The sub-nanomolar binding of DNA-RNA hybrids by the single chain Fv fragment of antibody S9.6

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Abstract

The monoclonal antibody S9.6 binds DNA-RNA hybrids with high affinity, making it useful in research and diagnostic applications, such as in microarrays and in the detection of R-loops. A single-chain variable fragment (scFv) of S9.6 was produced, and its affinities for various synthetic nucleic acid hybrids were measured by surface plasmon resonance (SPR). S9.6 exhibits dissociation constants of approximately 0.6 nM for DNA-RNA and, surprisingly, 2.7 nM for RNA-RNA hybrids that are AU-rich. The affinity of the S9.6 scFv did not appear to be strongly influenced by various buffer conditions or by ionic strength below 500 mM NaCl. The smallest epitope that was strongly bound by the S9.6 scFv contained six base pairs of DNA-RNA hybrid.

Keywords

Monoclonal antibody; scFv; antibody sequence; DNA-RNA hybrids

Introduction

Monoclonal antibody S9.6 was originally generated in mice by immunization with a ΦX174 bacteriophage-derived synthetic DNA-RNA antigen. It was characterized as having high specificity and affinity for DNA-RNA hybrids [Boguslawski et al. 1986]. Initially, S9.6 was used as an enzyme-labeled reagent in solution-based nucleic acid hybridization assays [Yehle et al. 1987; Miller et al. 1988; Casebolt and Stephensen 1992]. A study of small *Escherichia coli* RNAs by DNA microarray techniques showed the utility of S9.6 for detecting DNA-RNA hybridization without needing to amplify the RNA samples [Hu et al. 2006]. An extensive transcriptome analysis in *Schizosaccharomyces pombe* by microarrays depended on S9.6 for hybrid detection, which eliminated the need for, and potential bias introduced by, reverse transcription and PCR amplification [Hu et al. 2006; Dutrow et al. 2008]. More recently, S9.6 has been found to be useful in identifying and localizing DNA-

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RNA hybrids in normal and stressed eukaryotic cells. For example, S9.6 was used to show the presence of extensive DNA-RNA hybrid intermediates during replication of mitochondrial DNA [Pohjoismaki et al. 2010]. Several investigators have used S9.6 to detect and image R-loops, which are regions of genomic DNA that are annealed to RNA and that have been linked to genomic instability and the prevention of silencing of CpG island promoters by CpG methylation [Szekvolgyi et al. 2007; El Hage et al. 2010; Gan et al. 2011; Skourti-Stathaki et al. 2011; Ginno et al. 2012]. Additionally, several groups have used S9.6 detection of DNA-RNA hybrids as a system for the development of biosensor systems [Sipova et al. 2010; Qavi et al. 2011]. Polyclonal DNA-RNA hybrid specific antibodies are a key component of human papillomavirus (HPV) molecular diagnostics developed by Digene and now marketed by Qiagen. Because of the renewed use of mAb S9.6 as a research tool and its potential use in diagnostic reagents, we sought to fully characterize S9.6. In this work, we cloned and sequenced the S9.6 cDNA, and then produced a monovalent scFv S9.6 construct through expression in bacteria. The interactions of the S9.6 scFv with nucleic acid hybrids of various lengths and compositions were measured in a variety of conditions.

Materials and Methods

Synthesis of nucleic acid hybrids

DNA-DNA hybrids were synthesized by the FDA Facility for Biotechnology Resources (Bethesda, MD). RNA-RNA and DNA-RNA hybrids were synthesized by Integrated DNA Technologies (Coralville, IA). All hybrids were synthesized with a 5′ biotin-tetra-ethylene glycol (TEG) linker. Oligonucleotide sequences and descriptions are provided in Table 1. The image in Figure 1 was generated using the UNAFold software on the Integrated DNA Technologies website (http://www.idtdna.com/UNAFold).

Isolation and expression of the S9.6 IgG and scFv

The hybridoma that produces the S9.6 IgG, HB-8730, was purchased from the ATCC (Manassas, VA); this cell line is not being distributed by the ATCC at this time. S9.6 hybridoma cells were grown in DMEM supplemented with 10% fetal bovine serum, 2 mM Glutamax, 1 mM sodium pyruvate, and 50 μg/mL gentamycin (Life Technologies, Carlsbad, CA). S9.6 IgG was purified from culture supernatants using HiTrap Protein G columns (GE Healthcare Life Sciences, Piscataway, NJ) as previously described [Hu et al. 2006]. For cloning of the antibody genes and construction of an scFv expression vector, hybridoma cells were grown to 80–90% confluence, then were centrifuged at 1000 rpm for 10 min, supernatant was decanted, and the cell pellet was resuspended in RNAlater (Life Technologies) and placed at 4°C overnight. Total RNA was isolated using TRIzol (Life Technologies), and reverse transcription was performed using avian myeloblastosis virus reverse transcriptase (AMV-RT) with an anchored oligo(dT)20 primer from Integrated DNA Technologies. The V_L and V_H genes were amplified and fused into the scFv format in the vector pAK400 as previously described [Krebber et al. 1997]. The two initial PCR reactions to amplify the variable genes used a pool of 23 degenerate primers for amplification of the V_L gene, and another 23 degenerate primer pool was used to amplify the V_H gene in a separate reaction. The third, subsequent PCR reaction fused the two variable genes together in the order of V_L-linker-V_H into an scFv construct. The primers and the linker sequence

were used as previously described [Krebber et al. 1997]. The ligation mixture was electroporated into *E. coli* strain XL1-Blue, and 48 individual colonies were picked and inoculated into 200 μL/well Luria Broth (LB) with 2% glucose (w/v) and 30 μg/mL chloramphenicol in a 96-well plate, placed at 30°C, and shaken overnight. The next day, a fresh plate was prepared with a 1:10 inoculation of the overnight cultures into the same medium and it was shaken at 30°C for 3 h. The plate was then centrifuged, and the bacterial pellets were resuspended in 200 μL of LB with 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and 30 μg/mL chloramphenicol and shaken at 25°C overnight. After centrifugation the following morning, bacterial pellets were resuspended in 20% BugBuster HT (EMD Millipore, Billerica, MA) in PBS and shaken for 1 h at 25°C. Cell debris was pelleted by centrifugation, and the scFv-containing supernatant was transferred to a new plate and stored at 4°C until screening, as described below. A bacterial clone selected as described below was used for large-scale S9.6 scFv protein purification by immobilized metal affinity chromatography and size exclusion chromatography, as described previously [Radjainia et al. 2010]. The S9.6 heavy and light chains of the original S9.6 hybridoma cells were also independently determined by sequencing of the hybridoma RNA (LakePharma, Belmont, CA). Heavy and light chains of the S9.6 Fab, which were produced by papain cleavage of the IgG, were subjected to N-terminal sequencing by Edman degradation (Research Technologies Branch, NIAID/NIH).

Surface plasmon resonance analysis

Surface plasmon resonance (SPR) experiments were performed using a ProteOn XPR36 instrument (Bio-Rad, Hercules, CA). The standard running buffer used in experiments and analysis was 10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 7.4 (1x HBS-T). Other buffers used to test the effect of pH are described in Table 3. Screening of *E. coli* cell lysates from potential S9.6 scFv-producing clones was accomplished by a 60 sec injection of the supernatant at 50 μL/min across surfaces of a neutravidin SPR chip (NLC chip, Bio-Rad, Hercules, CA) coated with oligonucleotide 23MDR (Table 1) immobilized at four different densities, and dissociation was measured over a 300-second period. Single stranded DNA (30ssD, Table 1) and a neutravidin surface lacking bound oligonucleotides were negative controls. Regeneration was accomplished with a 30-sec injection of 1 M NaCl at 50 μL/min.

For kinetic analysis of purified S9.6 scFv, all flow channels were first rinsed and equilibrated with running buffer. Each nucleic acid hybrid was separately resuspended in doubly-deionized water and then diluted to a concentration of 6.25 nM in running buffer. These biotinylated hybrids were immobilized at a flow rate of 50 μL/min for 30 sec onto separate vertical lanes of a neutravidin-coated chip (NLC chip, Bio-Rad, Hercules, CA). Purified S9.6 scFv was diluted in running buffer to concentrations of 6.25, 12.5, 25, 50, and 100 nM, and the concentration series and a buffer blank was applied across horizontal lanes at 100 μL/min for 60 sec with a dissociation time of 600 sec, allowing for the simultaneous detection of S9.6 scFv interactions with multiple types of hybrids. Regeneration was accomplished with a 30-sec injection of 1 M NaCl at 50 μL/min, followed by equilibration in running buffer until the baseline stabilized. The neutravidin surface lacking oligonucleotides between each immobilized lane, termed the "interspot," was used as a

control for analysis. ProteOn Manager software was used to measure kinetic parameters using the Langmuir model of interaction.

Results

Production and purification of the S9.6 scFv

After the S9.6 variable regions were placed in the scFv format, it was found that supernatants from 28 of the 48 clones tested showed specific binding to the 23MDR DNA-RNA hybrid. After sequencing the 28 plasmids from the clones, one of them was picked that represented the consensus protein sequence of the samples. The scFv protein sequence encoded by this plasmid is presented in Figure 2 and this plasmid was used for expression, purification, and all further analyses. Large scale expression and purification of the S9.6 scFv from the *E. coli* strain XL1-Blue resulted in 555 μg of purified protein from 1 L of bacterial culture.

To confirm the fidelity of this sequence, the S9.6 cDNA from the S9.6 hybridoma was independently sequenced. The light chain cDNA sequence predicted a V domain that was almost identical to the one selected as a consensus, with the N-terminal sequence of D**VL**MTQTP (bolded and underlined residues indicate differences from the consensus of Figure 2). The two altered amino acids observed in the scFv clone of DIVMTQTP were likely introduced by the degenerate primers. However, two different heavy chain V domains were detected. These heavy chain variants showed differences only in framework region 1 (FR1) and were identical from amino acid position 15 onward. The first heavy chain variant sequence began with **E**VQLQQSGPELVKP, and thus differs from the scFv consensus sequence only at the E at the N-terminus. The corresponding N-terminal Q in the scFv sequence was likely introduced by the degenerate primers used during the initial PCR amplification step while constructing the scFv. The second heavy chain variant sequence began with QV**H**LQQSGPE**M**V**RL**. The variant amino acid residues at positions 3, 11, 13, and 14 in this sequence occur at low frequencies in these positions in all antibodies, as determined by reference to the Abysis database (http://www.bioinf.org.uk/abysis/). In contrast, the residues present at the same positions in the first heavy chain variant sequence are the most common at each location. Subsequent N-terminal sequencing of the S9.6 variable heavy and light chains revealed that only the first heavy chain sequence, **E**VQLQQSGPELVKP, was produced by the hybridoma. Therefore, the scFv and IgG V_H sequences are identical except for the N-terminal amino acid. The N-terminal sequence of the V_L domain was confirmed as D**VL**MTQTP, which differs from the scFv sequence at positions 2 and 3, as mentioned earlier.

S9.6 scFv specificity and affinity

Nucleic acid hybrids of varying sizes and sequence were synthesized for use in characterizing the binding of the S9.6 scFv (Table 1 and Figure 1). Each hybrid consisted of a single strand of deoxyribo- and ribo- nucleotides with a biotin residue at the 5′ end. Four thymidines (uracils in RNA-RNA) were located between the complementary sequences so that they formed a loop of non-pairing nucleotides. The name of each hybrid molecule includes the number of base pairs formed in the hybrid.

SPR was performed between the purified S9.6 scFv and 23-base pair DNA-DNA, RNA-RNA, and DNA-RNA hybrids in standard running buffer (1x HBS-T). All three types of hybrids were synthesized and tested in A-T (A-U) and G-C-rich forms to assess whether nucleotide composition affects affinity. For these two 23-base pair DNA-RNA hybrids, addition of S9.6 scFv in 1x HBS-T generated sensorgrams characteristic of high affinity binding. As expected, neither of the DNA-DNA hybrids nor the GC-rich RNA-RNA hybrid showed binding to the scFv. However, S9.6 scFv bound AU-rich RNA-RNA, although with a weaker affinity than to the DNA-RNA hybrids. When S9.6 IgG, instead of the scFv, was used under the same conditions, it also displayed binding to AU-rich RNA-RNA (data not shown).

The 23-base pair AT-rich DNA-RNA, GC-rich DNA-RNA, and AU-rich RNA-RNA hybrids were further analyzed by SPR to measure S9.6 scFv affinity and to assess the influence of their nucleotide composition. For the interaction of S9.6 scFv with each hybrid, data from three trials were combined to calculate the average association rates (k_a), dissociation rates (k_d), and the equilibrium dissociation constants (K_D). K_D values for the AT-rich, GC-rich, and mixed 23-base pair DNA-RNA hybrids were 0.6 ± 0.1 nM, 0.8 ± 0.2 nM, and 0.47 ± 0.08 nM, respectively (Table 2). The K_D for AU-rich RNA-RNA was 2.7 ± 0.4 nM.

S9.6 scFv affinity and epitope length

DNA-RNA hybrids of six different lengths were tested via SPR to determine the minimum size of the epitope that S9.6 would bind. The S9.6 scFv displayed similar affinities to hybrids having 8, 10, 15 and 23 base pairs, with affinities averaging 0.5 nM (Table 2 and Figure 3), consistent with those noted above for the hybrids having 23 base pairs. The affinity decreases to 1.9 nM for a hybrid of six base pairs, while no binding could be detected for the four base pair hybrid (Table 2, Figure 3).

Affinity in various ionic, pH, and buffer conditions

Several other running buffers were tested to characterize solvent effects on the affinity of S9.6 scFv for nucleic acid hybrids. The buffers tested included ionic or pH variations of the standard 1x HBS-T and two other buffer systems at pH 7.4 (Table 3). Nearly all the hybrids that previously had shown binding to S9.6 scFv were included, with the exceptions of the two shortest hybrids. In general, there was minimal variation in K_D for the DNA-RNA hybrids between buffers prepared at pH 7.4 and slightly more variation across different levels of pH. The affinities were significantly weaker for the AU-rich RNA-RNA hybrid across all conditions relative to DNA-RNA hybrids (Figure 4A and B). All samples resulted in higher K_D values (weaker binding) in 1x HBS-T containing 500 mM NaCl, which was expected in a higher salt environment.

Discussion

Elucidation of the protein sequence of the S9.6 scFv allows for analysis of this particular antibody in relation to previously characterized antibodies. CDR H3, which is known to significantly affect the specificity of antibodies, is ten amino acids in length in S9.6. This

corresponds well with a previous analysis of mouse antibodies, which revealed that the peak length of CDR H3 was eleven residues for DNA binding antibodies [Johnson and Wu 1998]. When comparing the amino acid sequences of the S9.6 scFv against the Kabat database, only two residues are distinctive at their given positions. The lysines located at light chain position 106A and heavy chain position 85 are unusually low in their frequency at these positions (0.5% and 0.4%, respectively) [Martin 1996]. Previous work has shown that although there is considerable variability in the protein sequences of CDR loops, the main chains of the hypervariable loops are found to inhabit a relatively few structural conformations [Chothia and Lesk 1987; Chothia et al. 1989]. The set of conformations for each CDR are referred to as Chothia canonical classes, and the analysis of the CDR loops of S9.6 by online resources provided by Dr. A.C.R. Martin (http://www.bioinf.org.uk/abs/chothia.html) reveal that CDR L2, L3, and H1 each conform to the Chothia canonical class one. CDR L1 is most similar to Chothia canonical class four, except that light chain residue 27B is isoleucine rather than leucine. CDR H2 is most similar to Chothia canonical class two, with heavy chain residues Leu52A, Asp55, and Ser71 differing from the expected residues.

The binding of the S9.6 scFv and IgG to AU-rich RNA-RNA hybrids was an unanticipated result that confirmed a previous report of the direct detection of RNA-RNA hybrids with the S9.6 IgG [Kinney et al. 1989]. This type of cross-reactivity has been reported for the siRNA binding protein, P19, isolated from tombusviruses [Silhavy et al. 2002]. P19 has been determined to bind to RNA-RNA hybrids with low nanomolar affinity [Jin et al. 2010], but it has also been used to detect DNA-RNA hybrids [Gullerova and Proudfoot 2012].

The kinetic measurements presented here confirm that S9.6 displays a strong preference for binding DNA-RNA hybrids, while also showing a weaker affinity for certain RNA-RNA hybrids. The measured K_D of about 0.6 nM (or 600 pM) for DNA-RNA hybrids appears quite different from the K_D of 12 pM originally reported for the bivalent IgG in a competition ELISA done in solution with the high molecular weight ΦX174 DNA-RNA hybrid [Boguslawski et al. 1986]. However, that assay was subject to complications due to avidity effects and to its different format, making a comparison of the affinities problematic.

The S9.6 scFv appears to interact with a nucleic acid duplex of at least six base pairs, with full occupancy of the antibody binding site occurring only for the duplex having eight base pairs. This can be rationalized structurally, as an A-form DNA-RNA hybrid of six or eight base pairs is 16.9 and 22.5 Å, respectively, in length and distances on a typical scFv molecule that span the entire binding face or only the CDR3 loops, both light and heavy, are on the order of 30 and 18 Å, respectively. Thus, when the DNA-RNA hybrid length drops below the prototypical core size of an scFv, the affinity is reduced. It is likely that S9.6 recognizes the A-form of nucleic acid hybrids, as DNA-RNA and RNA-RNA hybrids are believed to exist predominantly in this conformation. The viral protein P19 similarly cross-reacts with both of these hybrids, and the crystal structure of the P19-double stranded RNA complex reveals that P19 binds to the A-form of double stranded RNA in a sequence-independent manner [Vargason et al. 2003].

Conclusions

We examined the specificity and affinity of the monoclonal antibody S9.6 using its monovalent scFv fragment. Isolation of the S9.6 scFv and SPR binding analyses provided a value for the monovalent affinity of S9.6 in the range of 600 pM, as compared to the originally determined K_D of 12 pM. Because RNA-RNA hybrids were a secondary target, with five-fold lower affinity, it may be worthwhile to engineer the S9.6 scFv to exhibit more stringent specificity. The sequence of the S9.6 scFv, as presented here, should aid in that process.

Acknowledgments

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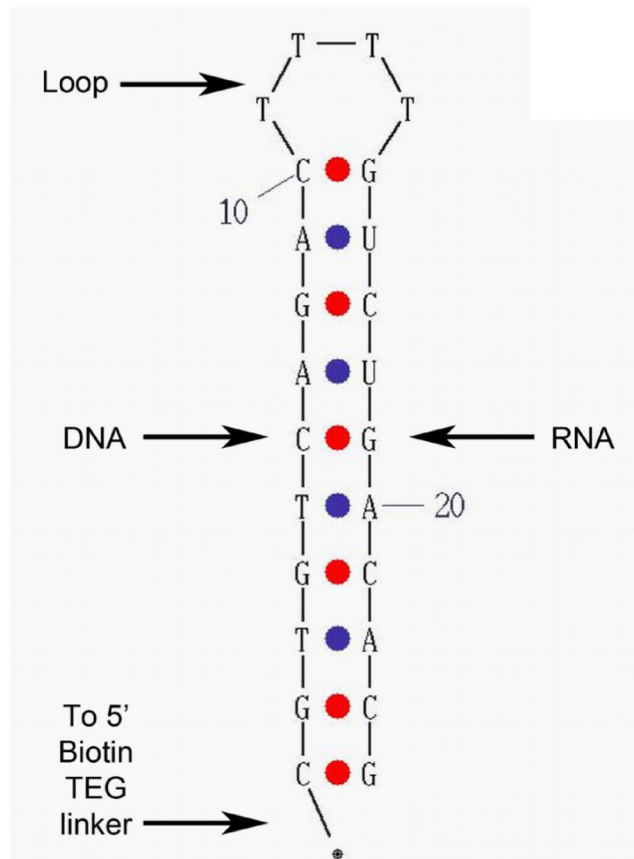
Figure 1.
Schematic of 10MDR, one of the 10-base nucleic acid hybrids used in this study. Ten bases of DNA-RNA hybrid extend from the base of the loop to the 5′ and 3′ ends of the oligonucleotide. Four thymidines, that remain single-stranded, form the hairpin loop. Other hybrids are similar in design, but differ in hybrid length, in percent GC content, and whether they are DNA-DNA, RNA-RNA, or DNA-RNA.
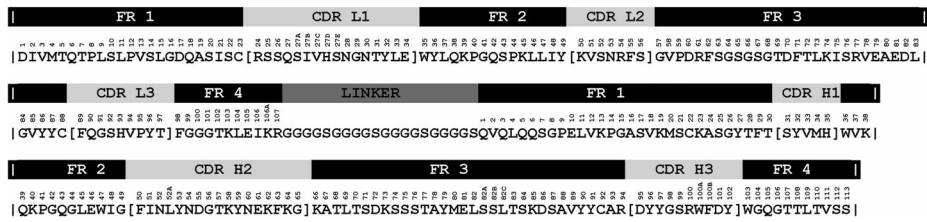
Figure 2.
Sequence of the S9.6 scFv protein displayed with the light and heavy variable domains, numbered by the Kabat scheme [Kabat et al. 1991] above the amino acid sequence (light chain residues 1 to 107 and heavy chain residues 1 to 113). The framework regions (FR), complementarity determining regions (CDR), and linker region (LINKER) are drawn above the numbered sequence.
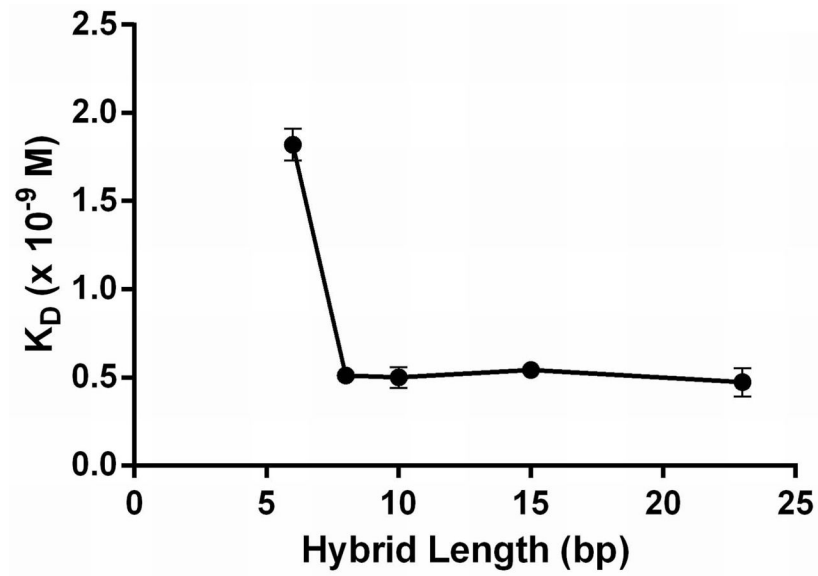
Figure 3.
Effect of DNA-RNA hybrid length on S9.6 scFv affinity as determined by SPR in 1x HBS-T buffer. The smallest detectable hybrid had six base pairs (6MDR in Table 1). A four-base pair hybrid was not detectable (4MDR in Table 1). Each point represents the average of three measurements. Error bars denote standard deviation.
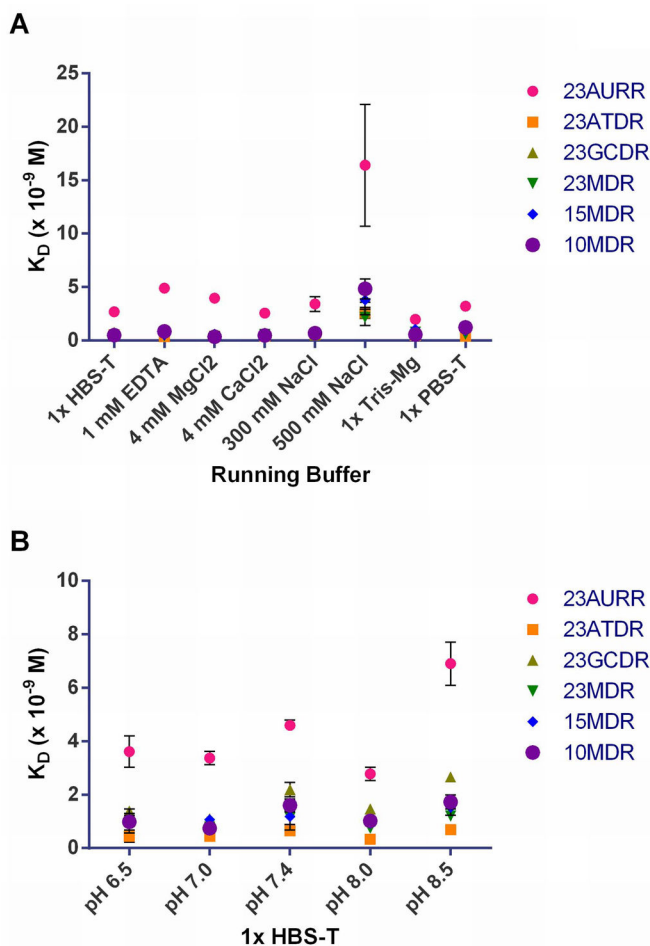
Figure 4.
Comparison of S9.6 scFv affinities under several conditions obtained via SPR. (A) Binding between S9.6 scFv and nucleic acid hybrids was measured in ionic strength variations of 1x HBS-T buffer, in the presence of Mg/Ca, in Tris-Mg, and 1x PBS-T. The leftmost lane shows the binding in the standard 1x HBS-T buffer. Note that the RNA-RNA hybrid (23AURR) displayed lower affinity binding under every condition when compared to the DNA-RNA hybrids. (B) Binding interactions were tested in individual experiments with standard 1x HBS-T prepared at different levels of pH. Each point represents the average of three measurements. Error bars denote standard deviation.

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Table 1

Descriptions and sequences of nucleic acid hybrids used in SPR

Hairpin Length (bp)	Content	%GC[a]	Hybrid name[b]	Oligonucleotide Sequence[c,d]
0	Equal Mix	50	30ssD	CGGTGTGGTCGCTGTAATCAGAAAACGTGT
23	AT-rich	17.4	23ATDD	AATTACATTGATAGAATTATTAG**TTTT**CTAATAATTCTATCAATGTAATT
23	GC-rich	82.6	23GCDD	GGCGGTCCCAGCGAGGCCGCCGCCGA**TTTT**TCCGGCGGCCTCCGCTGGGACCGCC
23	AU-rich	17.4	23AURR	rArArUrUrArCrArUrUrGrArUrArGrArArUrUrArUrUrArG**rUrUrU**rUrCrUrArArUrArArUrUrCrUrArUrCrArArUrGrUrArArUrU
23	GC-rich	82.6	23GCRR	rGrGrCrGrGrUrCrCrCrArGrCrGrArGrGrCrCrGrCrCrGrCrCrGrA**rUrUrU**rUrUrCrCrGrGrCrGrGrCrCrUrCrCrGrCrUrGrGrGrArCrCrGrCrC
23	AT-rich	17.4	23ATDR	AATTACATTGATAGAATTATTAG**TTTT**rCrUrArArUrArArUrUrCrUrArUrCrArArUrGrUrArArUrU
23	GC-rich	82.6	23GCDR	GGCGGTCCCAGCGAGGCCGCCGCCGA**TTTT**TrUrCrCrGrGrCrGrGrCrCrUrCrCrGrCrUrGrGrGrArCrCrGrCrC
23	Equal Mix	52.2	23MDR	CGGTGTGGTCGCTGTAATCAGAA**TTTT**rGrUrCrUrGrArtUrtArCrArGrCrGrArtCrCrArCrtArCrtCrG
15	Equal Mix	53.3	15MDR	CGGTGTGAATCAGAC**TTTT**rGrUrCrUrGrArtUrtCrArCrtCrG
10	Equal Mix	60	10MDR	CGTGTCAGAC**TTTT**rGrUrCrUrGrArCrArCrG
8	Equal Mix	50	8MDR	GAGAACTG**TTTT**rCrArGrUrtCrUrtC
6	Equal Mix	50	6MDR	GAACTG**TTTT**rCrArGrUrtUrtC
4	Equal Mix	50	4MDR	GACT**TTTT**TrArGrUrtC

[a] GC content is calculated for the hybrid portion of the oligonucleotides, which does not include the four thymidine bases encompassing the turn. "Equal mix" denotes 40–60% GC content.

[b] For example, "30ssD" is an abbreviation of "30-nucleotide single stranded DNA".

AT = AT-rich, GC = GC-rich, DD = DNA-DNA hairpin, RR = RNA-RNA hairpin, DR = DNA-RNA hairpin, M=equal mix of AT and GC.

[c] Thymidines and uracils (**T**, **rU**) in bold and underline do not participate in base pairing.

[d] 5′(Biotin-TEG) linker is at the 5′ end of all sequences

Table 2

Kinetic-Langmuir approximations of the affinity of S9.6 scFv binding to hybrids obtained via SPR.

Nucleic Acid Hybrid	k_a ($\times 10^6$ 1/M·s)[a]	k_d ($\times 10^{-3}$ 1/s)	K_D ($\times 10^{-9}$ M, nM)
23AURR	0.44 ± 0.03	1.19 ± 0.07	2.7 ± 0.4
23ATDR	2.2 ± 0.3	1.2 ± 0.4	0.6 ± 0.1
23GCDR	1.3 ± 0.3	1.0 ± 0.1	0.8 ± 0.2
23MDR	3.22 ± 0.06	1.5 ± 0.3	0.47 ± 0.08
15MDR	2.6 ± 0.3	1.4 ± 0.2	0.54 ± 0.02
10MDR	3.2 ± 0.1	1.6 ± 0.3	0.50 ± 0.06
8MDR	3.1 ± 0.4	1.6 ± 0.2	0.51 ± 0.01
6MDR	1.00 ± 0.06	1.81 ± 0.04	1.82 ± 0.09

[a] Averages and standard deviations for all parameters are derived from 3 trials.

Table 3

Running buffers used to test the effects of differing ionic strength, divalent cations, and pH on binding in SPR.

10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 7.4 -- standard
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, 1 mM EDTA, pH 7.4
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, 4 mM $MgCl_2$, pH 7.4
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, 4 mM $CaCl_2$, pH 7.4
10 mM Hepes, 300 mM NaCl, 0.005% Tween 20, pH 7.4
10 mM Hepes, 500 mM NaCl, 0.005% Tween 20, pH 7.4
10 mM Tris HCl, 15 mM $MgCl_2$, 0.005% Tween 20, pH 7.4 (1x Tris-Mg)
1.06 mM KH_2PO_4, 5.60 mM Na_2HPO_4, 154 mM NaCl, 0.005% Tween 20, pH 7.4 ($1 \times$ PBS-T)
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 6.5
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 7.0
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 8.0
10 mM Hepes, 150 mM NaCl, 0.005% Tween 20, pH 8.5