Recognition of DNA by Synthetic Antibodies

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A general method for the generation of molecules capable of recognizing double-stranded DNA (dsDNA) with high affinity and specificity would have wide implications in chemistry, biology, and medicine. For instance, such reagents could be useful in genome mapping by blocking certain sites to enzyme action, in diagnostic procedures requiring identification of certain DNA sequences or in therapeutic regimes requiring control of gene expression. Forming the most comprehensive recognition/diversity system known, antibodies would appear promising candidate molecules for high-affinity sequence specific recognition of DNA. However, with some notable exceptions,[1] the generation of monoclonal antibodies to DNA by conventional immunization procedures has not been successful because of the very poor ability of dsDNA to elicit an immune response in normal animals.[2] To circumvent this problem, we have prepared large antibody libraries (of the order of 10^8 members) by a synthetic route in the laboratory. In these libraries, antibody molecules are expressed on the surface of phage allowing the selection and amplification of specific antibodies by affinity sorting of the libraries on immobilized antigen. We show here how this method can be readily used to generate antibodies with high affinity for dsDNA. One of the antibodies shows some sequence specificity which has the potential to be enhanced by mutagenesis.

Three combinatorial Fab libraries were prepared on the surface of phage using the pComb 3 system.[3] Each library used the same heavy chain with the CDR3 (complementarity determining region 3) of length 10 (library 1) or 16 amino acids (libraries 2 and 3) being randomized using synthetic oligonucleotides in library construction as described (refs 4; see Figure 1). The CDR3 of the heavy chain was chosen for randomization as it forms a loop which varies in sequence and length and generally provides the most significant contribution to binding antigen. Each library used the same light chain except library 3, which had a randomized CDR3 (Figure 1). The library sizes were each on the order of 10^8. The libraries were taken through rounds of panning against human placental dsDNA dried down on to plastic. Briefly, phage were layered onto the immobilized DNA, nonspecific phage were removed as far as possible by vigorous washing, specific phage were eluted at low pH, and the library was amplified in bacteria and then reapplied to DNA in the next round of panning. After four rounds of panning, supernates from the resulting clones were screened in enzyme-linked immunoassay (ELISA) for reactivity with dsDNA. Two Fabs giving a strong positive signal were identified. The amino acid sequences of the CDR3 regions of these Fabs are presented in Figure 1.

	HC CDR3	LC CDR3
Library E	GxxxxxxxxxxxxxDx	QQYGGSPW
Library F	GxxxxxxxDx	QQYGGSPW
Library κ10/E	GxxxxxxxxxxxxxDx	QQYxxxxxxT
Fab SD1	GRAYGGNGLKMMSLDN	QQYGGSPW
Fab SD2	GRGWSGSLDI	QQYGGSPW

Figure 1. Amino acid sequences of the variable domain CDR3 regions of the heavy chains (HC) and light chains (LC) constituting the semisynthetic libraries and the DNA-binding antibodies SD1 and SD2. x refers to a position which was randomized as described.[4] The complete sequences of the heavy and light chain variable domains from which the libraries were constructed are available from GenBank (accession numbers L22156 and L22157).

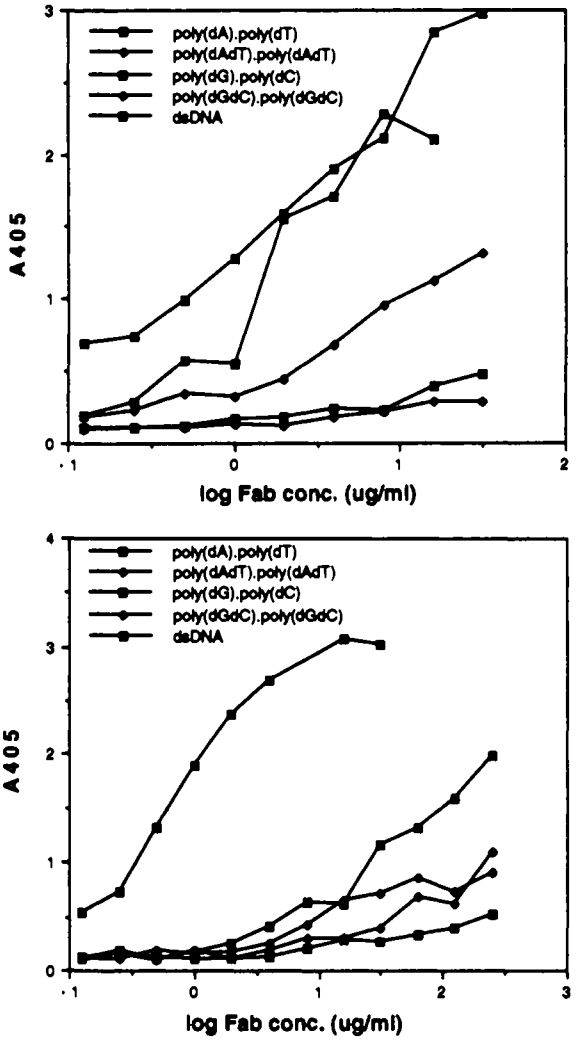

Figure 2. ELISA profiles of the reactivity of Fabs SD1 (a, top) and SD2 (b, bottom) with DNA and selected double-stranded oligonucleotides. The plots show the binding of Fabs monitored colorimetrically as a function of Fab concentration. ELISA wells were first coated with antigen. For human placental DNA, 10 μg of DNA/well was used. For the oligonucleotides, 0.5 μg/well was used. Plates were then washed five times with water, blocked in 100 μL of 3% BSA/PBS for 1 h at 37 °C, and then reacted with 25 μL of Fab in PBS for 1 h at 37 °C. After washing 10 times with water, 25 μL of a 1 in 1000 dilution of alkaline phosphatase-conjugated goat anti-human IgG $F(ab')_2$ (Pierce) was added and incubated for 1 h at 37 °C. Following 10 washes with water, 50 μL of *p*-nitrophenyl substrate was added and color development monitored at 405 nm.

The affinities and specificities of the Fabs were examined by ELISA. Both Fabs bind to double- and single-stranded DNA but not to the negatively charged polyelectrolyte dextran sulfate

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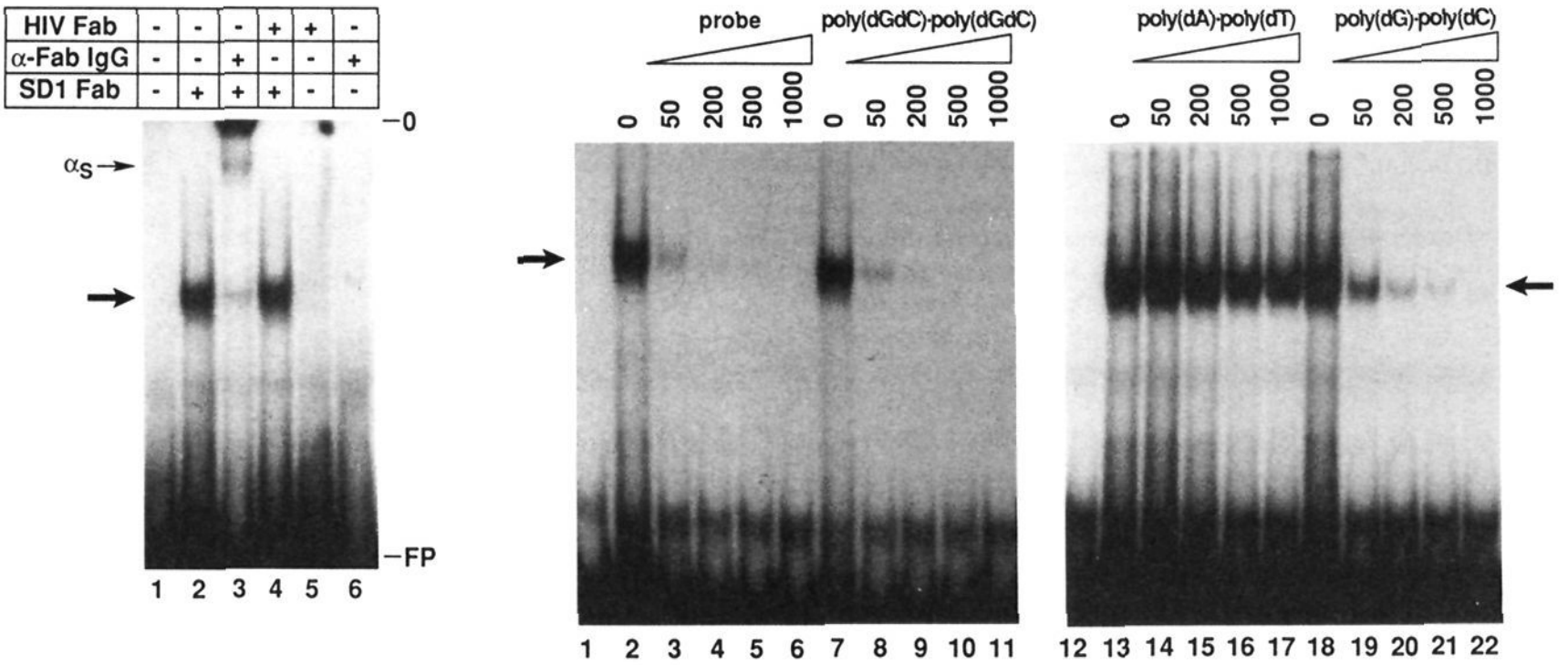

Figure 3. (a, left panel) Interaction between Fab SD1 and DNA in an electrophoretic mobility shift assay (EMSA). Lanes 1–6 contain 0.08 pmol of ^{32}P-labeled double-stranded probe (5′-AAT-GTA-TGC-GCG-CGC-GCT-TTA-GGG-GCC-CC-3′) with 0.2 pmol of Fab SD1 (lanes 2, 3, and 4). Lanes 3 and 6 included a preincubation with an anti-Fab reagent (α-Fab IgG). Lanes 4 and 5 included 0.2 pmol of a control Fab reacting with HIV-1 surface glycoprotein gp120 (HIV Fab). Binding and EMSA conditions were as described.[7] The thick arrow indicates the position of the specific Fab SD1 nucleoprotein complex, and the α_s arrow indicates the position of the supershifted Fab SD1 nucleoprotein complex. 0 marks the origin of migration and FP the position of the free probe. (b, right two panels) Specificity of binding of Fab SD1 to DNA. Lanes 1–22 contain 0.08 pmol of ^{32}P-labeled probe and 0.2 pmol of Fab SD1 in lanes 2–11 and 13–22, respectively. In addition, increasing concentrations of competitor oligonucleotide are included as shown. Numbers refer to molar excess of competitor oligonucleotide relative to the probe. Arrows indicate the position of the Fab nucleoprotein complex.

nor to lipid A. Competitive ELISA,[5] in which soluble double-stranded plasmid DNA competed with immobilized DNA for binding to Fab, showed half-maximal binding of Fab at approximately 10^{-9} M plasmid DNA, indicating a high-affinity interaction.[6] Figure 2a shows that Fab SD1 binds poly(dG)·poly(dC) as effectively as DNA, poly(dG-dC)·poly(dG-dC) less effectively, and poly(dA)·poly(dT) and poly(dA-dT)·poly(dA-dT) poorly. Fab SD2 binds all of the oligonucleotide duplexes considerably less effectively than DNA (Figure 2b).

Fab SD1 was further investigated by electrophoretic mobility shift assays. Figure 3a shows that the antibody retards a double-stranded oligonucleotide probe containing both GC- and AT-rich regions. Evidence that Fab SD1 is directly responsible for the formation of the retarded complex is provided by the lack of a shift with an Fab against HIV-1 surface glycoprotein gp120 and a supershift for Fab SD1 in the presence of an anti-Fab reagent. Figure 3b confirms the strong preference of Fab SD1 for GC over AT sequences in that poly(dG-dC)·poly(dG-dC) and poly(dG)·poly(dC) inhibit Fab binding to the probe whereas poly(dA)·poly(dT) is ineffective.

Comparison of the sequences of the heavy chain CDR3 regions for Fabs SD1 (16 residues) and SD2 (10 residues) reveals marked similarities within the stem sequences of the loops, with the former having an internal stretch of six extra residues (sequence NGLKMM, Figure 1). It may be that this stretch is responsible for the GC preference of the Fab SD1, and the feasibility of introducing mutations in this stretch to confer sequence specificity in DNA recognition is currently under study.

In conclusion, the studies described show that high-affinity antibodies to DNA can be isolated by antigen selection from synthetic antibody libraries. They also provide the basis for the selection of antibodies capable of sequence specific DNA recognition.

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(6) The plasmid used was a 5-kb derivative of pComb3.[3] Therefore a stoichiometry of 10–100 Fabs would indicate apparent affinities on the order of 10^7–10^8 M^{-1}. This is consistent with the data in Figure 2a,b showing half-maximal binding of Fabs in the range 10^{-7}–10^{-8} M. Recombinant Fabs have previously been found to be monomeric under typical conditions as judged by gel filtration analysis.

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