Selection of Antibody Single-chain Variable Fragments with Improved Carbohydrate Binding by Phage Display*

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Su-jun Deng, C. Roger MacKenzie, Joanna Sadowská, Joseph Michniewicz, N. Martin Young, David R. Bundle†, and Saran A. Narang‡

From the Institute for Biological Sciences, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada

A single-chain variable fragment (Fv) version of a murine monoclonal antibody, Se155-4, specific for Salmonella serogroup B O-polysaccharide, was used as a model system for testing monovalent phage display as a route for enhancing the relatively low affinities that typify anti-carbohydrate antibodies. Random single-chain Fv mutant libraries generated by chemical and error-prone polymerase chain reaction methods were panned against the serogroup B lipopolysaccharide. Panning of a randomly mutated heavy chain variable domain library indicated selection for improved serogroup B binders and yielded six mutants, five of which showed wild type activity by enzyme immunoassay. Two of these were apparently selected on the basis of better functional single-chain Fv yield in Escherichia coli. A heavy chain mutation (Ile77 → Thr) in one mutant, 3B1, appeared to have a particularly dramatic effect, resulting in yields of approximately 120 mg/liter of functional periplasmic product. The sixth mutant, 4B2, had complementarity determining region 1 (CDR1) and CDR2 mutations and demonstrated 10-fold improved binding, by enzyme immunoassay, relative to the wild type. Extensive analysis of antigen-antibody interactions indicated that the improved binding properties of 4B2 were attributable to a higher association rate constant and interaction with an epitope that is larger than the trisaccharide recognized by the wild type. None of the mutations involved known trisaccharide contact residues; this was consistent with analysis of wild type and mutant single-chain Fvs by titration microcalorimetry. Examination of the structure indicated that two mutations in the heavy chain CDR2 provided improved surface complementarity between the protein and the extended epitope encompassing 2 additional hexose residues. However, introduction of only the CDR2 mutations into the wild type structure failed to confer the improved binding properties of 4B2, indicating an indirect effect by the more distant mutations. Panning of randomly mutated light chain variable domain and full-length single-chain Fv mutant libraries did not yield mutants with improved assembly or binding properties.

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Intervention in recognition events in which carbohydrates participate has great potential in the prevention and therapy of various disease states because carbohydrates are primary markers in cellular recognition processes (1). A major challenge in this area is to overcome the problems associated with the relatively low affinities that are characteristic of most carbohydrate-binding proteins, a trait that also makes them obvious candidates for protein engineering from both theoretical and practical perspectives. We have used an antibody Se155-4, specific for Salmonella serogroup B O-polysaccharide, as a model system for investigating the molecular basis of carbohydrate binding by proteins. The advantages offered by this system include a well refined crystal structure (2), a detailed description of binding thermodynamics (3, 4), and an efficient Escherichia coli expression system for Fab and single-chain Fv (5, 6). An extensive site-directed mutagenesis study expanded our understanding of antigen binding by Se155-4 but also showed the limitations of predictive binding site redesign (7). In view of this, in vitro mimicry of the immune system, particularly its capacity for diversification under selective pressure, is an attractive alternative to a completely rational approach to redesign. By expressing antibody fragments on the surface of filamentous bacteriophages, it is possible to mimic both the antigen-driven selection and affinity maturation stages of the immune response (8).

In this report we describe the screening of randomly generated mutants of Se155-4 by the phage display technology that has recently been developed for the isolation of antibody fragments by expression of natural (9–11) or semisynthetic (12) variable-gene libraries on bacteriophage surfaces. The entire V<sub>H</sub> or V<sub>L</sub> domains and full-length scFv were subjected to mutation by chemical and error-prone PCR methods, and the resulting mutant libraries were panned against serogroup B lipopolysaccharide. Only the V<sub>H</sub> libraries yielded clones with improved binding to serogroup B lipopolysaccharide. The majority of the amino acid residues that contribute to binding pocket formation and participate in the antigen-antibody hydrogen bond network reside in this domain. However, it is possible that the heavy chain interactions with antigen can be improved since CDRH2, although involved in binding pocket formation, interacts only weakly with the antigen.

EXPERIMENTAL PROCEDURES

Materials, Strains, and General Methods—All DNA manipulations were carried out by standard procedures (13). E. coli strain XL1-Blue and plasmid pHBluescript II SK(+) were purchased from Stratagene, and helper phage M13K07 was purchased from Life Technologies, Inc. Serogroup B lipopolysaccharide, O-chain, BSA-antigen conjugates, and

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† Present address: Dept. of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada.

‡ To whom correspondence should be addressed. Tel.: 613-990-3247; Fax: 613-941-1327.
trisaccharide methyl glycoside were prepared as described elsewhere.1
Enzyme conjugates and substrates for immunoassays were obtained from
Calbiochem, Kirkegaard and Perry Laboratories and Bio-Rad.

Construction of scFv-gIII Expression Phagemid—The scFv gene con-
stant was mutated by treating with nitrous acid for 50 min at 24 °C. The
purified by phenol extraction followed by restriction enzyme digestion.

Due to the variable nature of the library, the phagemid expressing the highest level of fusion protein
was used as wild type scFv-gIII construct for mutation studies and was
designated as pSK4.

Library Construction and Panning—Random mutagenesis using
plasmid pSK4 as the template was performed by error-prone PCR (50 cycles)
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Production and Isolation of Soluble scFv—Mutants selected for fur-
ther study were digested with BglII for insertion of three termination
sites, and BSA-0-chain were immobilized in 10 mM sodium acetate, pH 4.5, at
room temperature. Results obtained with the enzyme-linked immu-

Screening and Sequencing—For genera-
was digested with BglII and HindIII and ligated with BglII-HindIII
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the association rate and dissociation rate constants using BIAalogue™ software (Pharmacia Biosensor AB).

**Titration Microcalorimetry**—The thermodynamics of antigen binding by selected mutants were determined using a Microcal Inc. (Northampton, MA) titration microcalorimeter (20). Single-chain Fv's at concentrations of approximately 100 μM in 50 mM Tris, pH 8, containing 150 mM NaCl were titrated with 2 mM solutions of trisaccharide antigen in the same buffer at 25°C. Thermogram data obtained with 20 injections of 5 μl were analyzed as described previously (3, 4).

**RESULTS**

**Library Generation and Panning**—Chemical and error-prone PCR techniques were selected as methods for constructing mutant scFv libraries. The objective was to introduce diversity throughout the gene without generating structural diversity that exceeded the practical limits of library size and the screening process. The monovalent display mode afforded by the phagemid/helper phage system (9, 21) was chosen for screening purposes in the expectation that it would select primarily on the basis of affinity. The size of the error-prone PCR V\(_h\) library was estimated to be 5 x 10\(^5\), and that of the other libraries was estimated to be 10\(^6\) in each instance. With all libraries, colony-forming unit counts indicated approximately 50-fold enrichment for serogroup B binders during the panning process.

**Analysis of Panned Libraries**—Serogroup B binders obtained by the enrichment process were screened by enzyme-linked immunosorbent assay and colony lift methods. Based on screening results for the V\(_h\) library, 29 clones were sequenced. Only one clone gave the wild type amino acid sequence. The remainder yielded the six mutant sequences shown in Table I. The chemical and error-prone PCR libraries each generated three of the mutant sequences. Sequencing of selected clones from the panned V\(_c\) and full-length scFv libraries revealed that all had the 3B1 or 4B2 sequences shown in Table I. It was obvious that these clones were contaminants from the V\(_h\) library because they carried the same silent mutations as these clones.

There was a surprisingly strong bias toward substitutions at certain positions with all six V\(_h\) mutants. All exhibited substitutions at one or more of residues 31, 55, 56, 77, and 109 (Table I). Mutations appeared at four CDR positions, three FR3 positions, and one FR4 position. Particularly striking were the FR4 mutation, Gly\(^{109}\) → Ser, which occurred in three of the six mutants and the CDR1 mutation, Asn\(^{31}\) → Asp, which occurred in half of the mutants. Mass spectrometry was used as a convenient and accurate means of confirming the expected masses of wild type and mutant molecules. All observed values fell within a range of 4–6 Da above the calculated masses (calculated molecular mass = 26,547 for the wild type).

**Single-chain Fv Yields**—Product yields obtained by affinity chromatography and indirect EIA analysis of periplasmic extracts indicated that at least two of the mutants were selected on the basis of increased yield of functional scFv. In particular, replacement of Ile\(^{77}\) by Thr or Asn appeared to be associated with increased yield. Whereas the wild type construct typically gave yields of active product in the 10–15 mg/liter range, mutants 3B1, with an Ile\(^{77}\) → Thr mutation and 4B21, with an Ile\(^{77}\) → Asn mutation, gave yields of approximately 120 and 50 mg/liter of soluble periplasmic scFv, respectively.

**Structural Interpretation**—With the exception of the position 109 mutations, substitutions were limited to FR3 and the CDR1 and CDR2 loops (Fig. 2). Ile\(^{77}\), an FR3 residue that is solvent-exposed, was replaced by Asn in two of the six mutants...
binding properties of 4B2 were entirely attributable to a higher affinity to the wild type (Fig. 4). Affinity chromatography elution conditions also showed enhanced binding by 4B2. Although the other five mutants, four showed EIA activity that was similar to that of the wild type (data not shown), whereas 3B1 displayed slightly weaker activity, particularly on BSA-©-chain, whereas 3B8 and 4B21 displayed approximately twice as fast as that of the wild type and other mutants (Fig. 5). The association rate constant of 4B2 compared with that of the wild type resembled wild type than 4B2 activity.

Site-directed Mutants—A site-directed mutant was constructed to analyze the contribution of the CDRH2 mutations to improved antigen binding by 4B2. Surprisingly this mutant (SG), with Asn©15 → Ser and Ser©26 → Gly mutations displayed an antigen binding profile characterized by biphasic binding to both BSA-trisaccharide and BSA-©-chain and affinity, K equals 9.0 × 10^5 M^-1 with the trisaccharide conjugate, that more closely resembled wild type than 4B2 activity.

A site-specific mutant was also constructed to determine the additive effects of introducing the mutations thought to be responsible for the high 3B1 yield and enhanced 4B2 binding in the same scFv. The resulting scFv showed better binding than 4B2 in immunoassays (Fig. 4), but SPR results indicated very similar kinetics (Table II).

Thermodynamic Analyses—In contrast to the immunoassay and biosensor results, titration microcalorimetry showed that the affinities of wild type (K equals 1.3 × 10^7 M^-1) and 4B2 (K equals 1.0 × 10^6 M^-1) for the trisaccharide and ©-chain conjugate, respectively, with the trisaccharide conjugate) were approximately twice as fast as that of the wild type and other mutants (Fig. 5). There was a striking difference in the association profile of 4B2 compared with that of the wild type and other mutants (Fig. 5). Whereas the 4B2 association phase on plates coated with BSA-trisaccharide and BSA-©-chain was classical, that of the other mutants and the wild type was distinctly biphasic. When sensorgrams showed biphasic binding, the association rate constants were calculated using the slower second phase.

Fig. 3. Stereo drawing of the antigen-antibody complex showing the region where unfavorable contacts exist between the wild type heavy chain CDR2 loop and the extended epitope. The antigen is a heptasaccharide in which the abequose has been removed from the second repeating unit.

and Thr in a third. There was a strong bias for Asn©15 → Asp and Gly©19 → Ser mutations, but reasons for these preferences were not apparent. Although Asn©15 is a heavy chain CDR1 residue, it is not within antigen contact distance (Fig. 3).

The CDRH2 substitutions in mutant 4B2 are in an area where in the absence of conformational changes the extended oligosaccharide epitope clashes with the wild type structure. The Asn©15 → Ser and Ser©26 → Gly mutations appeared to improve surface complementarity between the protein and the extended epitope encompassing two additional hexoses (rhamnose and galactose), although Ala©29 still clashes with the galactose moiety of the extended epitope (Fig. 3).

Immunooassay and SPR Analyses—Indirect EIA performed on plates coated with BSA-trisaccharide and BSA-©-chain indicated that, of the six mutants described in Table I, only 4B2 exhibited improved antigen binding characteristics. Assays employing BSA-trisaccharide ([Man-©-Gal] and BSA-©-chain ([Man-©-Gal-Rha]©) showed that 4B2 binding to these antigens was approximately 10-fold higher in each instance, relative to the wild type (Fig. 4). Affinity chromatography elution conditions also showed enhanced binding by 4B2. Although wild type scFv eluted from an ©-chain antigen column at pH 4.5, more acidic conditions were required for 4B2 elution. Of the other five mutants, four showed EIA activity that was similar to that of the wild type (data not shown) whereas 3B1 displayed slightly weaker activity, particularly on BSA-©-chain plates (Fig. 4).

SPR analyses (Table II) of representative mutants generally agreed well with the EIA results. Relative to the wild type, 4B2 displayed approximately 10-fold stronger affinity for BSA-trisaccharide, (K equals 4.5 × 10^7 M^-1 compared with 6.0 × 10^6 M^-1 for the wild type) and BSA-©-chain, whereas 3B8 and 4B21 displayed similar affinities, and 3B1 showed slightly weaker binding (K equals 3.1 × 10^6 M^-1 and 1.5 × 10^6 M^-1 with the trisaccharide and ©-chain conjugate, respectively). The improved binding properties of 4B2 were entirely attributable to a higher association rate constant (k equals 1.3 × 10^5 M^-1 s^-1) compared with the wild type (Fig. 4). The dissociation rates were quite similar, although the k equals values for 4B2/3B1 and 4B21 (3.4 × 10^-3 s^-1 and 3.7 × 10^-3 s^-1, respectively, with the trisaccharide conjugate) were approximately twice as fast as that of the wild type and other mutants (Table II, Fig. 5). There was a striking difference in the association profile of 4B2 compared with that of the wild type and other mutants (Fig. 5). Whereas the 4B2 association phase with BSA-trisaccharide and BSA-©-chain was classical, that of the other mutants and the wild type was distinctly biphasic. When sensorgrams showed biphasic binding, the association rate constants were calculated using the slower second phase.

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The observation that the phage display system selected for mutants which assembled more efficiently in *E. coli* is of general interest and has practical implications. The functional secretion in *E. coli* of foreign proteins, including antibody fragments, is often problematic, and the underlying reasons that govern success or failure are not understood. Changes at certain positions in the scFv mutants described here may be related to improved folding or better interactions with the linker. Replacement of Ile⁷⁷ in the V₅ heavy chain exposed residue, by either Asn or Thr resulted in substantially increased yields of functional scFv, suggesting that mutation to a nonhydrophobic residue at this position increases the productive folding of such constructs. The Gly⁶⁰→Ser mutation appears to allow for the formation of hydrogen bonds from Ser OH to the carbonyl oxygen of heavy chain residue 4 and the NH of heavy chain residue 8. The latter 2 residues form a β bulge, and the additional hydrogen bonds may stabilize the structure in the region where the linker approaches the V₅ domain.²

Se155-4 binds a trisaccharide epitope within the four-sugar repeating unit of the O-polysaccharide in a "pocket"-like site via a network of hydrogen bonds and van der Waals contacts (2). None of the contact points that confer recognition of this epitope was altered in 4B2, the mutant with the improved binding characteristics described here. The finding that it is difficult to change the fundamental characteristics of antigen binding by Se155-4 is in agreement with an earlier saturation mutagenesis study of heavy chain CDR3 (7), the major participant in the carbohydrate network.

It is thought that the main reason for improved antigen binding by mutant 4B2 relates to its ability to accommodate more easily an extended carbohydrate epitope because of reduced surface bulk in the heavy chain CDR2 region. However, the binding properties of the site-directed SG mutant indicated that this explanation is incomplete and that the 4B2 mutations which are more remote from the combining site also exert indirect effects that are partially responsible for the 4B2 binding properties. The observation that mutants 3B8 and 4B21, which contain some of the 4B2 mutations not associated with heavy chain CDR2, do not exhibit enhanced binding also supports the idea that the 4B2 mutations act in concert to produce the properties of this mutant. The indirect influence of noncontact residues on antigen binding has been reported for several other antibodies and appears to be a general phenomenon. In a site-directed mutagenesis study, Sharon (23) showed synergistic interactions between mutations that resulted in a 200-fold enhancement in affinity of an anti-μ-azoprophylarsonate antibody. Also, in an analysis of somatic mutations in an anti-lysozyme antibody, Lavoie et al. (24) observed that mutations involving residues not contacting antigen enhanced binding by indirect or long range effects. If the improved binding displayed by 4B2 is primarily related to a reduction of antigen-antibody steric clashes in the heavy chain CDR2 region, this would lead to...
improved binding of larger oligosaccharides or trisaccharide conjugates but not of the trisaccharide hapten itself. The thermodynamic data support this interpretation since the mutants show very little change in the binding constants for the trisaccharide ligand. In contrast, the EIA and SPR analyses with the BSA-trisaccharide and BSA-poly saccharide conjugates show a far greater range of mutant properties. With the BSA-trisaccharide conjugate, the BSA or the nine-car bon tether may still clash with the heavy chain CDR2 region, in the manner of the D-chain.

Anti-carbohydrate antibodies have not been extensively studied from the standpoint of antigen binding kinetics, but the evidence to date indicates that their relatively low affinities are largely attributable to slow on-rates. The association rates reported here, and elsewhere for an antibody specific for a pneumococcal polysaccharide (25), are in the $10^5$-$10^6$ s$^{-1}$ range and are at least 100-fold slower than those reported for most antigen-antibody systems (26). For the wild type and mutant scFv s described here, the association constants determined by SPR analyses of binding kinetics were considerably greater than those from the thermodynamic data for the trisaccharide. For the wild type protein, the increase was 60-fold and for the 4B2 and 4B2I3B1 mutants 400-fold. Although both techniques dynamically more accurate, whereas the SPR and EIA systems give direct interaction data, the microcalorimetry is thermody-

| K & | & | & |
|---|---|---|---|
| & | & | & |
| & | & | & |
| & | & | & |

**Table III**

Thermodynamic parameters for trisaccharide binding by Sc55-4 Fab, scFv, and heavy chain scFv mutants isolated by phage display

![Thermodynamic parameters for trisaccharide binding](image-url)

The positioning of termination codons between the scFv and gene I11 sequences resulted in a kg-Ser carboxyl-terminal extension.

**REFERENCES**

1. Drickamer, K., and Carver, J. (1992) *Curr. Opin. Struct. Biol.* 2, 653-654
2. Cysler, M., Bose, D. R., and Bundl, D. R. (1991) *Science* 253, 442-445
3. Sigurskjold, B., W., Altman, E., and Bundle, D. R. (1991) *Eur. J. Biochem.* 197, 239-246
4. Sigurskjold, B., and Bundle, D. R. (1992) *J. Biol. Chem.* 267, 8371-8376
5. Anand, N., Dubuc, G., Phipps, J., MacKenzie, C. R., Sadowska, J., Young, N. M., Bundle, D. R., and Narang, S. A. (1991) *Gene* (Amst.) 109, 39-44
6. Anand, N. N., Mandsal, S., MacKenzie, C. R., Sadowska, J., Sigurskjold, B., Young, N. M., Bundle, D. R., and Narang, S. A. (1991) *J. Biol. Chem.* 266, 21573-21579
7. Brunsmill, D. A., Sharma, V. P., Anand, N. N., Bilous, D., Dubuc, G., Michiewicz, J., MacKenzie, C. R., Sadowska, J., Sigurskjold, B., Witt, B. W., Sittott, B., Young, N. M., Bundle, D. R., and Narang, S. A. (1993) *Biochemistry* 32, 1180-1187
8. Marks, J. D., Hogenboom, H. R., Griffiths, A. D., and Winter, G. (1992) *J. Biol. Chem.* 267, 16007-16010
9. Barbas, C. F., Kang, A. S., Lerner, R. A., and Benkovic, S. J. (1991) *Proc. Natl. Acad. Sci. U. S. A.* 88, 7798-7802
10. Marks, J. D., Hogenboom, H. R., Bonupert, T., P., McCafferty, J., Griffiths, A. D., and Winter, G. (1991) *J. Mol. Biol.* 221, 557-567
11. McCafferty, J., Griffiths, A. D., Winther, G., and Chiswell, D. A. (1990) *Nature* 345, 525-524
12. Barbas, C. F., Bain, J. D., Hoeksten, D. M., and Lerner, R. A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* 89, 4457-4461
13. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
14. Leung, D. W., Chen, E., and Goeddel, D. V. (1989) *Methods Cell. Mol. Biol.* 1, 11-15
15. Diaz, J., Rhoads, D. D., and Roufs, D. A. (1991) *BioTechniques* 11, 204-211
16. Myers, R. M., Lerman, L. S., and Mainiatis, T. (1985) *Science* 229, 242-247
17. Cwirla, S. E., Peters, E. H., Barrett, R. W., and Dower, W. S. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 85, 6378-6382
18. Jonsson, U., Fagerstam, L., Ivarsson, B., Johnsson, U., and Jaton, J. (1977) *Biochemistry* 16, 408-415
19. Fagerstam, L., Ivarsson, B., Johnsson, U., and Jaton, J. (1977) *Biochemistry* 16, 408-415
20. Wietzorek, T., Willment, S., Brandt, J. F., and Lin, L. N. (1989) *Anal. Biochem.* 17, 131-137
21. Bass, S., Greco, R., and Wells, J. A. (1990) *Proteins Struct. Funct. Genet.* 8, 309-314
22. Herron, J. N., Krana, D. M., and Voss, E. A. (1988) *Biochemistry* 27, 4602-4608
23. Sharron, D. (1990) *Proc. Natl. Acad. Sci. U. S. A.* 87, 4814-4817
24. Lavoie, T. B., Drohan, W. N., and Smith-Gill, S. J. (1992) *J. Immunol.* 148, 505-513
25. Maeda, H., Schmidt-Kessen, A., Engel, J., and Jaton, J. C. (1977) *Biochemistry* 16, 4956-4968
26. Pecht, J. (1982) *Aspects Antibody Funct.* 6, 1-68
27. Anand, N. N., Phipps, J., andBundle, D. R. (1992) *J. Mol. Recognit.* 5, 9-18
28. Foote, J., and Milstein, C. (1991) *Nature* 353, 630-632
29. Lawrence, M. B., and Springer, T. A. (1991) *Cell* 65, 659-673
30. Deng, S. J., MacKenzie, C. R., and Narang, S. A. (1993) *Nucleic Acids Res.* 21, 4418-4419