



Research paper

Charges drive selection of specific antibodies by phage display

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ABSTRACT

Phage display technology has emerged as a leading approach to select proteins with improved properties for many different types of applications. The selection typically selects not only for improved binding properties but also for other factors such as efficiency of protein production and folding in *Escherichia coli*, the host in which the proteins and the phage are produced. Furthermore, the selection methodology is likely to influence the character of retrieved variants. We have now defined the extent whereby the charge of the displayed proteins influence the selection process, resulting in an increased average positive charge among selected proteins in comparison to the proteins that are harbored in the library before selection. Implications of and possible routes to minimize this effect are discussed.

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1. Introduction

Antibodies bind specifically and with high affinity to a multitude of antigens on viruses, bacteria and parasites that enter our body daily and that would otherwise cause disease. As a scaffold in adaptive immunity, antibodies establish diversity through mechanisms such as gene recombination, junctional diversity and somatic hypermutation. Altogether these processes create a very large B cell population that encodes antibodies with diverse binding specificities by displaying differences and flexibility in binding site topography, charge, hydrophobicity etc. In the laboratory this type of diversity or synthetically generated molecular diversity is used as a tool in extensive recombinant antibody development programs. Many large libraries of high quality have been created and successfully exploited for these purposes (Fellouse et al., 2007; Knappik et al., 2000; Pini et al., 1998; Sheets et al., 1998; Söderlind et al., 2000; Vaughan et al., 1996). It should be realized though that not all antibody binding sites in populations are created equally in the sense that they can be effectively

exploited in a selection process as it occurs *in vivo* or *in vitro*, reducing the functional size of the available binder population. In the laboratory this can be circumvented to some degree as selection from phage-displayed libraries under different conditions appears to utilize different parts of the available repertoire resulting in the selection of different sets of clones (Kehoe et al., 2006; Lou et al., 2001), thereby extending the likelihood of success in a given project.

To gain a systematic insight into the driving forces in phage display selection, the dominant selection approach in use today, we investigated the nature of sequences in a combinatorial antibody library before and after selection. For this purpose we used a large antibody fragment library based on a single framework with an origin in IGHV3-23 and IGLV1-47 germline genes. Diversity has been introduced into all six complementarity determining regions (CDR²) by grafting of gene sequences encoding these loops from B cells of diverse origin. As members of this library all employ the same framework, differences in selectability are likely largely caused by differ-

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E-mail address: mats.ohlin@immun.lth.se (M. Ohlin).¹ Current affiliation: Division of Medical Genetics, University of Washington, Seattle, WA, USA.² Abbreviations: BSA: bovine serum albumin; CDR: complementarity determining region(s); FITC-biotin: 5(6)-(biotinamido)hexanoylamido-pentylthioureidylfluorescein; NIP: 4-hydroxy-3-iodo-5-nitrophenylacetyl; PBS: phosphate buffered saline, pH 7.4; scFv: single chain antibody fragment(s).

ences within the antigen binding site. Sequences of randomly picked clones before and after selections were analyzed to assess the nature of diversity in antigen binding surfaces that is effectively selected for by antigen.

2. Materials and methods

2.1. ScFv-encoding sequences

Genes encoding 69 single chain antibody fragments (scFv) were picked at random among members of the n-CoDeR library (Söderlind et al., 2000). Sequences encoding one scFv per antigen were picked at random among clones that had been selected from the same library to bind specifically to 69 different antigens (52 proteins, 9 peptides, 3 liposaccharide, 3 carbohydrates, 1 hapten and 1 cell-bound antigen). Amino acid composition was assessed in parts of the genes (Fig. 1) that had been diversified by incorporation through CDR shuffling (Jirholt et al., 1998) of B-cell derived diversity into the basic scaffold originating from the IGHV3-23 and IGLV1-47 germline genes. To get an estimate of the charge of the scFv, a theoretical value was determined from their full amino acid sequences at neutral pH using the algorithm provided in the Omega DNA analysis software (Accelrys Inc., San Diego, CA).

2.2. Antibody fragments displayed on phage and produced in soluble form.

FITC8 is a fluorescein-specific scFv that in the past has been selected from the n-CoDeR library (Söderlind et al., 2000). A library cloned into a modified version of the pFab5c. His vector (Ørum et al., 1993, Cicortas Gunnarsson et al., 2004) was created incorporating diversity in 14 residues distributed in CDR1, 2 and 3 of the heavy chain and CDR1 and 3 of the light chain of this sequence (Fig. 1) by an approach similar to that described by Persson et al. (2006). Specific binders (F2, F4 and F6) were selected from this library by phage display technology using FITC-biotin (5(6)-(biotinamido)hexanoylamido)-pentythioureidylfluorescein (Sigma-Aldrich Inc., St. Louis, MO, USA) immobilized onto streptavidin-coated paramagnetic beads (Dynal A/S, Oslo, Norway) as the catching antigen. NIP (4-hydroxy-3-iodo-5-nitrophenylacetyl)-specific binders (NIP4 and NIP19) were similarly selected using NIP-bovine serum albumin (BSA)-biotin (Biosearch Technologies, Novato, CA, USA). Monoclonal scFv displayed on phage were produced using the VSCM13 helper phage (Stratagene, La Jolla, CA, USA). Soluble scFv carrying a FLAG-tag and a hexahistidine tag were, after cloning of the genes into pPICZα vector (Invitrogen, Carlsbad, CA, USA) as described by Lantto and Ohlin (2002), produced in *Pichia pastoris* strain X-33 (Invitrogen). Soluble scFv were purified using immobilized metal affinity chromatography on a HisTrap HP column (GE Healthcare, Uppsala, Sweden) charged with Ni²⁺ ions. The purified protein was eluted with 20 mM NaH₂PO₄, 0.5 M NaCl, 500 mM imidazole, pH 7.4.

2.3. Binding analysis

Enzyme-linked immunosorbent assay was performed by coating streptavidin onto polystyrene microtitre plates (Nunc A/S, Roskilde, Denmark). After removal of unbound protein,

biotinylated antigen (FITC-biotin or NIP-BSA-biotin) diluted in PBS containing 1% BSA (Sigma-Aldrich Inc.) and 0.05% Tween 20 (TPBSB) was added to saturate the biotin-binding sites on streptavidin. Phage stocks and soluble scFv, diluted in 10 mM sodium phosphate buffer pH 7.4 containing 0.05% Tween 20, 1% BSA and 0–2 M NaCl, were added and incubated for 1 h at 37 °C. Bound scFv were detected with anti-FLAG M2 antibody (Sigma-Aldrich Inc.) followed by horse-radish peroxidase labeled rabbit anti-mouse immunoglobulins (DAKO A/S, Glostrup, Denmark). Bound phages were detected with horse-radish peroxidase labeled anti-M13 antibody (GE Healthcare). Orthophenylenediamine (Sigma-Aldrich Inc.) was used as chromogen.

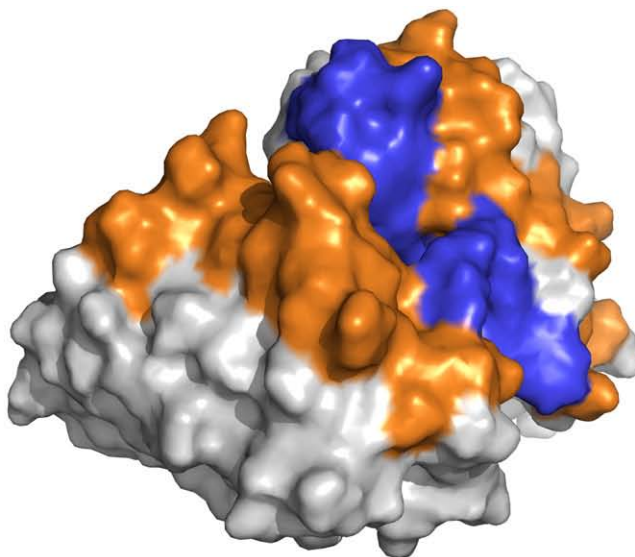
For Biacore analysis (Biacore AB, Uppsala, Sweden), streptavidin was immobilized onto CM5 sensorchips as recommended by the manufacturer. Biotin-binding sites were saturated with FITC-biotin. The binding of soluble scFv diluted to a concentration of 1–2 μM in 10 mM phosphate buffer pH 7.4 containing 0–1.5 M NaCl was investigated.

3. Results

3.1. Residues positively and negatively selected in antibody fragment phage display

The library used in this study encodes products with a common framework but with highly diverse paratopes as established by the many different hypervariable loops. Grafting of CDR into a framework, the mode whereby this library was created (Söderlind et al., 2000), may destabilize the members of the library if residues important for the structure of the domains are modified in inappropriate ways. Core residues considered to be of importance for the stability of the immunoglobulin scaffold (Ewert et al., 2003) are however conserved in the library at frequencies similar to those seen in human IgG found *in vivo* (Table 1) suggesting that a large fraction of members of the library is stable and well folded, in line with the observed behavior of the library. Consequently, a comparative study of the population of molecules before and after selection is not likely affected by poor stability of a part of the library. The population of binding sites was analyzed by sequencing of clones randomly picked from the library. Sequences of binders selected on 69 different antigens were used to determine how the selection process affected the makeup of binding sites. From this analysis it is apparent that the representation of many residues does not change substantially as a consequence of the selection process (Table 2). For instance, the frequency of tryptophan and tyrosine in selected clones did not differ in comparison to their frequencies in the population before selection. There was thus no tendency of these residues, which are known to be very important in interaction hot-spots (Bogan and Thorn, 1998), to be further enriched during antibody phage display selection. However, the frequency of valine, leucine and threonine, side chains that are underrepresented in protein–protein interaction hot-spots (Bogan and Thorn, 1998), were reduced by approximately 9–23% following selection. The most dramatic effect was seen for glutamate that was reduced in frequency in the selected relative to the unselected population by 52%. This residue has been reported to be disfavored at protein–protein interaction

A



B

HEAVY

	10	20	30						50
FITC8	EVQLLES	GGGLVQ	PGGSLRL	SCAAS	GFTF	---	SNY	WMSWVR	QAPGKGL
F2	EVQLLES	GGGLVQ	PGGSLRL	SCAAS	GFTF	---	SNY	HMTWVR	QAPGKGL
F4	EVQLLES	GGGLVQ	PGGSLRL	SCAAS	GFTF	---	SNY	WMSWVR	QAPGKGL
F6	EVQLLES	GGGLVQ	PGGSLRL	SCAAS	GFTF	---	SNY	HMSWVR	QAPGKGL

	60		70	80	90	100
FITC8	EWVSGISGN	-GGGYTY	FADSVK	-DRFTISR	DNSKNTLY	LQMNSLRAEDTA
F2	EWVSGISGN	-GGHTTF	FADSVK	-DRFTISR	DNSKNTLY	LQMNSLRAEDTA
F4	EWVSGISGN	-GGHTLY	FADSVK	-DRFTIPR	DNSKNTLY	LQMNSLRAEDTA
F6	EWVSGISGN	-GGGYTH	FADSVK	-DRFTISR	DNSKNTLY	LQMNSLRAEDTA

			120		
FITC8	VYYCAGGD	G---	SGWSFWGQGT	LVT	VSS
F2	VYYCAGGD	A---	GGWSFWGQGT	LVT	VSS
F4	VYYCAGGD	S---	SGWSFWGQGT	LVT	VSS
F6	VYYCAGGD	S---	GGWSFWGQGT	LVT	VSS

LIGHT

	10	20	30						50
FITC8	QSVLTQPPS	-ASGTPG	QRVTI	ISCTG	SSSNIG	---	AGYDV	HWYQ	QLPGTAP
F2	QSVLTQPPS	-ASGTPG	QRVTI	ISCTG	SSSNIG	---	AGH	DVHWY	QLPGTAP
F4	QSVLTQPPS	-ASGTPG	QRVTI	ISCTG	SSSNIG	---	AGYDV	HWYQ	QLPGTAP
F6	QSVLTQPPS	-ASGTPG	QRVTI	ISCTG	SSSNIG	---	AGYDV	HWYQ	QLPGTAP

	60	70	80	90	100
FITC8	KLLIYGN	-----	NNRPSGVP	-DRFSGSK	-SGTSASLAISGLRSEDEA
F2	KLLIYGN	-----	NNRPSGVP	-DRFSGSK	-SGTSASLAISGLRSEDEA
F4	KLLIYGN	-----	NNRPSGVP	-DRFSGSK	-SGTSASLAISGLRSEDEA
F6	KLLIYGN	-----	NNRPSGVP	-DRFSGSK	-SGTSASLAISGLRSEDEA

			120					
FITC8	DYYCAAW	DDSS	--LS	GRVF	GGGT	KLT	VL	G
F2	DYYCAAY	DDSS	--IT	GRVF	GGGT	KLT	VL	G
F4	DYYCAAY	DDT	--V	GRVF	GGGT	KLT	VL	G
F6	DYYCAAW	DDSS	--LS	GRVF	GGGT	KLT	VL	G

Fig. 1. Illustration of the parts of scFv sequences derived from the n-CoDeR library (Söderlind et al., 2000) that were diversified and analyzed for amino acid content of 69 clones before and after selection. This is shown on a model (Söderlind et al., 2000) of FITC8 scFv in which heavy and light chain CDR3 are highlighted in blue and other residues introduced into the scaffold by transplantation (Jirholt et al., 1998) of sequences encoded for by B-cell derived mRNA are shown in orange (A). These residues are also underlined (black) in heavy and light chain variable region sequences of the set of fluorescein-specific scFv used in this investigation (B). CDR as defined by the IMGT nomenclature (Lefranc et al., 2003) are highlighted with a grey line. Residue numbers are given as defined by the IMGT nomenclature (Lefranc et al., 2003). The proteins represented by these sequences were either selected directly from the n-CoDeR library (FITC8) or after diversification of FITC8 and subsequent selection on immobilized antigen (F2, F4 and F6). Residues deliberately modified in the library from which F2, F4 and F6 were selected are highlighted by asterisks. Additional modifications are likely to have been introduced during the polymerase chain reaction-based construction of the library. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1Frequency of immunoglobulin core residues^a in an antibody library and in IgG.

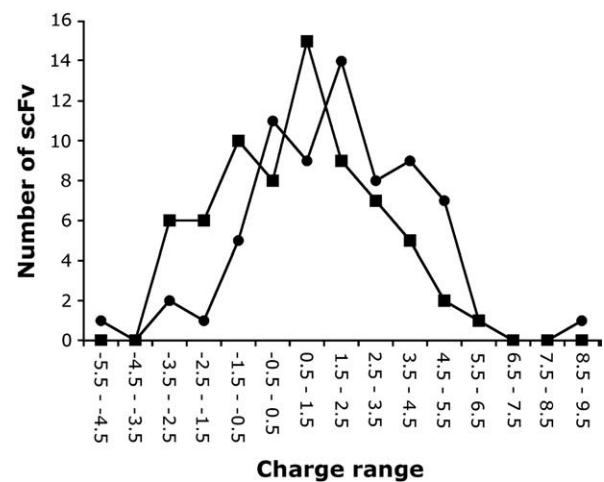
Sequence position ^b	Frequency of germline-encoded residue (%)																		
	Central core							Upper core							Lower core				
	6	23	41	104	43	51	75	95	97	98	2	4	L	A	F	S	I	V	G
IgHV3-23 residue	E	C	W	C	R	E	R	R	E	D	V	L	A	F	F	S	I	V	G
Naïve n-CoDeR library (n = 69)	99	100	100	100	93	100	99	100	100	100	100	100	100	100	100	100	100	100	100
Selected from n-CoDeR (n = 69)	100	100	100	100	100	100	99	100	100	100	100	100	100	99	100	100	100	100	100
IgG derived from IGHV3-23 (n = 32)	nd ^d	100	97	100	100	94	100	91	81	100	nd	nd	nd	91	100	88	90	100	97
IGHV1-47 residue	Q	C	W	C	Q	K	R	R	E	D	S	L	G	S	A	A	A	V	F
Naïve n-CoDeR library (n = 69)	100	99	100	100	100	100	100	100	100	100	100	100	100	88	96	96	100	100	100
Selected from n-CoDeR (n = 69)	100	100	100	100	100	100	100	100	100	100	100	100	100	100	96	100	100	100	100
IgG derived from IGLV1-47 (n = 7)	100	100	100	100	100	100	100	100	100	100	nd	nd	nd	83	86	100	100	100	100

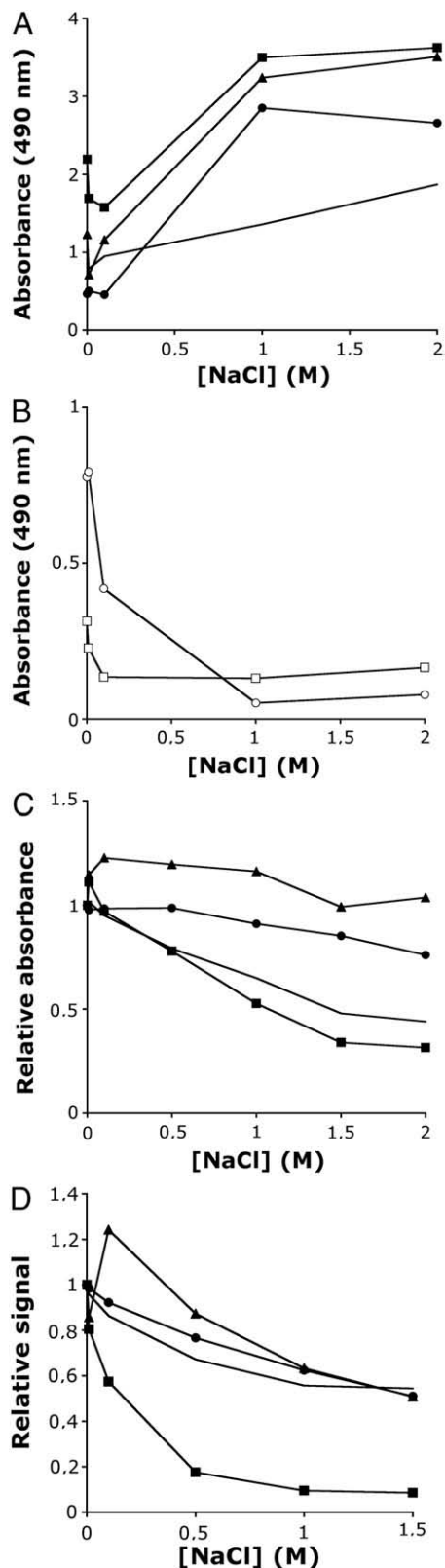
^a Diversity in core regions (Ewert et al., 2003) of the n-CoDeR scaffold before and after selection and in IgG originating from the same germline genes (de Wildt et al., 1999).^b Numbering according to the IMGT nomenclature (Lefranc et al., 2003).^c IGHV3-23 carries K in this position but it is e.g. as a consequence of the rearrangement process often mutated. Therefore R, commonly found in many other IGHV3 subgroup members as well as in mutated sequences, is also included in this analysis.^d nd = not determined.**Table 2**
Sequence diversity before and after selection from phage-displayed library.

Residue ^a	Before selection		After selection		Change (%)
	n ^b	Frequency (%)	n	Frequency (%)	
A	422	7.7	431	8.1	5
C	155	2.8	141	2.6	−7
D	380	6.9	333	6.2	−10
E	58	1.1	27	0.51	−52
F	71	1.3	80	1.5	16
G	611	11.2	654	12.3	10
H	84	1.5	76	1.4	−7
I	203	3.7	194	3.6	−2
K	139	2.5	114	2.1	−16
L	117	2.1	104	1.9	−9
M	90	1.6	92	1.7	5
N	396	7.2	383	7.2	−1
P	181	3.3	180	3.4	2
Q	131	2.4	135	2.5	6
R	346	6.3	376	7.0	12
S	948	17.3	1019	19.1	10
T	194	3.5	145	2.7	−23
V	372	6.8	298	5.6	−18
W	190	3.5	184	3.4	−1
Y	386	7.0	370	6.9	−2
Unknown	2	0.04	0	0	
Total	5476		5336		

^a Standard one-letter code defining amino acids.^b Number of each residue found in analyzed regions (defined in Fig. 1) of 69 scFv.

sites, potentially as a consequence of the high flexibility and thus unfavorable entropic contribution to binding of its side chain. Altogether, an average scFv contained 1.1 fewer negatively charged residues (aspartate and glutamate) and 0.1 additional positively charged residues (accounted for by an increase in arginine and a decrease in lysine residues) after as compared to before selection contributing to a statistically significant (Student's *t*-test, $p < 0.05$) increase in theoretical positive charge after selection. Nevertheless, the two populations substantially overlapped (Fig. 2) with respect to charge

**Fig. 2.** Charge distribution of 69 scFv identified at random in the n-CoDeR library (squares) and after selection on 69 different antigens (circles). Since all of the analyzed clones utilize the same framework, the differences seen between clones are mainly due to the number of charged residues in or close to the antigen binding site.



suggesting that it was not a minor part of the library that was efficiently utilized but rather that there was a trend towards better selection of a specific segment of the library.

3.2. Diverse effects of salt on scFv binding depending on context

To elucidate the effect of charged residues in antibody fragments, one set of model antibodies was investigated for binding under conditions of different ionic strengths. These scFv bind to fluorescein. One of them (FITC8), had been selected from the n-CoDeR library (Söderlind et al., 2000), and the others, F2, F4 and F6, had been further evolved by mutagenesis and subsequent selection for antigen binding. As a consequence, the sequences of these scFv differed only in a few positions mostly within or close to the CDR (Fig. 1). They did not differ in any of their charged residues (Asp, Glu, Arg and Lys). They all carried a conserved arginine in the penultimate position (position 116 according to the IMGT nomenclature (Lefranc et al., 2003)) of light chain CDR3. If this residue was mutated to valine or tryptophan the resulting product lost antigen binding (data not shown) suggesting that this charged residue was important for antigen interaction.

When investigating the ability of these fluorescein-specific scFv displayed on phage to bind antigen we noticed that the interaction was substantially enhanced at higher buffer ionic strengths (Fig. 3A) suggesting that repulsive electrostatic interactions may be shielded under such conditions. In contrast, the binding of these soluble scFv, i.e. in the absence of the highly charged phage particle, in ELISA (Fig. 3C) as well as in Biacore (Fig. 3D) assays was either not affected by an increase in ionic strength (and thus shielding of electrostatic attractive or repulsive forces) or was negatively affected under such conditions. Consequently, it appeared that the effect of salt on antigen binding behavior of these scFv on phage was not caused by the scFv–antigen interaction itself but depended on the presence of the charged phage particle. In contrast, scFv specific for NIP displayed on phage behaved quite opposite to the fluorescein-specific binders (Fig. 3B), suggesting that other factors dominated the binding of that particular set of scFv to its antigen.

Taken together our data propose that the affect of ionic strength on antigen binding is very much dependent on context, i.e. the specific nature of the antibody and its presence on phage particles in combination with the targeted epitope and its particular local environment.

4. Discussion

In the present investigation we demonstrate that different parts of antibody libraries will be harvested for their content of specific binders with different efficiencies based on their content and sign of charge. For any given epitope, specific binders displaying high positive charge may not be the optimal

Fig. 3. Binding of scFv displayed on phage (A, B) and in soluble form (C, D) in buffers with different concentrations of NaCl to antigen as determined by ELISA (A–C) and Biacore (D) technology. ScFv specific for fluorescein (A, C, D) and NIP (B) were analyzed. They include scFv FITC8 (no symbol), F2 (closed squares), F4 (closed circles), F6 (closed triangles), NIP4 (open circles) and NIP19 (open squares).

choice, but in the global context positively charged scFv fair better in the selection process at least when carried out in buffers close to physiological conditions, as is common practice.

Key side chains likely to participate in molecular interactions and interaction hot-spots (Bogan and Thorn, 1998), like tryptophan and tyrosine, did not differ substantially in frequency in selected clones over the frequency seen in the naïve repertoire. The herein investigated library was created using CDR-encoding gene sequences transplanted from immunoglobulin-encoding transcripts derived from human B lineage cells. A fraction of these CDR are likely to have undergone an antigen-selection process *in vivo*, a fact that likely contributes to the compatibility of these sequences with antigen binding. As CDR carry a higher frequency of tryptophan and tyrosine residues as compared to proteins or protein surfaces in general (Janin et al., 1988; Ofra et al., 2008) it seems that antibody CDR as found in this library already carry a sufficient level of these residues to create efficiently selected paratopes. Hence, an increased frequency of these side chains provides no further selective advantage.

Arginine is a residue that is able not only to participate in electrostatic interactions but also to contribute with hydrogen bonding as well as with hydrophobic and aromatic-type interactions. Consequently, arginine is overrepresented in many protein–protein interaction surfaces in comparison to other protein surfaces (Bogan and Thorn, 1998) and its frequency is, in contrast to that of tyrosine and tryptophan, further increased after as compared to before selection. Although arginine may be linked to cross-reactive binding properties (Hande and Manser, 1997; Birtalan et al., 2008) its overrepresentation has also been linked to successful library performance on targets like negatively charged carbohydrates (Schoonbroodt et al., 2008).

Several explanations for the increased or decreased frequency of certain residues in *in vivo* or *in vitro*-selected repertoires can be envisaged. Not only are, as outlined above, different side chains capable of effectively participating in binding surfaces to different degrees but other mechanisms may contribute as well. Immune repertoires selected on platelet-specific auto-antibodies in idiopathic thrombocytopenic purpura (Roark et al., 2002) are enriched for positively charged variable domains, just as are those selected by phage display on a multitude of antigens as demonstrated in this study. It was suggested that the enrichment of positively charged antibody variable domains on trombocytes was promoted by the strong negative charge of its surface. As a B cell's surface is negatively charged, too, it was postulated that mainly positively charged antibodies would be able to efficiently overcome the charge repulsion by the cells' surfaces. Consequently, positively charged binders would preferentially be selected against antigens found in such a negatively charged context. Phage particles also carry a substantial negative charge (Marvin et al., 1994) suggesting that charge repulsion and attraction may be acting under certain selection conditions. Our data suggest that modification of ionic strength during the selection process may substantially affect the selection outcome. It seems that the effect of modification of the ionic strength may be difficult to predict and that the context in which the antibody fragment and the antigen are presented to each other will affect the outcome of their encounter. It appears that shielding of electrostatic interactions will affect e.g. the

selection process by promoting or counteracting different binders in different ways. Inclusion of high concentrations of NaCl does not, however, affect only electrostatic interactions. It also serves to strengthen hydrophobic interactions (Ghosh et al., 2005; Thomas and Elcock, 2006). Together the effects of salt on selection may thus be multifactorial. Nevertheless, simple modification of ionic strength beyond commonly employed isotonic conditions may be used as a tool to diversify selected populations and to improve the efficiency whereby different parts of the specific repertoire contained within a library can be sampled. This agrees well with finding by Lou et al. (2001) that demonstrated the importance of using different selection approaches to fully harvest the specific diversity contained in a library.

A common procedure used in phage display involves retrieval of antigen-bound phages on streptavidin-coated paramagnetic beads such as Dynabeads. These beads are negatively charged at neutral pH. This particular bead type has been used in one or several steps in many of the selection procedures that resulted in the 69 different selected sequences analyzed in this study. It is quite possible that, in similarity to the effect seen on antigen-specific repertoires selected on trombocytes *in vivo* (Roark et al., 2002), the bead surface that negatively charged phages have to approach to allow retrieval of a specific binder may affect the outcome of selection. This effect, if present, is likely reduced by the common incorporation of higher concentrations of salt in the buffer used for selection. Modification of the charge of the major phage coat protein or neutralization of charge interactions by the addition of oligo-lysine has also recently been shown to reduce unwanted interactions between phage particles and selection targets (Lambooy et al., 2008). Collectively, the use of these approaches has the potential to minimize overrepresentation of positive charges in selected populations and thus to exploit certain parts (in terms of charge) of antibody space more efficiently.

Reasons other than those directly affecting the binding of a displayed molecule to a target may, however, be involved in preferential selection of certain sequences. It is possible that certain CDR motives, such as those rich in arginine or lacking aspartate or glutamate, contribute to the stability of scFv or their ability to be displayed on phage, thereby affecting the selectability of these sequences. Secondly, certain CDR, in particular those derived from other gene subgroups, may not always be perfectly incorporated into a given scaffold (Lantto and Ohlin, 2002; Kügler et al., 2009) such as that used in the n-CoDeR library, causing differences in the population of molecules before and after selection. However, CDR inserted into the library derived largely from the same heavy and light chain subgroups as the framework scaffold. As a result, residues in the charge cluster and upper and lower cores, structures that are likely to influence stability (Ewert et al., 2003), are conserved at levels similar to IgG produced by human B cells (Table 1). We conclude that the majority of the genes encode proteins with a core compatible with folding. It is nevertheless possible that some sequences fit less well than others in the framework affecting the efficiency whereby they are displayed. In our view, a range of effects both related to propensity to display and to bind intended targets are likely to contribute to the shift in charge properties seen after selection.

In contrast to the behavior of populations of clones, individual antibody fragments may, as illustrated in this

study, behave differently dependent on the context in which the antigen is presented. We conclude that it is imperative that proper choices of conditions with respect to selection and screening technique are made to isolate clones with properties well suited for the intended application.

In conclusion, this study demonstrates the effect of charges for the interaction of antibody fragments displayed on filamentous phage and their cognate antigen. It suggests that efforts to modify the level of such interactions may expand the diversity and nature of specific binders selected by this technology.

5. Disclosure statement

MO has received research grants from BioInvent Int. AB, the owner of the n-CoDeR library technology. LD is employed by and holds stocks in BioInvent Int. AB.

6. Role of the funding source

This study was supported by grants from the Swedish Research Council and BioInvent Int. AB. All genes and proteins encoded by them are owned by BioInvent Int. AB. Representatives of BioInvent Int. AB have been allowed to review the contents of the manuscript prior to submission.

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