



Monoclonal antibody proteomics: Use of antibody mimotope displaying phages and the relevant synthetic peptides for mAb scouting



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ABSTRACT

Monoclonal antibody proteomics uses nascent libraries or cloned (Plasmascan™, QuantiPlasma™) libraries of mAbs that react with individual epitopes of proteins in the human plasma. At the initial phase of library creation, cognate protein antigen and the epitope interacting with the antibodies are not known. Scouting for monoclonal antibodies (mAbs) with the best binding characteristics is of high importance for mAb based biomarker assay development. However, in the absence of the identity of the cognate antigen the task represents a challenge. We combined phage display, and surface plasmon resonance (Biacore) experiments to test whether specific phages and the respective mimotope peptides obtained from large scale studies are applicable to determine key features of antibodies for scouting. We show here that mAb captured phage-mimotope heterogeneity that is the diversity of the selected peptide sequences, is inversely correlated with an important binding descriptor; the off-rate of the antibodies and that represents clues for driving the selection of useful mAbs for biomarker assay development. Carefully chosen synthetic mimotope peptides are suitable for specificity testing in competitive assays using the target proteome, in our case the human plasma.

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1. Introduction

In the search for disease-specific biomarkers decoding of human proteome(s) has become crucial [1]. Current proteome profiling technologies are mainly developed on around the use of mass spectrometry (MS) [2], and different affinity reagents-centered technology platforms [3] or a combination of the two [4,5]. MS-based technologies suffer from some technological issues resulting from the wide dynamic representational range of different proteins in the examined proteome(s), which, in case of the human plasma, covers more than 10 orders of magnitude [6]. Also the lack of appropriate affinity reagents for downstream verification and development of clinically useful diagnostic tests makes it difficult to bring MS-platform-discovered diagnostics to the bedside [7].

Affinity reagents based approaches use a wide spectrum of specific binders such as polyclonal poly- and mono-specific antibodies [8], monoclonal antibody derived recombinant antibodies [9,10], or antibody fragments [11] as well as the non-antibody constituted reagents; affibodies [12] and aptamers [13]. The affinity reagent based approach has demonstrated its potential in several clinical applications [14,15] and it has the promise, that it can quickly bring its discovery to the bed-site. Limitations of these technologies are the pre-selected targets, determined by the inclusion of the antibodies with known specificity, and the fact that resolution of these techniques directly correlates with the number of the applied reagents which is presently a key bottleneck of these procedures.

Global proteome survey (GPS) combines features of MS and affinity proteomics, using context-independent motif specific antibodies to enrich motif containing peptides from the digested proteome(s) and a highly specialized MS technique for the analysis [16]. With GPS it is possible to increase MS output, to carry out pre-conception independent screening, but the search is limited by the use of motif-specific antibodies and the technology probes

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a digested proteome, where PTI-s and protein–protein interactions cannot be followed.

Monoclonal antibody proteomics [17–19] is a technology that profiles epitopes of complex proteomes like the human plasma, and this process may also produce pre-validated clinical assay as the outcome of the discovery procedure. Epitope profiling provides an advantage over other methods because it measures protein levels as well as posttranslational or other protein modifications that affect epitopes and are apparent in disease conditions. The technology has recently shown unique success in identifying lung cancer associated [18] and other biomarkers [17,19] using high throughput ELISA or microchip based assays for the discovery. Monoclonal antibody libraries are complex, and in the case of nascent libraries include thousands of mAbs [17]. These antibodies are primarily planned to be used in experiments where no pre-conception is set toward antibody specificity and the resolution of such proteome profiling efforts correlate directly with the number of antibodies included. Initially, during the mAb proteomics process in the absence of known antigen, very little information is available to rank mAbs based on specificity and binding characteristics. Finding the antigens for each of the thousands of mAbs in the library with presently applied methods would be a daunting task but it might be circumvented by finding mimotopes for them.

Libraries of peptides displayed on the surface of bacterial phages have been frequently used for mapping antibody epitopes, for characterizing or inhibiting protein–protein contacts, or selecting peptide mimics for non-peptide ligands. The technique has gained huge popularity since George P. Smith first described it [20]. A random library of peptides coding double stranded DNA fragments are inserted into the genome of (most frequently Ff class) filamentous phages and the encoded peptides are displayed as fusion proteins on the surface of the phage. Connection between genetic material and selectable function, such as mAb binding allows rapid identification of variants with the desired properties [21]. The most frequently applied method of screening is bio-panning [22], where phages which are able to bind to the immobilized interacting partner are enriched by repeated and alternating “propagation” and “binding” cycles. Binding of the phages to the partners are verified usually with an immunoassay and subsequent sequencing of the relevant phage DNA reveals the peptides displayed [23].

Epitope mapping of antibodies greatly helps understanding the nature of antigen–antibody interactions [24] which is important not only to elucidate the etiology of different (e.g. autoimmune) diseases [25], but to develop better, more effective vaccines [26] or diagnostics [27,28]. Considering that most of the epitopes recognized by antibodies are not linear [29], the usefulness of linear peptides as antibody epitopes has limitations. Since linear continuous epitopes are often only a few amino acids in length the screening of libraries might affinity select peptides that exactly match the primary structure of the epitope [30,31], however, in most of the cases phage display peptide libraries can only help to identify critical residues within a continuous epitope that are involved in antibody binding.

Screening phage display peptide libraries can affinity select mimotopes, which are peptides that mimic discontinuous epitope structures [32]. Mimotopes may not have similarity to any linear sequence of the antigen and may represent conformation dependent interaction of the epitope with the antibody. Several analytical tools are available now to map the native epitope based on sequences of the selected mimotopes and the three dimensional structure of the antigen [33,34]. Testing affinity of the phage-displayed peptides directly is a rarely used technology compared to the widely applied mimotope selection. Surface Plasmon Resonance (SPR) or other methods are rather used to demonstrate binding of the antibody to the phages expressing specific peptides than to determine affinity [35–37] and the technique is not often

applied in determining phage(expressed peptide)–protein affinity at all [38]. Because of the lack of this information the selection of the peptides to be synthesized can only be based on the phage display results and not on binding data. The most likely reason which limits the wider use of this potentially suitable method is the technical difficulties which lay in the application of phages in SPR experiments.

Here, we present and devise a workflow of phage display and SPR experiments and show that these provide clues for scouting of mAbs based on key features of binding and specificity in the absence of cognate epitope and purified cognate protein antigen.

The usefulness of the methodology we describe here is in the selection of candidates for the development of protein biomarker assays such as ELISA or mAb microchips during the mAb proteomics technology.

2. Materials and methods

2.1. Antibodies

Monoclonal antibodies (Bsi0080, Bsi0086 and Bsi0088, all IgG-s) were derived from our human plasma proteome specific mAb library recognizing accessible epitopes on human plasma proteins. The mAbs were produced by immunizing animals with selectively depleted and normalized human plasma using the classical somatic cell fusion procedure. Antigen binding of the purified antibodies was verified in a capture enzyme immune-assay where the binding of biotin-labeled plasma samples to immobilized antibodies were determined, as described before [18].

2.2. Phage display

Phage display system Ph.D.TM-12 Phage Display Peptide Library Kit (New England Biolabs, Ipswich, MA) which contains approx. 10^9 individual clones was used according to the vendor's manual. The library contains random peptide 12-mers fused to a minor coat protein (pIII) of M13 phage and the displayed peptide 12-mers are expressed at the N-terminus of pIII, with the sequence followed by a short spacer (Gly–Gly–Gly–Ser). The 10^9 individual clones covering only a minor fraction of the possible 4×10^{15} sequences. The panning protocol was executed in 96-well Nunc Maxisorp plates. The plates were coated with antibodies at $10 \mu\text{g}/\mu\text{l}$ concentration diluted in 0.5 M NaHCO_3 , 0.5 M NaCl pH 8.6 buffer and incubated o/n at 4°C . The empty surface of the plate was blocked using 5 mg/ml casein dissolved in 100 mM NaHCO_3 , 100 mM NaCl $0.02 \text{ Na}_2\text{S}_2\text{O}_3$ pH 8.6 for 1 h at 4°C . The remaining blocking protein was washed away 6 times with TBST buffer (TBS with 0.1% added Tween-20). The phage mixture (for the first panning cycle the phage library, for the remaining cycles the amplified and purified phages from the previous cycle) was added then and incubated with constant shaking at 250 rpm for a hour at room temperature. The unbound phages were washed 6 times with TBST buffer. The specifically bound phages were eluted with 0.2 M glycine pH 2.2 then immediately neutralized with 1 M Tris pH 9.1. For amplification of the phage particles *E. coli* ER2738 strain was used. The culture was grown for 5 h at 37°C . The phages were precipitated from the supernatant by 1/6 volume of 20%PEG/2.5 M NaCl and resuspended in TBS. The precipitation procedure was executed twice. The phage particles purified by this method were used for the latter panning cycles. The eluted phages after the third panning round were used for the subsequent steps. 24 individual phage clones were picked up and their binding to the antibodies were checked by phage-ELISA. Positive clones were selected for DNA preparation and sequencing. For each tested mAb DNA/peptide sequences of at least 12 independent phage clones were determined.

Table 1

Summary of the sequences selected for Bsi0080, Bsi0086 and Bsi0088 mAbs.

Antibody name	Peptide name in study	Sequence	Frequency of phage display selection	Used in SPR experiments
Bsi0080	080A	SSPTIPWEQHPF	8/14	
	088A-Bio	SSPTIPWEQHPF-GGGS-K-B [*]	NA	Pep-AB
	080B	TSQVTDWLYHPY	6/14	
	088B-Bio	TSQVTDWLYHPY-GGGS-K-B [*]	NA	Pep-AB
Bsi0086	–	TYAHRGPHLNH	4/16	
	086A	AWITHPTQLIQI	4/16	AB-Phage
	086C	ETWYISTSYVVS	3/16	AB-Phage
	–	HIYYISESLPM	1/16	
	–	EWYVSTSLPLP	1/16	
	–	SYTYTTHLLPLA	1/16	
	086B	TFYYNTSLLPIP	1/16	AB-Phage
	–	HDDWYISTSHTD	1/16	
Bsi0088	088B	TSQVTDWLYHPY	8/16	AB-Phage; Pep-AB
	088B-Bio	TSQVTDWLYHPY-GGGS-K-B [*]	NA	
	088A	SSPTIPWEQHPF	6/16	AB-Phage; Pep-AB
	088A-Bio	SSPTIPWEQHPF-GGGS-K-B [*]	NA	
	088C	DPYLLWENHHAG	1/16	AB-Phage
	–	YTNPMTLTLQLR	1/16	

AB-Phage: immobilized antibody, phage used as analyte.

Pep-AB: immobilized peptide, antibody used as analyte.

^{*} C-terminal Biotinylated peptides.

2.3. Peptide displaying phage selection for the studies

The peptides specified by the phage display are listed in Table 1. Both with Bsi0088 and Bsi0086 three phages were selected for binding studies displaying peptides that were selected with high (2 phages for each antibody) or with low (1 for each) frequency out of the 16 individual phages.

2.4. Determination of phage concentration

Phage titer by plaque formation was quantified according to the Phage Display Kit manual with the slight modification of using 96 deep-well plates. PhageTitration ELISA kit (Progen, Heidelberg, Germany) was used for the ELISA quantification of phages.

2.5. Peptide synthesis for studies

Two peptides were chosen for the studies. These peptides were selected with high frequency by the phage display in case of Bsi0088 and with almost similar frequency in case of an independent antibody Bsi0080. The biotinylated peptides were synthesized at JPT Peptide Technologies (GmbH, Berlin, Germany). Synthetic peptides contained a GGGS linker sequence similarly to the phage displayed peptide, which was fused to the N terminus of pIII minor coat protein of M13 phage via this linker. In the synthetic biotinylated peptide the biotin was linked to the linker through an additional C-terminal lysine (Table 1).

2.6. Surface plasmon resonance (SPR) measurements

All binding experiments were executed on a Biacore X (Biacore, GE Healthcare Life Sciences, Uppsala, Sweden) instrument. Experiments were done in HBS-EP buffer (10 mM HEPES, 0.15 M NaCl, 3 mM EDTA, 0.005% Surfactant P20 (=Tween 20), pH 7.4). During the analysis of the phage–antibody interaction the flow rate was 10 µl/min for both the association and dissociation phase.

2.7. Antibody immobilization on the chip surface

All mAbs were covalently immobilized on C1 sensor chips using the Amine Coupling Kit. The surface matrices were activated by a

7-minute injection of an aqueous solution of 0.2 M 1-ethyl-3-(3-dimethylpropyl)-carbodiimide and 0.05 M N-hydroxysuccinimide. Then the antibody at 50 µg/ml concentration in 0.01 M Na-acetate (pH 5) was injected into the sensor cell (30–60 µl). All coupling reactions were done at a flow rate of 5 µl/min. Remaining N-hydroxysuccinimide-ester groups were inactivated by injection of 35 µl 0.1 M ethanoleamine-HCl.

2.8. Determination of binding curves using different dilutions of the phages

For SPR studies phages were amplified according to the manual with the slight modification of using HBS-EP as buffer. The phage preparations from each clone were diluted 5-, 10-, 20- and 40-fold (12.5–100 nM) in HBS-EP. Calculation of dissociation constants was done from data collected for 300 s after termination of ligand injection.

2.9. Peptide capture on the chip surface

For the capture of peptides BiaCore precoated streptavidin chips were used. Before capture the surface was pretreated with 1 M NaCl, 50 mM NaOH solution three times to remove any contamination. For capture 10 µl of 10 µg/ml (5 µM) biotinylated peptide solution was used to reach saturation on the cell surface.

2.10. Determination of binding curves using different dilutions of the antibodies

The antibody preparations were diluted to 1333 nM, 666 nM, 266 nM, 133 nM, 66 nM and 26 nM in HBS-EP. Calculation of dissociation constants was done from data collected for 120 s after termination of ligand injection, which lasted also for 120 s (20 µl of analyte).

2.11. Competition experiments

The binding specificity of antibodies to immobilized peptides was further investigated through plasma competition assays. During the experiments three kinds of runs were executed: mAb at a fixed concentration (133 nM) alone; a serial dilution from the

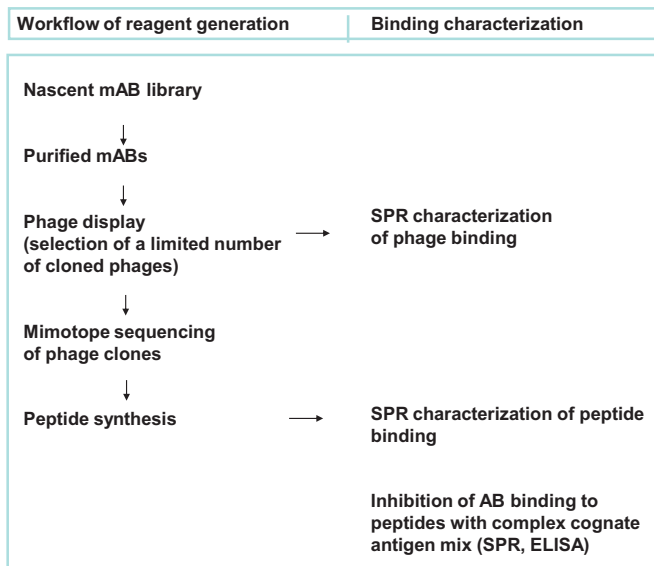


Fig. 1. Workflow of reagent generation and testing.

plasma alone (non-specific signal); and a serial dilution of the plasma with the fixed concentration of antibody. During analysis we recorded the response changes for the plasma-mAb and the plasma experiments then we calculated the difference of these responses at each plasma dilution, and normalized it to the response of the plasma-free mAb. For the analysis we determined the dynamic range of the competition, above which addition of higher amount of plasma will not change the level of competition. Usually 0–5% of plasma provided the dynamic range.

2.12. Determination of the K_D values

BIAevaluation is a stand-alone software package for presentation and evaluation of sensorgram data from SPR analyses. This program was used for the determination of the K_D values. For the tested interactions 1:1 Langmuir model was applied. This is the simplest model for 1:1 interaction between the analyte and immobilized ligand and is equivalent to the Langmuir isotherm for adsorption to a surface.

3. Results and discussion

Initially, during the monoclonal antibody proteomics profiling process the nascent libraries may contain multiple thousands of mAbs. At this stage, no other information is available about antibody specificity than verified binding to the complex analyte mix, but in subsequent steps antibodies are selected based on biomarker quality criteria [17–19]. At the same time, in order to select antibodies for assay development, clues for antibody quality and specificity are necessary. Based on the experiments described here, Fig. 1 shows a workflow for the generation of useful information on

antibody quality in the absence of epitope specificity and purified cognate protein. The technology is proposed for application during the proximal phase of the monoclonal antibody proteomics profiling process we described earlier [17,18].

3.1. SPR characterization of phage binding

Previously, in a seminal paper Houshmand [36] has shown that for phage–antibody interaction the C1 chip is the most suitable due to the readily accessible surface for phage particles. The C1 sensor chip has a matrix-free carboxymethylated surface instead of the commonly used CM chip surfaces, where carboxymethylated dextran is attached covalently to the gold surface. The flat surface of C1 allows interactions to take place closer to the surface. These features can be beneficial when working with multivalent interaction partners, large molecules, molecular complexes and particles. Our pilot experiments confirmed these findings and therefore we used the C1 chips in the experiments reported here.

Under the conditions described, the signals produced by phage binding to antibodies could easily be differentiated from those created by non-binders. Although, the shape of the binding curves differed significantly from each other, depending on the antibody and the specific phages, the dose dependence of the responses was obvious in all the cases, and we determined k_{on} , k_{off} and K_D values, which are summarized in Table 2. The determined affinities varied in a range of approximately 1 order of magnitude.

We attempted to find an explanation for the variability in cognate phage species number (binding to the same mAb but carrying different sequences). In our present phage display experiments one or two leading peptide sequences were selected, with (Bsi0088) or without (Bsi0080) additional sporadic peptides. Our first working hypothesis was that the affinity (K_D) of the peptide sequence for a particular mAb determines the number of phages selected carrying this sequence. However, we found that the dissociation constant (k_{off}) of the phage–antibody interaction correlates with the number of cognate phage species recognized by a particular mAb (Fig. 2). We found that the same rule applies to other antibody phage pairs as well (not shown). The correlation between the dissociation constant and the frequency of cognate phage species means that those phages will be selected more frequently during the panning procedure which dissociate slowly from the antibody independently of their on rate (k_{on}). As in addition to the low K_D , the low k_{off} are also important, independent and preferential characteristics of good mAbs, our results suggest that complexity of independent cognate phage species in phage display provides a potentially valuable clue for selecting useful antibodies.

3.2. SPR characterization of mimotope peptide binding

For the peptide–antibody binding experiment we used two biotinylated peptides which represented the sequences of phages 088A and 088B and were named as 088A-bio and 088B-bio respectively. Binding characteristics of several mAbs were tested at high concentration on both peptides. The binding and nonbinding pairs could be distinguished easily, just as in the case of phage–antibody

Table 2
Phage binding parameters on antibodies Bsi0088 and Bsi0086.

Antibody	Phage identifier	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)	Standard deviation of K_D (M)
Bsi0088	088A	1.73E+05	7.87E−04	4.55E−09	8.18E−10
Bsi0088	088B	1.17E+05	2.90E−04	2.48E−09	1.25E−09
Bsi0088	088C	1.70E+05	3.70E−03	2.18E−08	6.27E−09
Bsi0086	086A	2.92E+05	8.10E−04	2.77E−09	1.60E−10
Bsi0086	086B	5.85E+05	2.65E−03	4.53E−09	3.80E−09
Bsi0086	086C	3.01E+05	1.94E−03	6.45E−09	1.83E−09

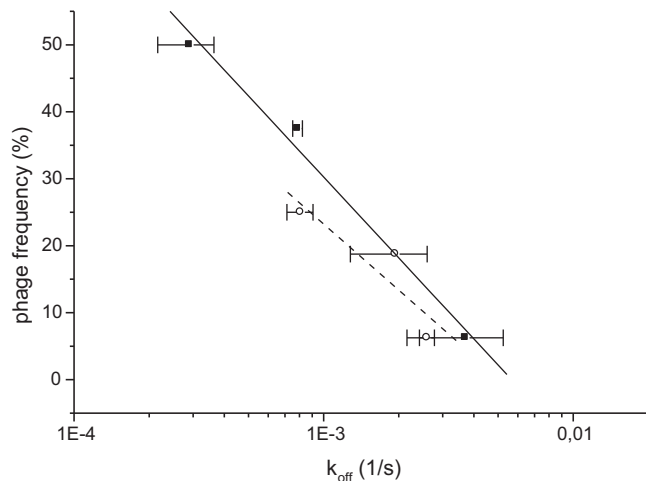


Fig. 2. Correlation between the frequency of phages carrying a certain peptide sequence and their k_{off} rate determined in SPR experiments for the interaction between the phages and their respective antibodies (Bsi0088 solid squares with solid line, $r^2 = 0.986$; Bsi0086 open circles with dashed line, $r^2 = 0.795$). Plot was generated using data from Table 2.

interaction. To characterize quantitatively the binding pairs we analyzed the binding parameters at four mAb concentrations, and to study the reproducibility of the technique we repeated each experiment 2–4 times.

The binding parameters (k_{on} , k_{off} , K_D) were calculated for each measured antibody concentration. The average and standard deviation of the four values are summarized for each series in Table 3.

The quality measures of the interactions between the immobilized biotinylated peptides and antibodies are reproducibly obtainable using the setup. The kinetic and affinity constants are accurate and reproducible giving a good view on the mode of interaction between monoclonal antibodies and mimotope peptides. The dissociation parameters of binding (k_{off}) were in good agreement with those measured between immobilized antibodies and phages, however we noticed that association is significantly slower in the case of peptide–antibody interactions. This slower association might have been resulted from the use of different chips for the antibody–phage vs. peptide–antibody interactions (C1 vs. SA-coated chips respectively) as the quality of Biacore surface matrix is known to affect affinity and binding kinetics of antibodies [39]. Similarly the antigen–antibody binding might have been influenced by the different electrostatic properties of the antigens. Engineering electrostatic attraction between protein–complex partners resulted in hundreds of fold faster binding rates compared to wild type partners [40] and using the same principle antibody fragments with increased association rates have been designed [41]. The agreement between the dissociation parameters, which are the main determinants of the interactions in focus, however supports our suggestion from the phage binding experiments, that the mere complexity of different mimotope carrying phages is a valuable clue for selecting mAbs peptide partners with low k_{off} .

Table 3
Bsi0088 binding parameters on peptide 088A-bio and 088B-bio.

Peptide	Antibody	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)	Standard deviation of K_D (M)
088A-bio	Bsi0088	1.00E+04	1.58E−04	2.07E−08	1.64E−08
088B-bio	Bsi0088	4.30E+03	6.82E−04	1.59E−07	2.14E−08

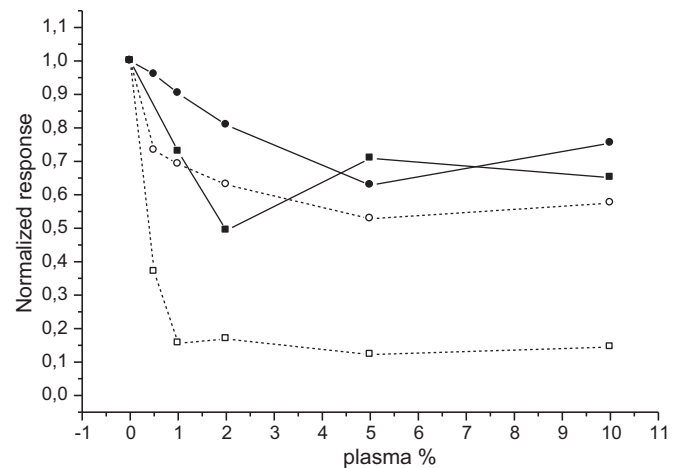


Fig. 3. Plasma competition of the interaction between Bsi0088/Bsi0080 antibodies and the two immobilized peptides. Results are expressed as the fraction of the normalized (maximal) binding. The interactions involving Bsi0080 are shown with squares, Bsi0088 with circles, 088A-bio with open symbols and dashed lines, while 088B-bio with solid symbols and lines. Only the interaction between Bsi0080 and 088A-bio can be competed quantitatively with plasma.

3.3. Inhibition experiments with complex cognate antigen mix

The antibodies used in this study were made against available epitopes on plasma proteins, and their cognate antigen, i.e. the protein carrying this epitope is not known yet. Their specific recognition of biotinylated plasma proteins has been verified with different plasma preparations; however, it was of particular interest whether the specificity of the binding of antibodies to immobilized peptides could be verified with a kinetic approach via a plasma competition assay. For this type of data analysis the association and dissociation curves are not informative, but the amount of bound proteins (maximum response) can be used [42] for the calculation. In preliminary experiments the dynamic range of the competition has been determined, which proved to be between 0.5 and 5% plasma concentration. The peptides selected for this study, were different from each other, however they contained a well definable motif (xSxxxxWxxHPF/Y) (Table 1). The inhibition of the interaction between the antibody–peptide pairs showed dose-dependency but could not be competed off quantitatively for each interaction. The response at higher plasma concentration was still high, about half of the original response (Fig. 3) for several interactions. The incomplete competition suggests that the mimotope peptide does not completely mimic the epitope recognized by the mAb. It might be possible that the motifs represent some of the features of the original epitopes, and binding of the antibody to the peptide antigen happens through conformational changes (induced fit) [43,44]. The induced structural change materializes in a significant population of the antibody, and this interaction “locks” the peptide at the antibody binding site which hence cannot be competed off by the original antigen. As the induced fit trapped peptide might not be a real mimic of the original antigen, in this case the specificity of the interaction is questionable. The complete inhibition of the binding of mAb Bsi0080 to 088A-bio peptide indicates that the resemblance of the original epitope and the selected mimotope in case of this antibody peptide pair should be close to each other. We expanded the plasma competition experiments to other peptide–mAb pairs, and found that several of the interactions could be competed off fully, as an indication of the same binding site for the mimotope peptide and the natural antigen recurring in plasma. We suggest that phage display result derived peptide studies, if carefully and censoriously analyzed, provide additional information on the cognate antigenic site.

4. Conclusion

Here we conclude that complexity of selected individual mimotope carrying phages provides an early clue for the selection of high quality mAb–peptide pairs for further characterization of mAb specificity. We show that SPR experiments with phage particles are suitable to confirm the selection of mAbs and peptides with low k_{off} . These experiments correlate well with those obtained with synthetic peptides, which are then suitable to show specificity in context of the natural antigen and for the selection of mAb peptide pairs for the development of quantitative assays. We suggest that in mAb proteomics the workflow proposed here is a suitable initial tool in mAb scouting for candidate assay development and initial mAb characterization in the absence of cognate epitope and purified cognate antigen.

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References

- [1] Hanash S. Disease proteomics. *Nature* 2003;422:226–32.
- [2] Aebersold R, Mann M. Mass spectrometry-based proteomics. *Nature* 2003;422:198–207.
- [3] Haab BB. Applications of antibody array platforms. *Curr Opin Biotechnol* 2006;17:415–21.
- [4] Poetz O, Hoeppe S, Templin MF, Stoll D, Joos TO. Proteome wide screening using peptide affinity capture. *Proteomics* 2009;9:1518–23.
- [5] Wingren C, James P, Borrebaeck CAK. Strategy for surveying the proteome using affinity proteomics and mass spectrometry. *Proteomics* 2009;9:1511–7.
- [6] Anderson NL, Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. *Mol Cell Proteomics* 2002;1:845–67.
- [7] Rifai N, Gillette MA, Carr SA. Protein biomarker discovery and validation: the long and uncertain path to clinical utility. *Nat Biotechnol* 2006;24:971–83.
- [8] Uhlén M, Björling E, Agaton C, Szgyarto CA, Amini B, Andersen E, Andersson A, Angelidou P, Asplund A, Asplund C, Berglund L, Bergström K, Brumer H, Cerjan D, Ekström M, Elobeid A, Eriksson C, Fagerberg L, Falk R, Fall J, Forsberg M, Björklund MG, Gumbel K, Halimi A, Hallin I, Hamsten C, Hansson M, Hedhammar M, Hercules G, Kampf C, Larsson K, Lindskog M, Lodewyckx W, Lund J, Lundberg J, Magnusson K, Malm E, Nilsson P, Odling J, Oksvold P, Olsson I, Oster E, Ottosson J, Paavilainen L, Persson A, Rimini R, Rockberg J, Runeson M, Sivertsson A, Sköllerö A, Steen J, Stenvall M, Sterky F, Strömberg S, Sundberg M, Tegel H, Tourle S, Wahlund E, Waldén A, Wan J, Wernérus H, Westberg J, Wester K, Wrethagen U, Xu LL, Hober S, Pontén F. A human protein atlas for normal and cancer tissues based on antibody proteomics. *Mol Cell Proteomics* 2005;4:1920–32.
- [9] Mazor Y, Van Blarcom T, Mabry R, Iverson BL, Georgiou G. Isolation of engineered, full-length antibodies from libraries expressed in *Escherichia coli*. *Nat Biotechnol* 2007;25:563–5.
- [10] Holt LJ, Enever C, de Wildt RM, Tomlinson IM. The use of recombinant antibodies in proteomics. *Curr Opin Biotechnol* 2000;11:445–9.
- [11] Wingren C, Steinhauer C, Ingvarsson J, Persson E, Larsson K, Borrebaeck CAK. Microarrays based on affinity-tagged single-chain fv antibodies: sensitive detection of analyte in complex proteomes. *Proteomics* 2005;5:1281–91.
- [12] Löfblom J, Feldwisch J, Tolmachev V, Carlsson J, Ståhl S, Frejd FY. Affibody molecules: engineered proteins for therapeutic, diagnostic and biotechnological applications. *FEBS Lett* 2010;584:2670–80.
- [13] Gold L, Ayers D, Bertino J, Bock C, Bock A, Brody EN, Carter J, Dalby AB, Eaton BE, Fitzwater T, Flather D, Forbes A, Foreman T, Fowler C, Gawande B, Goss M, Gunn M, Gupta S, Halladay D, Heil J, Heilig J, Hicke B, Husar G, Janjic N, Jarvis T, Jennings S, Katilius E, Keeney TR, Kim N, Koch TH, Kraemer S, Kroiss L, Le N, Levine D, Lindsey W, Lollo B, Mayfield W, Mehan M, Mehler R, Nelson SK, Nelson M, Nieuwlandt D, Nikrad M, Ochsner U, Ostroff RM, Otis M, Parker T, Pietrasiewicz S, Resnicow DI, Rohloff J, Sanders G, Sattin S, Schneider D, Singer B, Stanton M, Sterkel A, Stewart A, Stratford S, Vaught JD, Vrkljan M, Walker JJ, Watrobka M, Waugh S, Weiss A, Wilcox SK, Wolfson A, Wolk SK, Zhang C, Zichi D. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One* 2010;5:e15004.
- [14] Carlsson A, Wingren C, Kristensson M, Rose C, Fernö M, Olsson H, Jernström H, Ek S, Gustavsson E, Ingvar C, Ohlsson M, Peterson C, Borrebaeck CAK. Molecular serum portraits in patients with primary breast cancer predict the development of distant metastases. *Proc Natl Acad Sci U S A* 2011;108:14252–7.
- [15] Sanchez-Carbayo M, Socci ND, Lozano JJ, Haab BB, Cordon-Cardo C. Profiling bladder cancer using targeted antibody arrays. *Am J Pathol* 2006;168:93–103.
- [16] Olsson N, Wingren C, Mattsson M, James P, O'Connell D, Nilsson F, Cahill DJ, Borrebaeck CAK. Proteomic analysis and discovery using affinity proteomics and mass spectrometry. *Mol Cell Proteomics* 2011;10: M110.003962.
- [17] Csanky E, Olivova P, Rajnavolgyi E, Hempel W, Tardieu N, Katalin ET, Jullien A, Malderez-Bloes C, Kuras M, Duval MX, Nagy L, Scholtz B, Hancock W, Karger B, Guttman A, Takacs L. Monoclonal antibody proteomics: discovery and prevalence of chronic obstructive pulmonary disease biomarkers in a single step. *Electrophoresis* 2007;28:4401–6.
- [18] Guergova-Kuras M, Kurucz I, Hempel W, Tardieu N, Kadas J, Malderez-Bloes C, Jullien A, Kieffer Y, Hincapie M, Guttman A, Csanky E, Dezso B, Karger BL, Takacs L. Discovery of lung cancer biomarkers by profiling the plasma proteome with monoclonal antibody libraries. *Mol Cell Proteomics* 2011;10: p. M111.010298.
- [19] Szebeni J, Weiszhar Z, Rozsnyay Z, Martinsky T, Kadas J, Lazar J, Takacs L, Kurucz I. Plasma proteome profiling with monoclonal antibody libraries: a pilot biomarker analysis for nanomedicine-induced complement activation. *Adv Nanoparticles* 2013;2:133–44.
- [20] Smith GP. Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 1985;228:1315–7.
- [21] Smith GP, Petrenko VA. Phage display. *Chem Rev* 1997;97:391–410.
- [22] Pande J, Szewczyk MM, Grover AK. Phage display: concept, innovations, applications and future. *Biotechnol Adv* 2010;28:849–58.
- [23] Böttger V, Böttger A. Epitope mapping using phage display peptide libraries. *Methods Mol Biol* 2009;524:181–201.
- [24] Zheng L, Li B, Qian W, Zhao L, Cao Z, Shi S, Gao J, Zhang D, Hou S, Dai J, Wang H, Guo Y. Fine epitope mapping of humanized anti-IgE monoclonal antibody omalizumab. *Biochem Biophys Res Commun* 2008;375:619–22.
- [25] Woo M, Heo C, Hwang H, Ko J, Yoo H, Cho E. Optimization of phage-immobilized ELISA for autoantibody profiling in human sera. *Biotechnol Lett* 2011;33:655–61.
- [26] de Moura J, Felicori L, Moreau V, Guimarães G, Dias-Lopes C, Molina L, Alvarenga LM, Fernandes P, Frézar D, Ribeiro RR, Fleury C, Nguyen C, Molina F, Granier C, Chávez-Olórtegui C. Protection against the toxic effects of *Loxosceles intermedia* spider venom elicited by mimotope peptides. *Vaccine* 2011;29:7992–8001.
- [27] Casey JL, Coley AM, Parisi K, Foley M. Peptide mimics selected from immune sera using phage display technology can replace native antigens in the diagnosis of Epstein-Barr virus infection. *Protein Eng Des Sel* 2009;22:85–91.
- [28] Wu D, Li C, Qin C, Ren X. Phage displayed peptides to avian H5N1 virus distinguished the virus from other viruses. *PLoS One* 2011;6:e23058.
- [29] Barlow DJ, Edwards MS, Thornton JM. Continuous and discontinuous protein antigenic determinants. *Nature* 1986;322:747–8.
- [30] Fack F, Hügler-Dörr B, Song D, Queitsch I, Petersen G, Bautz EK. Epitope mapping by phage display: random versus gene-fragment libraries. *J Immunol Methods* 1997;206:43–52.
- [31] Geysen HM, Rodda SJ, Mason TJ. A priori delineation of a peptide which mimics a discontinuous antigenic determinant. *Mol Immunol* 1986;23:709–15.
- [32] Geysen HM, Mason TJ, Tribbick G, Edmundson AB. Mimotopes-mimics of antibody epitopes. In: Lernmark A, Dryberg T, Terenius L, Hokfelt B, editors. *Molecular mimicry in health & diseases*. Amsterdam: Elsevier Pub; 1988. p. 102–10.
- [33] Huang J, Gutteridge A, Honda W, Kanehisa M. Mimox: a web tool for phage display based epitope mapping. *BMC Bioinformatics* 2006;7:451.
- [34] Mayrose I, Shlomi T, Rubinstein ND, Gershoni JM, Ruppin E, Sharan R, Pupko T. Epitope mapping using combinatorial phage-display libraries: a graph-based algorithm. *Nucleic Acids Res* 2007;35:69–78.
- [35] Ferrières G, Villard S, Pugnieri M, Mani JC, Navarro-Teulon I, Rharbaoui F, Laune D, Loret E, Pau B, Granier C. Affinity for the cognate monoclonal antibody of synthetic peptides derived from selection by phage display. Role of sequences flanking the binding motif. *Eur J Biochem* 2000;267:1819–29.
- [36] Houshmand H, Fröman G, Magnusson G. Use of bacteriophage t7 displayed peptides for determination of monoclonal antibody specificity and biosensor analysis of the binding reaction. *Anal Biochem* 1999;268:363–70.
- [37] Khurana S, Kennedy M, King LR, Golding H. Identification of a linear peptide recognized by monoclonal antibody 2d7 capable of generating ccr5-specific antibodies with human immunodeficiency virus-neutralizing activity. *J Virol* 2005;79:6791–800.
- [38] Soykut EA, Dudak FC, Boyaci IH. Selection of staphylococcal enterotoxin b (seb)-binding peptide using phage display technology. *Biochem Biophys Res Commun* 2008;370:104–8.
- [39] Drake AW, Tang ML, Papalia GA, Landes G, Haak-Frendscho M, Klakamp SL. Biacore surface matrix effects on the binding kinetics and affinity of an antigen/antibody complex. *Anal Biochem* 2012;429:58–69.
- [40] Schreiber G, Shaul Y, Gottschalk KE. Electrostatic design of protein–protein association rates. *Methods Mol Biol* 2006;340:235–49.
- [41] Marvin JS, Lowman HB. Redesigning an antibody fragment for faster association with its antigen. *Biochemistry* 2003;42:7077–83.
- [42] Jelonek M, Natarajan K, Margulies D. Physical measurements of the interaction of MHC molecules and t-cell receptors. In: Nagata K, Handa H, editors. *Real-time analysis of biomolecular interactions*. Tokyo: Springer-Verlag; 2000. p. 115–25.
- [43] Colman PM. Structure of antibody–antigen complexes: implications for immune recognition. *Adv Immunol* 1988;43:99–132.
- [44] Rini JM, Schulze-Gahmen U, Wilson IA. Structural evidence for induced fit as a mechanism for antibody–antigen recognition. *Science* 1992;255:959–65.