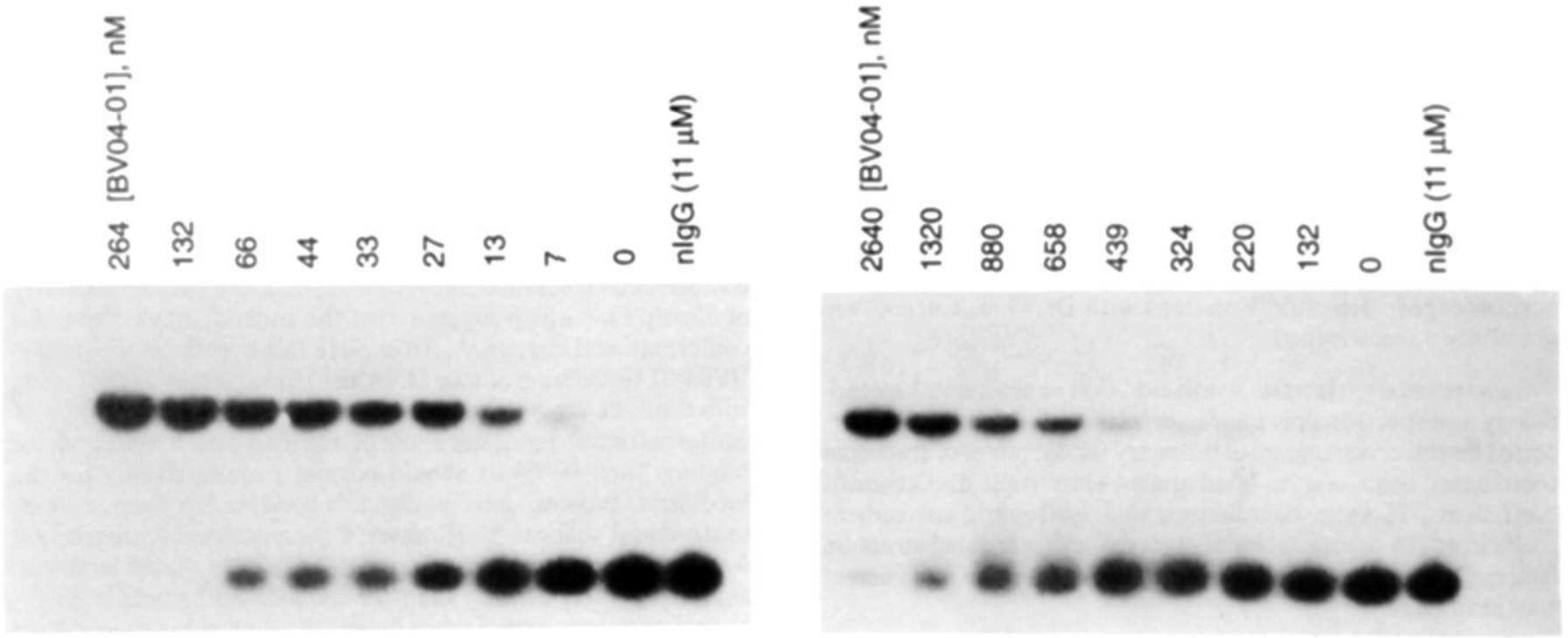
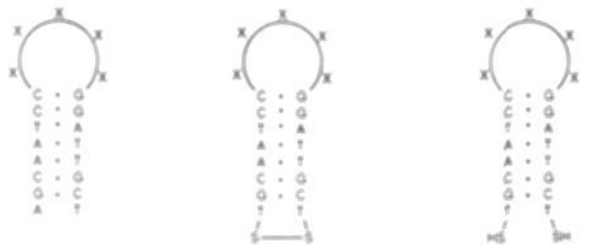


Figure 1. Representative gel shift data. (Left) Hairpin **1**. (Right) Hairpin **5**. [^{32}P] end-labeled hairpin (0.1 nM, ~2300 cpm) was titrated with antibody in TBE at pH 8.3. After equilibration for 1 h at 25 °C the mixtures were loaded onto a 6% polyacrylamide gel (9:1 acrylamide:bisacrylamide) at 300 V for 90 s and then electrophoresed at 106 V for 30 min at 4 °C. The high cross-linking ratio affords a nonrestrictive gel that permits the complex to enter the matrix.[20] The gels were then autoradiographed at −70 °C for 18 h. To determine K_d values, DNA concentrations were quantified by densitometry and the data were fit to the binding isotherm for the equilibrium Ab + DNA ⇌ Ab-DNA by nonlinear least-squares regression. The protein concentration at which half the DNA is bound corresponds to the apparent K_d.



	unmodified		crosslinked		reduced	
X= T	34 ± 2	(1)	523 ± 37	(5)	31 ± 5	(9)
C	48 ± 3	(2)	> 3000	(6)	49 ± 7	(10)
A	53 ± 4	(3)	> 3000	(7)	48 ± 4	(11)
G	104 ± 8	(4)	788 ± 34	(8)	108 ± 9	(12)

Figure 2. Apparent dissociation constants for BV04-01·hairpin complexes (K_d per IgG binding site, nM). Each K_d is the average of at least three separate measurements with the associated error representing error to the fit.

tinguishable from binding to **1**–**4**.[21] To provide further support that BV04-01 alters the structure of DNA upon binding, we synthesized **13** and **14**, which are less stable than **1**.[22] If induced

13 **14**

fit is important for complexation, then less binding energy will be needed for structural reorganization of these new hairpins, resulting in tighter binding to the antibody. Gel shift measurements showed that both **13** and **14** form tighter complexes than **1** with BV04-01 (K_d(**13**) = 17 ± 3 nM and K_d(**14**) = 22 ± 4 nM). However, the differences in stability between **1** and **13** and **1** and **14** are not fully expressed as added binding energy, which suggests that the hairpins may not be completely unfolded when bound to the antibody.

To illuminate the specificity of anti-DNA, future studies must focus on examining both free- *and* bound-state DNA geometries. Such data will hopefully guide the synthesis of molecules that specifically block autoantibody recognition of DNA and form the basis for new and effective therapies to combat SLE.

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(21) NOESY spectra indicate that the cross-link does not alter the conformation of native DNA structure. Cain, R.; Glick, G. D., unpublished results.

(22) T_m(**1**) = 55.8 °C, T_m(**13**) = 47.7 °C, T_m(**14**) = 49.5 °C. ΔG(**1**) = 5.3 kcal/mol, ΔG(**13**) = 4.2 kcal/mol, ΔG(**14**) = 4.4 kcal/mol (from van't Hoff analysis of UV thermal denaturation curves).

Formation of a Stable Metallacyclobutene Complex from α-Diazocarbonyl and Alkyne Substrates

Joseph M. O'Connor,*,† Hongli Ji, and Mahzad Iranpour

Department of Chemistry (216), University of Nevada Reno, Nevada 89557

Arnold L. Rheingold*

Department of Chemistry, University of Delaware Newark, Delaware 19716

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The metal-catalyzed reactions of α-diazocarbonyl compounds with alkenes[1] and alkynes[2] are widely employed in the synthesis of hetero- and carbocyclic organic molecules. A central mechanistic consideration in these reactions is the nature of the inter-

† After July 1, 1993, address correspondence to this author at: Department of Chemistry (0506), University of California at San Diego, La Jolla, CA 92093-0506.

(1) For recent leading references and a review, see: (a) Doyle, M. P.; Pieters, R. J.; Martin, S. F.; Austin, R. E.; Oalmann, C. J.; Müller, P. *J. Am. Chem. Soc.* **1991**, *113*, 1423. (b) Doyle, M. P. *Chem. Rev.* **1986**, *86*, 919. (c) Maxwell, J. L.; Brown, K. C.; Bartley, D. W.; Kodadek, T. *Science* **1992**, *256*, 1544. (d) Taber, D. F.; Hoerrner, R. S. *J. Org. Chem.* **1992**, *57*, 441.

(2) For recent leading references, see: (a) Padwa, A.; Krumpe, K. E.; Kassir, J. M. *J. Org. Chem.* **1992**, *57*, 4940. (b) Hoye, T. R.; Dinsmore, C. J. *J. Am. Chem. Soc.* **1991**, *113*, 4343. (c) Hoye, T. R.; Dinsmore, C. J. *Tetrahedron Lett.* **1992**, *33*, 169. (d) Davies, H. M. L.; Cantrell, W. R., Jr.; Romines, K. R.; Baum, J. S. *Org. Synth.* **1991**, *70*, 93. (e) Mykytka, J. P.; Jones, W. M. *J. Am. Chem. Soc.* **1975**, *97*, 5933. (f) Petiniot, N.; Anciaux, A. J.; Noels, A. F.; Hubert, A. J.; Teyssie, Ph. *Tetrahedron Lett.* **1978**, 1239.

0002-7863/93/1515-1586$04.00/0